Biology of the Blue Mussel
(*Mytilus edulis*)

A Manual Prepared for Participants of the Water Quality Monitoring Programme for Mussel Growers*

Prepared by
M. Brylinsky

Publication No. 9 of the
Acadia Centre for Estuarine Research
Wolfville, Nova Scotia
January, 1989

*Supported by DSS (Contract No. FP630-7-8219/01-0SC) and
ERDA

Table of Contents

INTRODUCTION

This text has been prepared as part of an extensive water quality monitoring programme presently being developed for mussel growers in the Atlantic Maritime Provinces. Its purpose is to introduce non-biologists, specifically practicing mussel culturists, to the subject of mussel biology as it applies to the culture of the blue mussel, *Mytilus edulis*. Its intent is to describe, in layman's terms, the major biological concepts necessary to understand how mussels live, grow, and reproduce, and the major environmental factors that determine the rate at which these processes occur, so that growers participating in the monitoring programme will better understand the logic and concepts behind the various monitoring tasks. It does not deal with the technical aspects of mussel culture.

The manual is organized into three major sections. The first section deals with the general biology of mussels in terms of their taxonomy, anatomy and physiology. The concepts presented here are important to understanding the way mussels are affected by the environment in which they live and how they in turn affect their environment. The second section deals with the ecology of mussels and the physical, chemical and biological environmental factors currently thought to be most important in controlling the settlement, growth and survival of mussels. Measurement of these factors constitutes the core of the monitoring programme. The last section provides a general description of the techniques being employed in the monitoring programme, but does not attempt to provide step-by-step instructions. The latter is the subject of another manual that should be considered supplementary to this one.

We have attempted to present this information in layman's terms, assuming that most mussel growers will not have had a strong background in biology, but with enough detail to cover the topics in a more than superficial way. There are many technical terms used by biologists. No attempt has been made to avoid the use of these terms since it is important that mussel growers are familiar with them and understand the concepts behind their use. Only in this way will growers be able to converse intelligently with biologists and gain a better understanding of the biological and environmental factors important to the successful cultivation of mussels. In addition, familiarity with scientific terms and concepts will allow growers to better convey to biologists the many invaluable observations they make during the long hours spent in the field while growing mussels.

To help ease the task of learning new terminology, all scientific and technical terms have been gathered together in a glossary presented at the end of the text. When a scientific term is first used it is underlined and the page on which it is first used is indicated in the glossary. In this way the glossary also serves as a simple index.

ACKNOWLEDGEMENTS

A number of persons helped in preparing this manual. Dr. G.R. Daborn of the Acadia Centre for Estuarine Research, Mr. L. MacLeod of the Nova Scotia Department of Fisheries, and Dr. R. Thompson of the Marine Science Laboratory of Memorial University provided numerous constructive comments on an early draft of the manual. Ms. Sharon McGladery of the Department of Fisheries and Oceans in Moncton and Dr. G. Johnson of the University of Prince Edward Island Veterinary College contributed the section on diseases and parasites of mussels. Mr. J. Monchamp and Ms. P. Crawford Kellock prepared the figures and diagrams, and Ms. D. Feener typed the manual. I thank all of these individuals for their contribution.

PART I

THE GENERAL BIOLOGY OF *Mytilus edulis*

A. Taxonomy and Evolution

The blue mussel, *Mytilus edulis*, belongs to a large group of organisms collectively referred to as molluscs. The term mollusc, translated from latin, literally means soft or plastic, and stems from the fact that members of this group have a soft, pliable body, although in many instances this body is encased in a hard outer shell. The molluscs are an extremely diverse and successful group of animals that includes some of the slowest moving marine animals, such as the snails, mussels, oysters and clams, as well as some of the fastest moving marine animals, such as the squids. No one really knows how many living species of molluscs exist in the world today--estimates range from 80,000 to 150,000 species. There is also a large number of extinct species of molluscs, on the order of 35,000, that are known only from the fossil remains they have left behind.

Within the molluscs, the blue mussel belongs to the class Bivalvia. Members of this group include the mussels, clams, oysters and scallops and are characterized by having two "valves" or shell pieces that enclose the body. Table I presents a detailed account of the complete taxonomy of *Mytilus edulis* along with some notes indicating the general characteristics that unite members of each taxonomic division. Taxonomic divisions are constantly changing as we learn more about the relationships between various species of organisms. As a result, there are more than one classification scheme. Table I presents one of the more commonly used taxonomies, but others exist.

Table I. Taxonomy of the Blue Mussel (*Mytilus edulis*)

Phylum Mollusca - unsegmented, soft-bodied animals having a foot, mantle and gills enclosed in a hard shell.
- Class Bivalvia - laterally compressed molluscs with the soft body enclosed in a rigid shell of two parts, thin plate-like gills, head absent.
  - Subclass Lamellibranchia - gill filaments folded and adjacent filaments attached by ciliary or tissue junctions.
    - Order Anisomyaria - gills of the filibranch type with interlamellar junctions. Usually sessile. Foot small. Anterior adductor mussel reduced or absent. No siphons.
      - Genus *Mytilus*
        - Species *edulis*

From: Barnes, R.D. 1974. Invertebrate Zoology. W.B. Saunders Co.

Most living molluscs are thought to have evolved from an ancient mollusc that resembled present-day limpets or chitons. In the case of bivalves it is thought that a series of evolutionary changes occurred that allowed these organisms to burrow into soft substrates. The most striking change involved enclosure of the body entirely within a shell of two parts that could be opened and closed at will, and that was compressed into a wedge shape to facilitate burrowing. Another important change was the development of a foot that could be extended from the shell and used to burrow into soft bottoms. Both of these modifications can be easily seen in present-day burrowing clams. *Mytilus edulis*, however, does not burrow and it is thought that it has retained most of these characteristics after evolving from a burrowing mollusc to one which lives attached to a hard substrate. The wedge shaped body and two shell valves remain, but the foot has become modified from a form used for burrowing to one that secretes a thread-like material that functions mainly in anchoring the shell to a substrate.

B. Geographic Distribution

On a world-wide scale, the geographic distribution of most species of marine organisms is controlled mainly by temperature. Although some species can withstand both warm and cold waters, most are adapted to live in either one or the other, but not both. *Mytilus edulis* is no exception--it prefers cold water and is found mainly in the more northern areas of the Atlantic and Pacific Oceans. Along the Atlantic coast of North America it has been found as far north as Greenland and Labrador where during winter it can survive frozen in ice at temperatures as low as -20°C for periods as long as six to eight months. Its southern distribution coincides with an average annual sea temperature of about 27°C. This limits its southerly distribution to the Carolinas along the western Atlantic coast and to the Mediterranean Sea and the North African coast along the eastern Atlantic coast. In the Pacific, *M. edulis* can be found as far north as the White Sea and as far south as California and Japan.

C. Habitat and General Ecology

An organism's habitat refers to the type of place in which it is most commonly found and has become adapted to live in. The blue mussel is a sessile organism, which means that as an adult it has very limited abilities to move from one place to another. As a result, it cannot easily change its habitat once settled and if it happens to settle in an unsuitable habitat it will not survive.

The most important elements of a suitable habitat for *M. edulis* are a hard substrate, such as rocks or compacted sand or mud, on which it can attach itself, and sufficient water movements to bring food and other nutrients and to allow wastes and eggs and sperm to be carried away. Although *M. edulis* is sometimes found in deeper waters attached to rocks, pilings, buoy lines and similar submerged substrates, it seldom does well here, perhaps because of predation by other animals such as starfish and crabs, and its most common habitat is the intertidal zone of coastal shorelines. The intertidal zone is the area of coastline that alternately undergoes periods of exposure to air and submergence by water as a result of the action of tides. Its upper limit is set by the high tide mark and its lower limit by the low tide mark.

The intertidal zone is not an easy environment to live in, particularly along open shorelines that are subject to strong wave action. Although *M. edulis* is commonly found along open coastlines, it does best in intertidal areas having less exposure to wave action, such as the shorelines of estuaries and enclosed bays. In its natural environment, mussels tend to grow together in clumps formed by anchoring themselves together with byssal threads. By forming clumps the mussels reduce the amount of exposed surface area and are better able to withstand strong wave action. They also tend to orient themselves so that the narrow edge of their wedge-shaped shells is positioned toward the direction of incoming waves thus further reducing the chance of becoming dislodged by strong waves.

Aside from the problem of being washed away by strong wave action, there are other difficulties faced by organisms that live within the intertidal zone. During the winter, ice often tends to scour the shoreline and can sometimes completely remove mussels that have settled during the previous year. One of the greatest problems faced by organisms living within the intertidal zone is coping with the wide variations in environmental conditions caused by alternate exposure to air and submergence by sea water. During the summer air temperatures are often much higher than water temperature and in the winter the reverse occurs which means the mussels must be able to adapt to rapid changes in temperatures. In addition, since mussels are not very well adapted to breathing in air, they must be able to withstand periods during which there is little oxygen available. These factors, together with being able to feed only when submerged, makes the intertidal zone one of the most stressful marine habitats and those organisms that do manage to survive there, such as *M. edulis*, are generally very hardy organisms.

In contrast to mussels growing intertidally, mussels attached to long lines as under culture conditions, live under less stressful conditions and are usually able to grow much faster. The greatest advantage to mussels growing attached to long lines is that they always remain submerged and can therefore feed continuously. In addition, they are less subject to sudden changes in temperature and salinity and therefore use less energy to adapt to these changes. As a result they have the opportunity to collect more food while expending less energy than intertidal mussels and, as a consequence, grow much faster. Another important advantage to living suspended within the water column away from the bottom is that there is less chance of being preyed upon by animals that typically live on the sea bottom such as starfish and crabs.

D. Anatomy

Introduction

The anatomy, or general shape and structure of an organism, depends to a large extent on the type of life style it lives. It was previously noted that mussels are sessile organisms as adults. This characteristic is common to many marine organisms--as can be seen if one explores almost any coastline having a stable substrate to which organisms can either attach themselves, such as rocks, or burrow into, such as soft sands and muds. In addition to mussels and other bivalves like oysters and clams, barnacles and sea anemones are good examples of other sessile marine animals.

There are many advantages to having a sessile life style. By eliminating the need for movement, there is little need for a complex skeleton for support and leverage or an intricate muscular system. Nor is there need for a complex nervous system for coordination of movements and sensory perception of the environment into which the organism is moving. Furthermore, since movement is limited there is no requirement for complex respiratory and circulatory systems to rapidly provide nutrients to the various body organs. As a result, sessile organisms tend to have a relatively simple body plan and do not expend large amounts of energy for body maintenance and locomotion. This allows them to put a greater amount of their food intake into growth and reproduction.

Along with all the above advantages of being a sessile organism, there are, however, a number of disadvantages. Since sessile organisms cannot move quickly, it is difficult to escape predators. As a result most sessile organisms have had to enclose their soft body parts in a protective external skeleton of some kind. As in *M. edulis*, this skeleton commonly not only affords protection from predators, but also allows the organism a place of refuge to withdraw into when external environmental conditions become unfavorable.

Perhaps the greatest disadvantage to being sessile is that these organisms must rely on water currents to bring food materials and nutrients to them as well as for dispersion of metabolic wastes. Where complexity in body structure does occur in sessile organisms, it is usually associated with the feeding structures required to obtain food particles from the surrounding water. This is particularly true of *Mytilus* as we shall see when we discuss the feeding and digestive systems (section IE).

One other important problem associated with being sessile concerns that of colonizing new habitats. Since most sessile animals cannot change their location to any great extent, they must have another means of dispersal. This is accomplished in most instances by having a life cycle that involves external fertilization and a free-swimming <u>planktonic</u> larval stage. External fertilization solves the problem of males and females having to find each other, and the free-swimming larval stage allows offspring to be distributed over large areas by oceanic water currents.

Owing to their sessile life style, bivalves are perhaps the most modified in structure when compared to other molluscs. Their general anatomy is characterized by a well developed external shell that encloses a large <u>mantle cavity</u> containing the gills and other soft body parts. In body plan, mussels exhibit <u>bilateral symmetry</u>. This means that if a mussel is cut into two halves along the midline there will be two parts, each of which is a mirror image of the other. Mussels also have an <u>anterior</u> (head region), <u>posterior</u> (tail region), <u>dorsal</u> surface (back region) and <u>ventral</u> surface (belly region). Figure 1 illustrates the location of these areas along with some of the other terms used by biologists to describe the external anatomy of a mussel. Note that under natural conditions, mussels attach and orient themselves so that their posterior points upwards, which means that they literally stand on their heads.

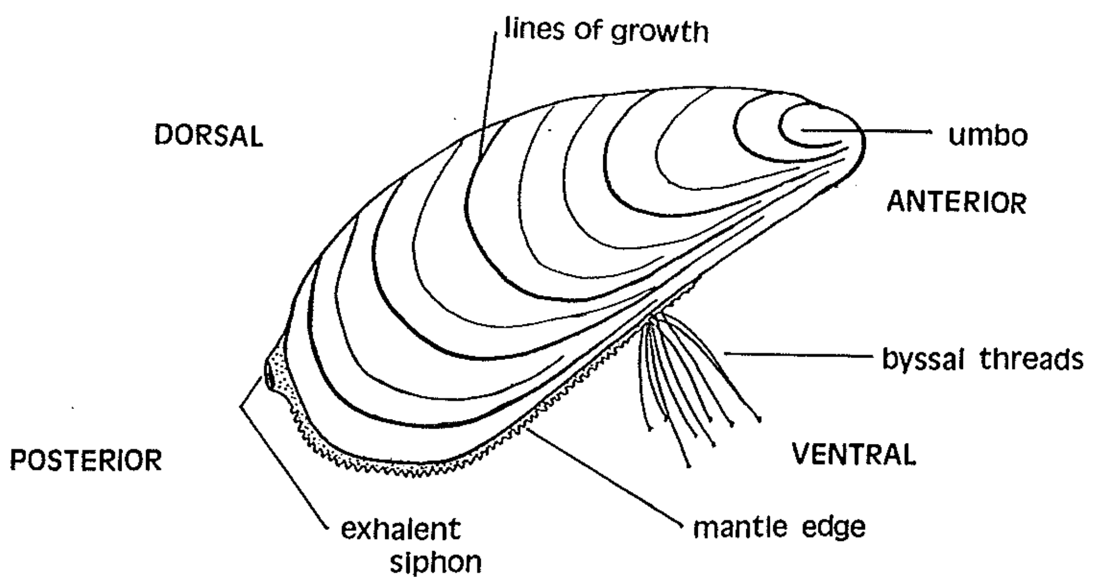

Figure 1. The external anatomy of *Mytilus edulis*.

The Shell

The most obvious external feature of a mussel is its shell. The shell consists of two valves which are attached along the midline on the dorsal side. Although each shell is more or less ovoid in shape, shell shape is variable and depends not only on environmental conditions but also on the age of the mussel, older mussels tending to have heavier and wider shells. Of the numerous environmental factors affecting shell shape, the most important is the degree to which the mussel is subjected to wave action. Mussels growing on open shores exposed to strong wave action tend to have rounder and thicker shells than those growing in more sheltered areas. Mussels growing on longlines under culture conditions are generally not subjected to strong wave action and tend to have thin, elongated shells. Density also appears to be important in controlling shell shape. Under conditions where mussels grow in dense clusters the shell tends to become elongated, perhaps in an effort to grow above and away from neighbouring mussels in order to compete better for food materials.

Each valve has a rounded protuberance at the anterior end called an umbo. This is the oldest part of the shell and it is surrounded by a series of concentric lines often referred to as "growth lines." These are somewhat similar to the lines that can be observed in a cross section of a tree stump and, to one who is well experienced, they can give an indication of the age of a mussel as well as how its growth rate has varied with time and season.

Along the dorsal midline, the two valves are attached by an elastic ligament called the hinge. This ligament consists of a horny material and is constructed so that when the valves are closed the outerpart is stretched and the inner part is compressed (Figure 2). As a result of this arrangement, when the internal muscles holding the shell closed are relaxed, the ligament causes the valves to spring open. Two muscles, the anterior and posterior adductors, when contracted hold the valves in a closed position. In *M. edulis*, the anterior adductor muscle is very small and hardly noticeable, the posterior adductor muscle being most important in keeping the valves closed. This muscle can be easily observed if the valves are spread apart, and the point of attachment of the posterior adductor is obvious if one examines the interior surface of a valve.

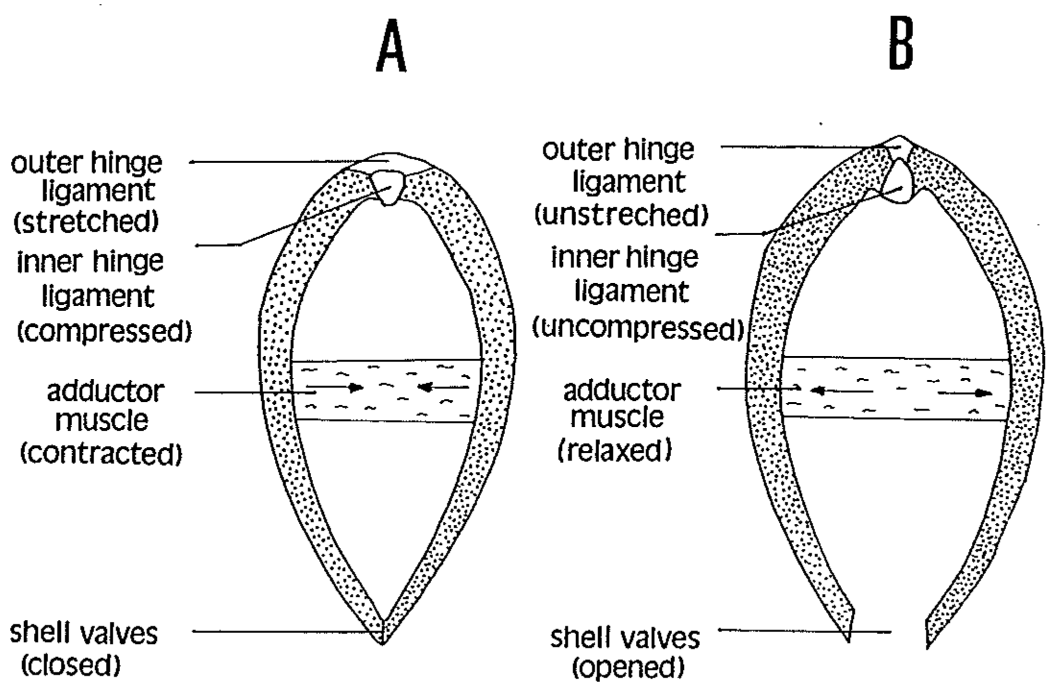

Figure 2. Section through a mussel showing hinge ligaments and adductor muscle. A. valves closed. B. valves open.

The shell is composed of a number of layers which differ mainly in the relative proportion of protein and calcium carbonate (limestone) of which the shell is composed. The outer layer, called the periostracum, contains a high proportion of a horny brown protein which gives the shell its dark color. The inner nacreous layer in contrast contains a high proportion of calcium carbonate which gives it a smooth, irridescent, pearly texture.

The Mantle

Immediately beneath the shell is a large sheet of tissue which envelops the soft body parts contained within the shell. This structure is called the mantle and it serves a number of important functions. The outer surface secretes the materials that compose the inner nacreous layer of the shell. This layer is continuously being secreted by the mantle and tends to become thicker as the mussel ages. If a foreign particle, such as a sand grain, lodges between the mantle and shell, the mantle begins to cover it with a layer of nacreous material. Sometimes the particle will become completely imbedded in the shell, but if it moves about while being covered, a spherical pearl may be formed.

The outer edge of the mantle contains three lobes (Figure 3). The outer lobe is responsible for forming the outer surface layer of the shell (the periostracum) as the mussel grows. The middle lobe contains a number of sensory organs that enable the mussel to respond to touch, various chemical stimuli, and light. The most inner lobe is the largest and contains mainly muscle. When the inner lobe is contracted the body parts enclosed within the mantle are sealed off from the outside. This often occurs when external conditions are unfavorable, such as when the surrounding water contains high amounts of silt or is low in oxygen content. In clams and some other bivalves that burrow into the substrate, this inner lobe is often modified to form the siphons that protrude above the surface. Since mussels do not burrow they have no siphons, but the inner lobe of the mantle is somewhat modified to form two channels, one in which water enters and another through which water leaves.

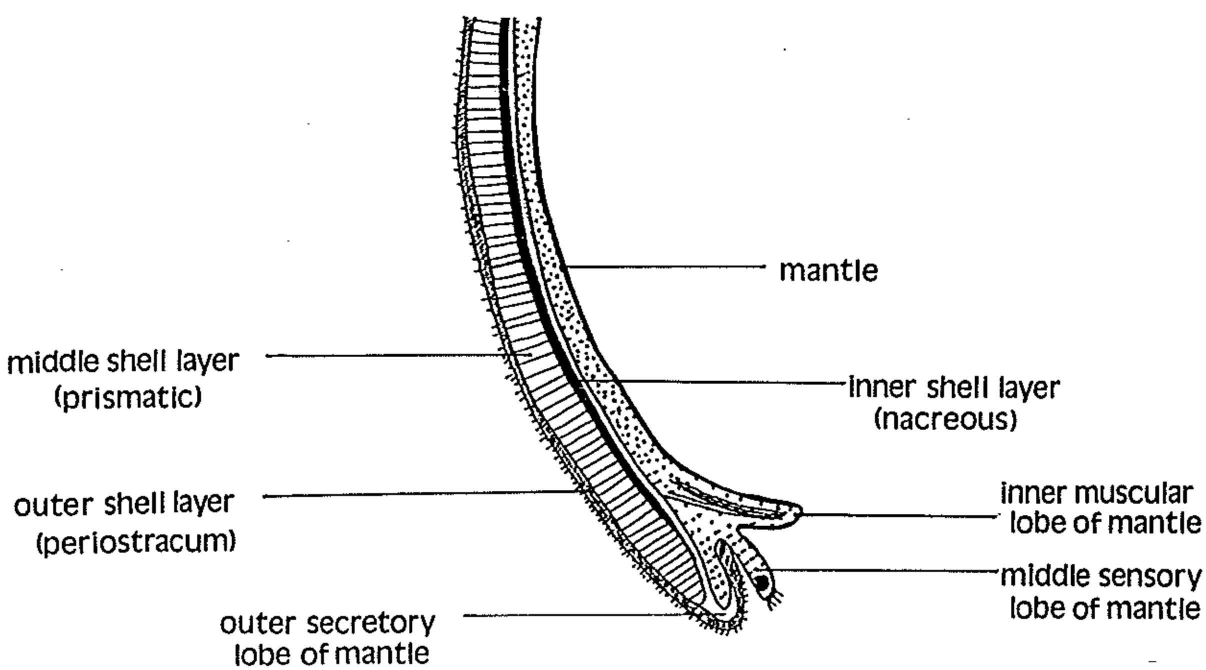

Figure 3. Section through outer edge of mantle.

The Mantle Cavity, Gills and Visceral Mass

The internal space created by the mantle is referred to as the mantle cavity. Within the mantle cavity are the soft body parts of the mussel. These consist mainly of the visceral mass containing the digestive tract and other internal organs, and the delicate gills used for both breathing and feeding (Figure 4). The mantle cavity provides a space into which water enters and from which it exits. It is divided into a lower inhalent chamber and an upper exhalent chamber by the gills which extend from the body of the mussel to the surface of the mantle. Food materials and other nutrients enter the lower portion of the mantle cavity and waste materials and reproductive products (eggs and sperm) exit via the upper mantle cavity.

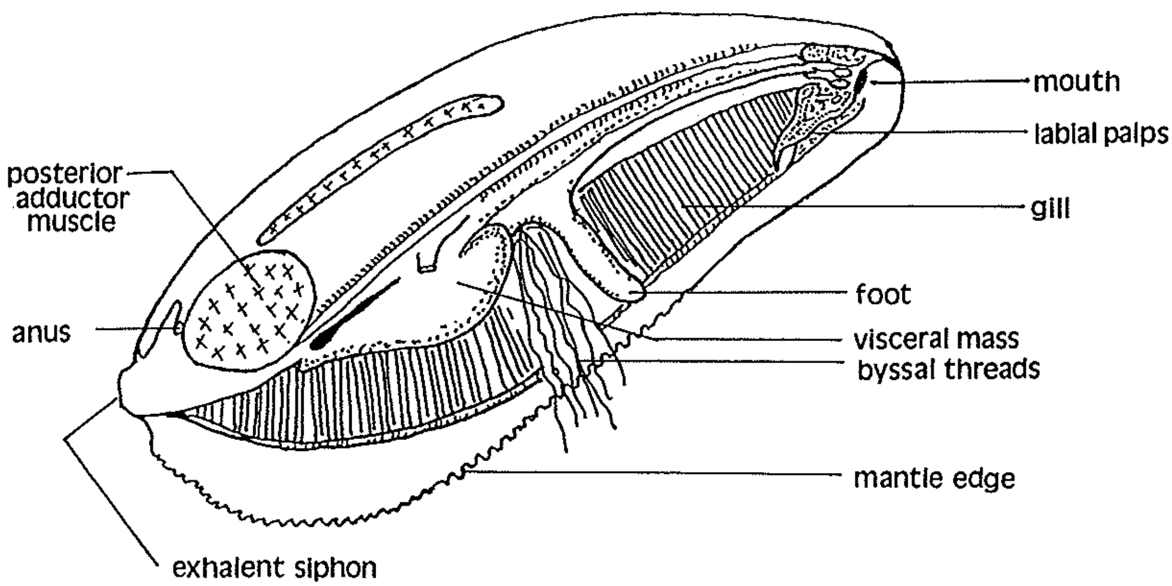

Figure 4. External anatomy of *Mytilus edulis* with shell removed.

The gills are a pair of complex W-shaped structures composed of a series of filaments (Figure 5). Along the surface of each filament are numerous hair-like structures called cilia that continuously wave back and forth in unison to create the water movements that bring materials into and out of the mantle cavity. The cilia form a sieve-like structure which, together with mucous secreted by the gills, filters out particles contained in the incoming water. Once trapped, the particles are incorporated into a mucous string that is carried to the mouth of the mussel by beating cilia. More will be said about this when we discuss the feeding and digestive systems.

The mantle and gills surround the visceral mass which contains the digestive gland and other internal organs. The digestive gland or liver appears as a green, pulpy material. The gonads can often also be seen as a yellowish or whitish material surrounding the visceral mass and, when well-developed, permeating the mantle tissue. Mussels have no well defined head, but if one looks near the anterior region of the visceral mass two small fleshy projections, the labial palps, can be found. These palps surround the mouth and function in transferring the mucous strands containing food materials from the gills to the mouth.

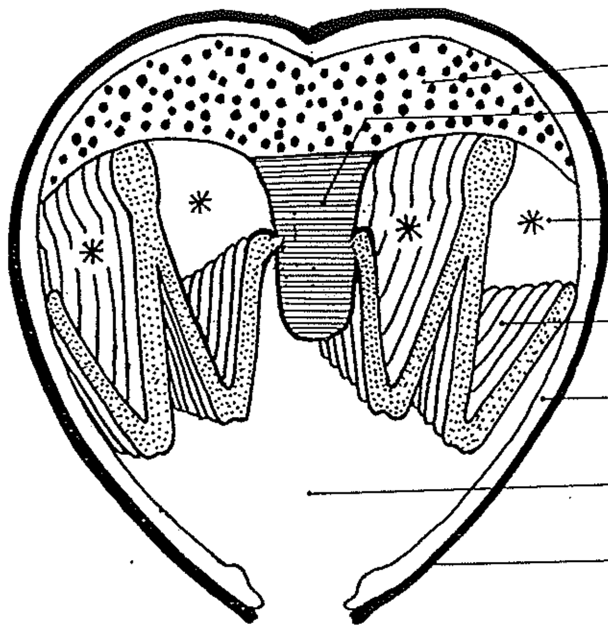

Figure 5. Cross-section through *Mytilus* showing arrangement of gills and separation of mantle cavity into exhalent and inhalent chambers.

The Foot

Located below the palps on the ventral surface of the visceral mass is the foot of the mussel. Most bivalves that live buried in a soft substrate have a large foot that is used for burrowing. In use, the foot is extruded from the shell into the substrate and then enlarged by forcing body fluids into it. This is followed by contraction of the foot and the bivalve is pulled into the substrate. In mussels, which do not burrow, the foot is much smaller and it has become modified to function primarily in positioning and attaching the byssal threads used to anchor the mussel to a hard substrate. At times, however, particularly in its early life stages when still small in size, the foot can be used for movement over short distances by being extended, attached to a substrate, and then contracted to move the mussel forward. This process is often aided by producing a temporary byssal attachment to pull against. Movement in this manner is slow however, the muscle responsible for contraction of the foot having a contraction time on the order of 5 seconds and a relaxation time on the order of 15 seconds.

The primary function of the foot in mussels is for attachment to a hard substrate. To do this the foot is extruded from the shell by the combined action of the pedal protractor mussels and engorgement of the foot with body fluids. The extended foot is then pressed against the substrate and a protein material, secreted from a gland at the base of the foot, flows down a groove to the tip of the foot. There are actually two materials secreted, one from the "white gland" which contains mainly protein, and another substance from the "purple gland" which contains a material that causes the protein to harden. (This process is very similar to that which forms the horny periostracum covering the outer surface of the shell). When the two secretions are mixed and exposed to sea water the byssal thread is formed and hardens. The foot is then withdrawn, leaving the byssal thread behind. This process only takes a few minutes and is repeated until a mass of threads is formed firmly anchoring the mussel to its substrate. To detach itself, the mussel places its foot against the substrate and pries itself loose.

E. Physiology

Physiology

The term physiology refers to the way in which an organism's body parts function in carrying out the various processes vital to life. In this section a brief description of the physiology of mussels is presented.

Feeding and Digestion

Mussels are ciliary-mucus-filter-feeders. This means that they obtain food by filtering small particles from seawater brought into the mantle cavity by water currents created by beating cilia, and then entrap the particles within mucous strands. All of this takes place on the surface of the gills which have become greatly modified in size and structure to serve as both feeding and respiratory organs. The mechanism by which a mussel creates water currents and filters out food particles can best be understood by closely examining the fine structure of the gills. The gills are composed of a series of gill filaments held together by tufts of interlocking cilia. Figure 6 shows a cross-section through one of the two gills of a mussel and illustrates the arrangement and location of cilia, the direction in which collected food is moved, and the places where food is accumulated.

Cilia on the surface of the gill beat in a coordinated fashion to move water from the lower inhalent chamber of the mantle cavity through the gill filaments and then into the upper exhalent chamber of the mantle cavity. At the same time a thin mucous film is continuously secreted by the gills. Particles contained in the inhalent water are screened out by cilia and become bound into mucous strands that are moved by other cilia, first to the food grooves, and then to the labial palps. At the labial palps some sorting of the trapped particles occurs. Heavier and coarser particles, consisting mainly of sands and silts, are passed to the margins of the palps and rejected at the tip. The lighter and finer particles, consisting mainly of organic matter, move across the labial palp to the mouth. Under conditions when more particles are trapped than can be handled by the digestive tract, there is an overspill from the food grooves and the labial palps. This material, together with the materials sorted and rejected by the labial palps, constitutes what is termed pseudofeces. This is ejected from the mantle cavity by rapid opening and closing of the shell which expells the particles.

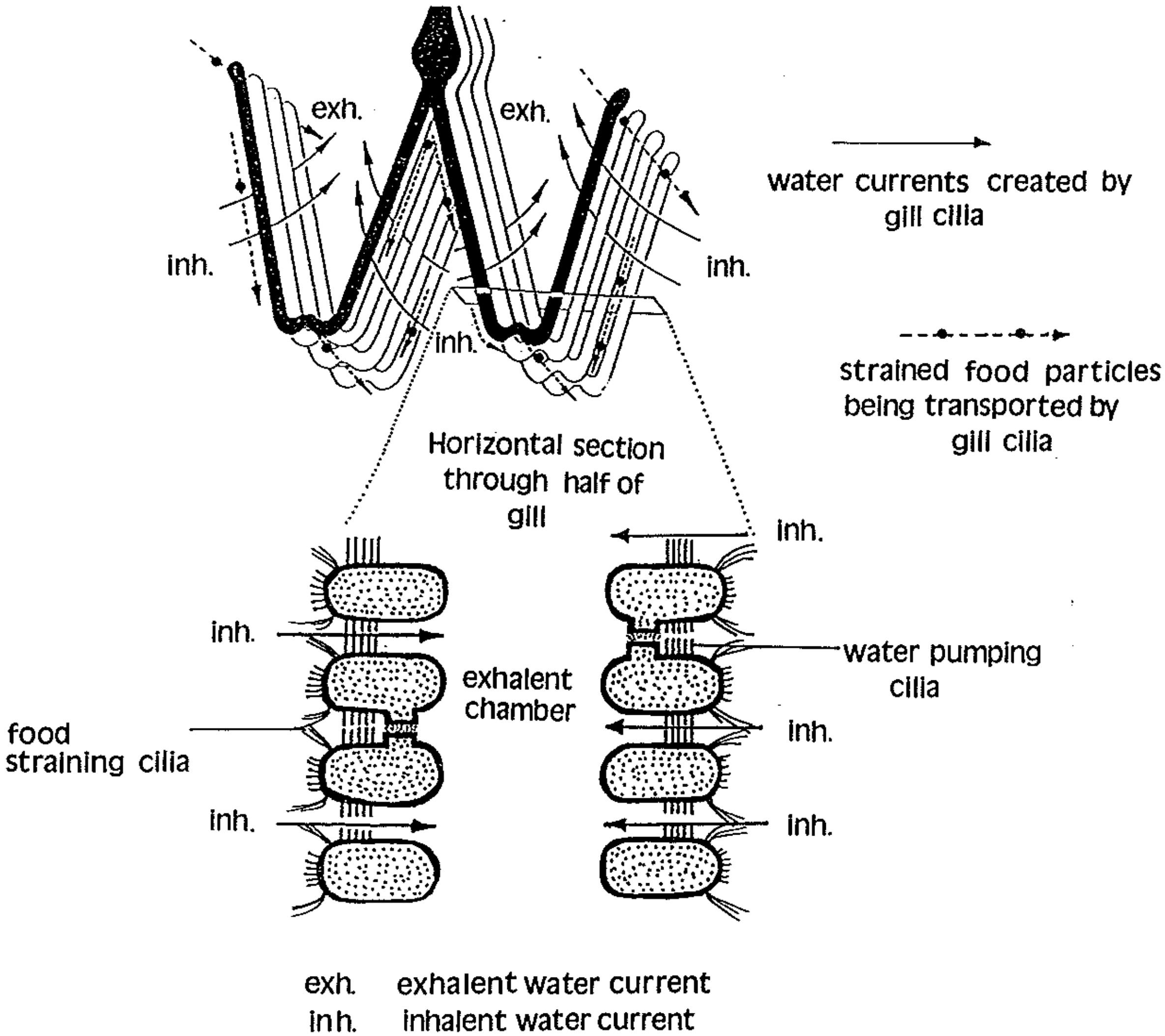

Figure 6. Structure of gills showing path of food particles and location of cilia.

The mucous strands collected by the labial palps are passed to the mouth and drawn into the stomach by a remarkable process. Located within the stomach is a very specialized structure called the crystalline style (Figure 7). This is a rod-shaped structure that continuously revolves within the stomach. As it turns the mucous strand passed to the mouth by the labial palps is continuously wrapped around the rod and thereby pulled into the stomach. Here particles contained within the mucous strand are sorted into digestible (organic) materials and indigestible (inorganic) materials. The digestible materials then travel to the digestive gland surrounding the stomach where they become digested and absorbed. The indigestible materials are passed from the stomach to the intestine where they are formed into fecal pellets that eventually leave the digestive tract via the anus. (This material is called true feces as opposed to pseudofeces.) The

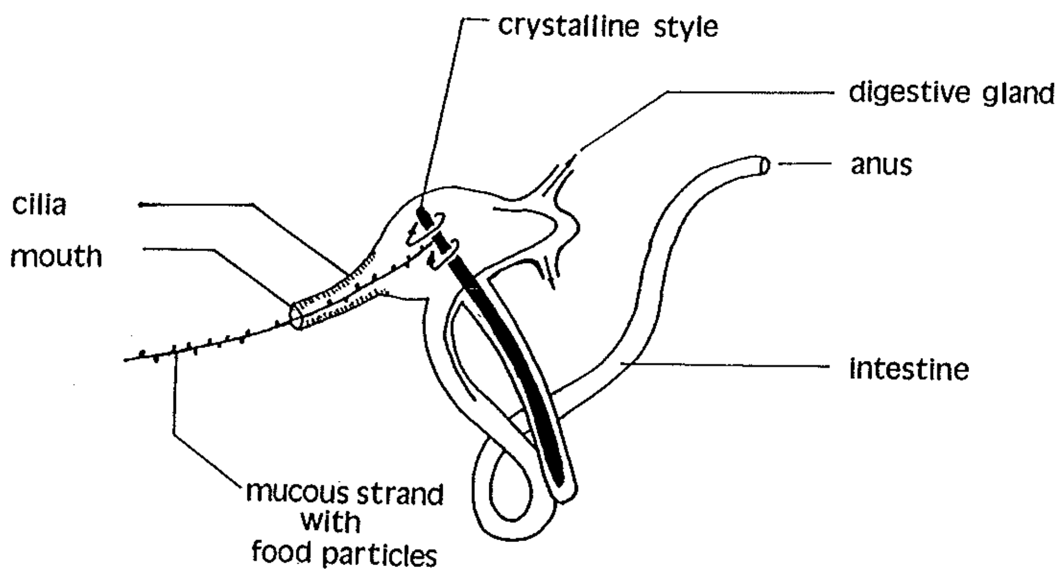

Figure 7. The stomach of *Mytilus edulis* showing the location and mechanism of the crystalline style.

anus empties into the exhalent chamber of the mantle cavity where the fecal pellets eventually become expelled by the exhalent water currents.

The filtering mechanism described above is very efficient in removing small particles from seawater. Virtually all of the small microscopic algae contained in seawater can be removed, and there is even some evidence that particles as small as a single bacterium can be removed. One problem, however, is that, although some sorting of digestible from indigestible particles does occur, if there are large amounts of indigestible matter, particularly fine sands and silts, much of it will be passed into the digestive tract. Under these conditions the amount of true food digested will be reduced, since only a limited amount of material can be ingested altogether.

The rate at which mussels filter water, and thereby collect food, is thought to be related to both water temperature and the amount of food present in the water. It has been estimated that an average size mussel filters somewhere between 30 to 60 times its own volume of water per hour. This means that a single market-size mussel filters about 15 to 30 liters of water per day.

Excretion

In addition to the undigested food materials that pass through the digestive tract and eventually out the anus, all organisms require some mechanism to remove waste products that result from the metabolic processes occurring within the cells and tissues that make up the various body organs. One of the most important metabolic waste products is ammonia, a by-product of the metabolism of proteins, which becomes very toxic if allowed to accumulate. Most food materials also contain various salts many of which are not required for metabolism and must be continuously removed. Mussels remove these waste products in much the same way as other animals by an organ commonly referred to as the nephridium in lower animals or the kidney in higher animals. The nephridia of a mussel are located near the heart and are closely associated with the circulatory system. Blood travels into the nephridia where waste products are removed and then transported via a tube into the exhalent chamber of the mantle cavity where they are swept out by the exhalent water current.

Circulation and Respiration

An organism's circulatory system is responsible for distributing nutrients to and removing waste products from the various organs of the body. The circulatory system is always closely associated with the respiratory system which functions in providing oxygen and removing carbon dioxide from these same organs. Although mussels do have a heart to pump blood around the body, except for a few arteries and veins associated with the head, foot, gills and visceral mass, they do not have a well-developed system of blood vessels. Instead, blood pumped from the heart travels into and out of the various organs through a series of spaces, called body sinuses, located within the organs. Mussels also lack a blood pigment, such as the hemoglobin that gives human blood its red color, and therefore their blood is colorless.

The lack of well-defined arteries and veins as well as the lack of a blood pigment, makes the circulatory system of mussels somewhat inefficient. They partly make up for this by having a large blood volume which accounts for as much as 50% of their body weight. More importantly, however, is that their sedentary life style does not require a highly efficient system capable of delivering nutrients quickly as would be the case for organisms that move rapidly.

The major respiratory organs are, of course, the gills. As water is pumped over the gills oxygen diffuses from the water into the gill filaments. Within each of the gill filaments is a blood vessel and here oxygen diffuses into the blood where it is transferred to other parts of the body. At the same time carbon dioxide diffuses out of the blood, across the gill filaments and into the water contained within the mantle cavity, where it leaves via the exhalent chamber. In addition to the gills, the mantle is also thought to be important in taking up oxygen and releasing carbon dioxide by diffusion.

The Nervous System and Sensory Organs

Being sessile animals, mussels do not require the complex nervous system typical of animals that move about. There is no real brain and there are only two major nerve cords, one of which controls the posterior adductor mussel responsible for rapid closing of the shell, and another which controls the creeping movements and spinning of byssal threads associated with the foot.

Although not well developed, mussels do have a number of sensory organs. Touch and light sensors located within the middle lobe of the mantle edge are probably important in detecting the presence of predators such as starfish and crabs. The foot contains an organ called a statocyst which senses gravity and allows 'up' and 'down' to be distinguished. This allows a mussel to properly orient itself during settling or after either moving or becoming dislodged. Finally, within the mantle cavity is an organ called the osphradium. This is located near the exhalent chamber and, although not known for sure, is thought to function in determining the quality of water passing through the mantle cavity.

The Reproductive System

Under extremely favorable growth conditions mussels can reach sexual maturity within one year of age, but under most natural conditions this does not usually occur until the second year. In *Mytilus*, the sexes are separate although there have been some reports of rare individuals capable of producing both eggs and sperm. The gametes (eggs and sperm) are produced from glycogen reserves that are stored in the gonads. The gonad is a diffuse organ that surrounds the digestive gland and sometimes covers part of the surface of the mantle. At spawning the gametes leave the gonad by a duct that empties into the mantle cavity. The gametes are then swept out of the mantle cavity by the exhalent water currents.

F. Reproductive and Life Cycles of *M. edulis*

The Reproductive Cycle

The reproductive cycle of *Mytilus* consists of a series of events that begin with the activation of the gonads to produce eggs or sperm and end with the recession (shrinking) of the gonads after spawning. It is often divided into five stages (Figure 8); (1) activation of the gonads during which food reserves, consisting mainly of glycogen (a sugar-like compound) stored within the gonads, become transformed into the materials of which eggs and sperm are composed; (2) a process called gametogenesis during which the egg and sperm cells are made; (3) spawning, which involves the expulsion of eggs and sperm into the surrounding water; (4) recession of the gonads; and (5) a resting period during which new energy reserves are accumulated and stored to produce more eggs or sperm for repetition of the cycle.

The factors controlling the beginning and end of each of these stages are not clearly understood but it is known that it involves the interaction of a number of key environmental variables, many of which appear to be related to the amount of food that becomes stored for use in producing eggs and sperm. Among these factors food availability is obviously important, but the amount of food stored depends also on the general environmental conditions under which the mussel is growing. If stressful, as may be the case when water temperatures are high or salinity variations large, much of the assimilated food will be used in overcoming these stresses, and therefore will not be available for storage as glycogen from which the eggs and sperm are ultimately made. In a good year a mussel may produce and spawn gametes two or more times while in a poor year this may happen only once, or perhaps not at all if conditions are extremely poor. There is also considerable variation in the number of gametes that are produced during

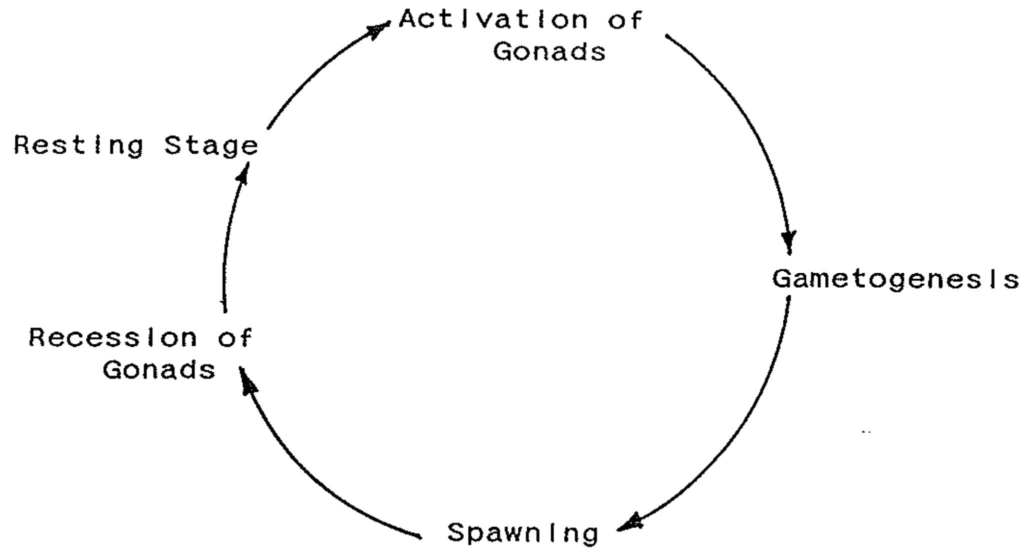

Figure 8. Major stages in the reproductive cycle of Mytilus.

each spawning period, depending on the amount of glycogen that is available for making gametes. Estimates of egg production by females range from as low as a few thousand to as high as several million per spawning.

The large number of gametes usually released is partly a result of fertilization being external rather than internal, since this greatly lessens the chance of an individual egg coming into contact with a single sperm. A major problem faced by organisms that release their eggs prior to fertilization is that males also have to release their sperm at about the same time females release their eggs. In addition, recently spawned eggs of mussels remain viable for only a short period, on the order of hours, and sperm maintain their motility for only a few hours, so it is important that spawning of both sexes occurs simultaneously. It is thought that many organisms having external fertilization, including *Mytilus*, release chemical substances along with the eggs that stimulate males to release their sperm, and in this way synchronous spawning is assured.

The exact stimuli that cause females to begin spawning are poorly understood. Some suggested factors include food availability, water temperature, and rapid changes in environmental conditions. Since fertilized eggs quickly develop into a larval form that feeds on small particles in the water, it is important for the survival of larvae that spawning occurs when food levels are high. In this context the idea that food availability may be an important stimulus seems reasonable and there is some evidence that chemical substances released by phytoplankton, an important food source of larvae, may act as a stimulus to spawning. With regard to water temperature, some studies indicate that degree days (i.e., the amount of heat accumulated over the growing season) rather than absolute water temperature may be the important factor.

Larval Stages and Development

Once spawning occurs, eggs that have been successfully fertilized begin undergoing a series of developmental stages that constitute the larval life cycle (Figure 9). Most of this occurs as part of the plankton*. Within a few hours of fertilization an embryo, called a trochophore larvae, begins to develop. The trochophore larvae is a small spherical organism, about 0.2 mm in diameter, with a band of cilia around its equator that is used for locomotion. The trochophore has the beginnings of a digestive tract with a mouth and anus and can feed, but exists mostly on the yolk reserves stored in the egg.

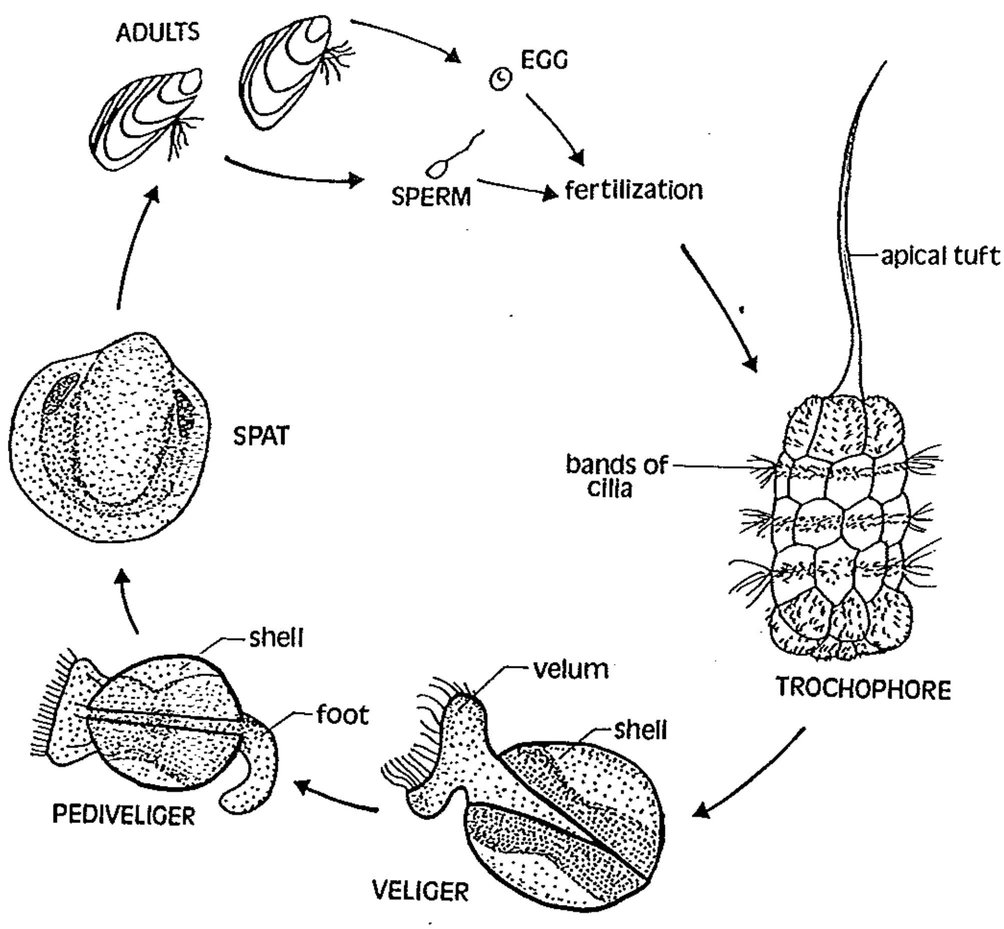

Figure 9. Larval stages of *Mytilus*.

---

*The term plankton is used to refer to organisms, usually microscopic, that live floating within the water and have only limited abilities to move independently of water currents. The term phytoplankton is applied to plant plankton, such as algae, and the term zooplankton is applied to animal plankton.

After about two days the trochophore develops into the veliger stage which may last anywhere from weeks to months depending on the environmental conditions it encounters during this period. The veliger contains a structure called a velum that is used in feeding and, to a limited extent, in locomotion. The velum consists of a pair of ciliated lobes on which food particles are collected and moved to the mouth by cilia. In some respects the veliger resembles an adult mussel in that it has the beginnings of a shell, mantle cavity and foot.

The next stage of the larval life cycle is the pediveliger. It differs from the veliger mainly in that the foot becomes more fully developed in preparation for settlement. At this time the larvae begin to exhibit a pattern of swimming and crawling behavior used in testing the suitability of a substrate for attachment. At the early pediveliger stage the larva is able to attach to a substrate temporarily by secreting a byssal thread and, if the substrate proves unsuitable, detach and swim away in search of a more suitable substrate. This behavior can be repeated for a number of days, but eventually the larva must settle or die.

Settling larvae seem to prefer substrates away from strong wave action that contain surface irregularities, particularly those created by algal films. They also seem to be attracted by the presence of other larvae and by the presence of byssal threads secreted by adult mussels. Some researchers believe that early pediveliger larvae often undergo a primary settlement, usually on a substrate that contains filamentous algae, and then later detach and undergo a secondary settlement, often in an area containing established mussel beds. It has been suggested that this behavior allows the younger larvae to first grow in an area where they are free from competition with adult mussels, but which for some reason may be unsuitable once they grow to a larger size.

After settlement, the pediveliger undergoes metamorphosis (a change in its basic form) and becomes what is commonly referred to as a spat and takes on the form of an adult mussel. This metamorphosis can be delayed for periods as long as one month if suitable environmental conditions are not found. There is some evidence that spat which settle late in the year, when growth conditions may be poor, may remain on a temporary substrate over the winter, and then leave when conditions become more favorable during the following spring.

The larval stages described above constitute one of the most difficult times in the life cycle of a mussel. Being planktonic, the larvae are vulnerable to heavy predation by zooplankton and small fish and, since they have little control over the area into which they are carried by water currents, they may find themselves in a location where environmental conditions are unsuitable for good growth and survival. The longer the larval stages last, the less chance the larvae have of reaching the spat stage. Of the original thousands or millions of eggs spawned by an individual mussel, only a very small percentage survive to become adults.

Being able to predict the timing of events in both the reproductive cycle and larval life stages of mussels is very important to aquaculturists. The general condition of a mussel, particularly in terms of its market quality, is very much dependent on the particular stage of the reproductive cycle it is in, and the time when larvae begin to settle and attach is important to know when collecting spat for culture. From the previous discussion it is obvious that we do not clearly understand the environmental factors, and the ways in which they interact, that control the timing of these events. Both the time of spawning and length of time larvae remain in the plankton can vary considerably from one locality to another. In addition, the fact that larvae may have been spawned at a site far from where they settle makes gathering information on the conditions leading to spawning and settling difficult to obtain. It is obvious that a good deal of basic research still needs to be done before we can hope to make accurate and reliable predictions of spawning and spatfall times for any particular site.

PART II

THE ECOLOGY OF *Mytilus edulis*

A. Seawater as a Medium for Life

Being animals that live on land, we tend to think of the ocean as being a somewhat hostile environment in which to live. Although there are some unique problems associated with living in seawater, the sea is actually a much easier environment than land in which to carry out the various processes necessary to life. Because seawater is much denser than air, there is little need for marine organisms to have a complex skeletal system for support, although the same factor does make it more difficult to move about. Obtaining and conserving water is obviously not the problem for marine organisms that it is for terrestrial organisms, and there is no need for complex systems, such as internal lungs and an impermeable skin covering, to prevent water loss from the body surface. In addition, the body fluids of most animals, whether terrestrial or marine, have about the same salt concentration as seawater. This means that marine animals have less difficulty than terrestrial animals in regulating the loss of salts and therefore can have simpler kidneys and expend less energy in maintaining the proper salt balance in their body fluids. Even reproduction is easier in an aquatic environment. Fertilization of eggs can be accomplished externally in the surrounding water which lessens the need for the complex sex organs and courting behavior that is typical of animals living on land. Perhaps the greatest advantage to living in an aquatic environment is that both daily and seasonal variations in environmental factors tend to be less extreme. In the case of temperature, for example, seawater seldom exceeds the range of -2 to 30°C as compared to the -40 to +40°C range typical of the terrestrial environment, and since water tends to both warm and cool more slowly than air, there tends to be less temperature variation on a daily basis as well.

The major disadvantage to living in seawater is that, under some conditions, oxygen can become limiting, particularly when water temperatures are high and the system is <u>stratified</u> (see Section IIIB). In terrestrial systems, because air contains about 20% oxygen and varies very little, oxygen is rarely in short supply.

Mussels are particularly well-adapted to the marine environment. They obtain their food materials by filtering water that is being continuously being replenished by water movements and therefore do not have to deal with the problem of moving themselves through a dense medium. They reproduce by shedding eggs and sperm into the water and therefore have not had to evolve the complex structures required for internal fertilization and brooding of eggs. The larvae that develop from fertilized eggs lead a planktonic life-style and are distributed by water currents. This allows for distribution into habitats other than that of the adults and eliminates the need for movement by adults to colonize new habitats.

One of the major disadvantages to mussels growing naturally, however, is that they occur mainly in intertidal areas and do not realize the complete benefits of the more constant environmental conditions that would occur if they were always submersed in seawater. Cultured mussels, however, when grown on longlines, do realize this advantage and this is the main reason why they grow and survive better than natural mussels.

B. Environmental Factors Controlling the Growth of Mussels

The rate at which a mussel grows, as well as the length of time it lives, depends on a complex set of environmental factors and interactions. This is especially true of mussels growing in their natural environment, the intertidal zone, which is characterized by strong gradients in environmental conditions. Here mussels are periodically exposed to water and air which often results in large variations in such important factors as temperature, salinity and food availability. Under these conditions the rate of growth, as well as the age of a mussel, is extremely variable.

When we speak of the growth rate of a mussel we are usually referring to the rate at which the mussel increases in shell length. However, mussels also grow by increasing the size of their meats (soft body parts). These two processes are often not directly related to each other. In fact, under some circumstances, such as after spawning or when food materials are low, the size of the meat may actually decrease. The shell size, however, never decreases, but its rate of growth does vary considerably during the lifetime of the mussel. It is for these reasons that the condition index (see Section III D) of a mussel varies according to both season and age of a mussel. It is becoming apparent, on the basis of a number of recent studies, that shell growth and meat growth are not entirely controlled by the same factors. Although food availability and temperature seem to be the most important factors for both, shell growth seems to be more strongly influenced by temperature than does meat growth, whereas meat growth is more strongly influenced by food availability than is shell growth. Understanding these relationships is of obvious importance to growing large, good quality mussels and is presently an active area of research among mussel biologists.

Figure 10 presents a diagram that attempts to summarize the way in which the major physical, chemical and biological environmental factors interact to influence the rate at which mussels grow, reproduce and die. In trying to understand how these factors control the growth rate of a mussel it helps to think of a mussel as a little machine whose purpose is to grow and reproduce more little machines like itself. The major input is food and the major output is more mussel and sperm or eggs.

Generally, the more food ingested the more mussel and reproductive products produced. However, not all of the ingested food material is used for growth and production of sperm and eggs. A good deal of the ingested food is consumed by the mussel in carrying out these processes. For example, processing food involves filtering it from the water, moving it through the digestive system, sorting it into digestible and indigestible materials, breaking down and absorbing the digestible materials, and chemically transforming the food materials into the stuff that mussels are made of. All of these processing steps require energy that ultimately comes from the ingested food materials.

The amount of food energy that is used to process food as well as carry out other functions, such as secretion of byssal threads, adapting to changes in salinity, etc., is reflected in the

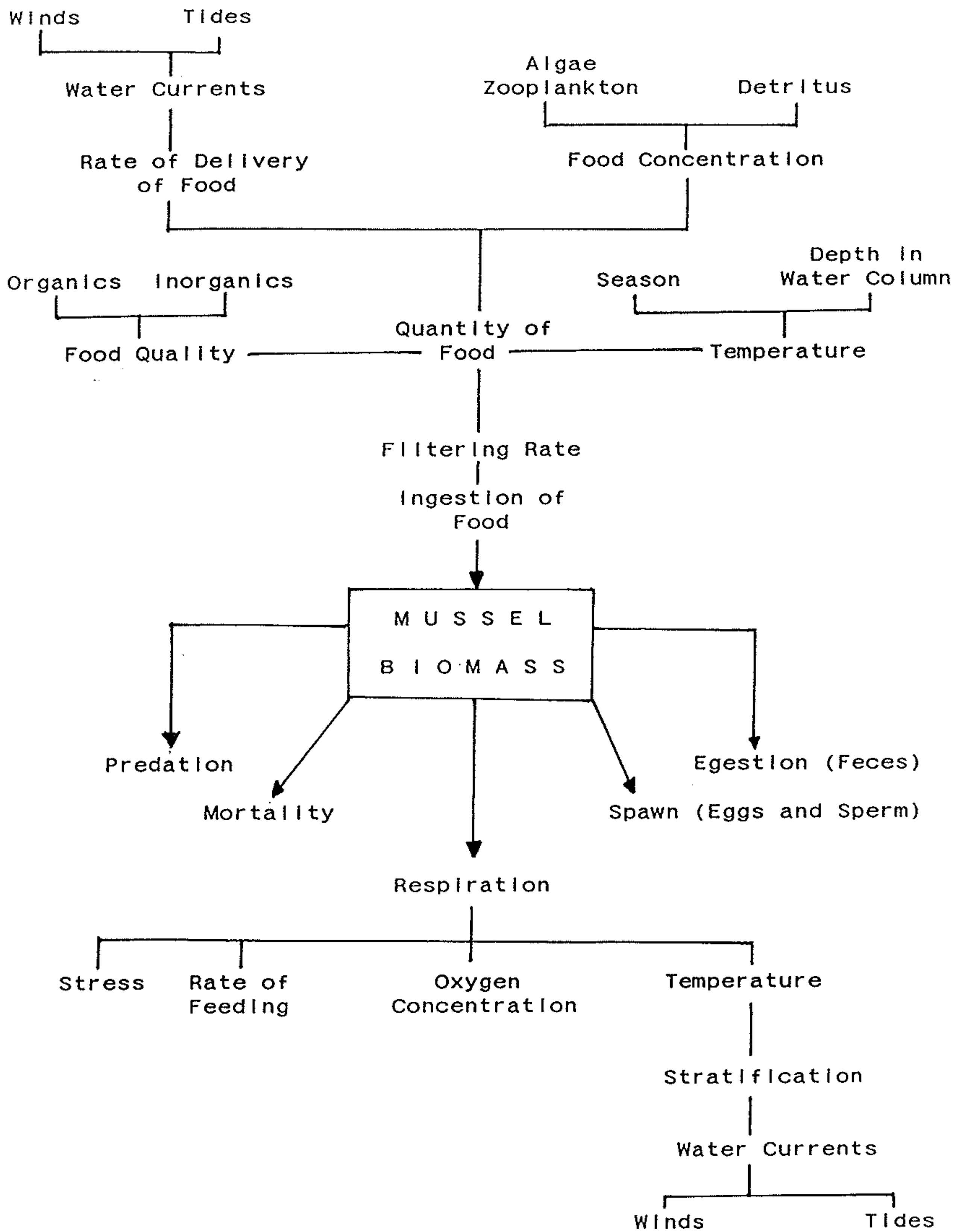

Figure 10. Summary of environmental factors affecting the growth of mussels.

respiration rate. The more the mussel does, the more it will respire. If food intake is low when respiration is high, as it may be in a stressful environment, the mussel may not be able to grow at all. If the environment is extremely unfavorable, as when temperatures are very high and food levels low, the mussel may actually shrink in size and, perhaps, eventually die.

It should be noted that the interactions summarized in Figure 10 are very generalized and simplified and the precise mechanisms by which these factors influence mussels is, in many instances, not clearly understood. One of the problems encountered in trying to understand these relationships is that most environmental factors operate at more than one level and have both direct and indirect effects on mussel biology. For example, temperature directly affects the amount of food digested because the rate at which mussels filter food and metabolize food materials is related to temperature. But temperature also has a number of indirect effects some of which may be beneficial to growth and some of which may be detrimental. At high temperatures the respiration rate of mussels increases. This means less energy is available for growth. In addition, warm water often contains less oxygen than cold water and if temperature becomes too high, a lack of oxygen may produce stressful conditions. Temperature also has an effect on the rate at which nutrients become available to phytoplankton and therefore the amount of phytoplankton, a major food of mussels, in the water. Numerous other examples of the direct and indirect effects of environmental factors could be given and it is obvious that the environmental characteristics of the system surrounding a mussel are quite complex and not easily understood in terms of how a particular change in one factor will affect a mussel. This is why biologists are often hard put to give a simple explanation of why mussels grow better in one place compared with another or why a sudden change has occurred. Hopefully, through constant monitoring of those variables thought to be most important and the development of a good data base on growth rates and environmental conditions we will be able to enhance our understanding and apply this knowledge to the development of improved culture techniques and strategies.

The following section discusses the environmental factors that are thought to be most important in controlling the growth and survival of mussels.

Physical Factors

- Water Movements

The general pattern of water circulation, and the rate at which water circulates, is probably the most important factor determining the general nature of a particular aquatic environment. Water currents produce turbulence that results in the distribution of suspended particles and dissolved materials, as well as heat, and strongly influences the environment of an organism. In aquatic systems where water movements are weak or absent, the environment soon becomes stagnant, often with the accumulation of toxic metabolic wastes, and becomes inhospitable for all but the most hardy of organisms.

Good water circulation is particularly important for sessile organisms like mussels. Mussels filter water and remove food particles and other nutrients at a high rate. If the water they filter is not constantly replenished by water movements, the food it contains would quickly become depleted and the mussels would soon starve to death. In addition, water movements that scour the sea bed redistribute food materials that have settled to the bottom and would otherwise be lost to the sediments. This process, called resuspension, is very important in determining the amount of food available to organisms living within the water column. This same process is also important in determining the degree of turbidity of the water column, which in turn influences the amount of light available for photosynthesis by the phytoplankton and benthic plants that form the basis of most marine food webs.

Water circulation is produced by a number of diverse processes. In coastal areas, the general circulation pattern and the rate at which water circulates depends mainly on the strength of the tides and winds. Tidal exchange is an extremely important process in flushing bays and estuaries and is usually the most important factor in mixing of coastal waters. Winds also move water and create currents, but are less regular than tides and the water movements they create are often limited to the upper few meters of the water column. Fresh water inputs from rivers entering the ocean are also often important in creating currents and mixing.

A less obvious, but often important, process that mixes water masses is water movement created by differences in the density of two water masses. A good example is the mixing of a stratified water column in the fall of each year. As the days become shorter and air temperatures cooler, the surface water cools faster than water near the bottom. As the surface water cools, its density increases and, if the density becomes greater than the bottom waters, it will sink to the bottom to be replaced by bottom water moving to the surface. Eventually this process leads to mixing of the entire water column and materials that have accumulated in the bottom water during the period of summer stratification will be redistributed to the surface waters. If this material is rich in nutrients it often results in a fall phytoplankton bloom.

- Water Temperature

In the case of cold blooded animals, such as mussels, temperature is the major environmental factor that controls metabolic rate. At low temperatures, most physiological processes are slow. As temperature increases there is a consequent increase in these processes which is reflected in a higher rate of respiration. There are limits however and, in the case of *M. edulis*, it appears that temperatures greater than 25°C become stressful and, if combined with other suboptimal conditions such as low oxygen concentrations or poor food availability, will often result in death.

The absolute lethal temperature depends on the age, size and general nutritional condition of the mussel as well as the length of time over which temperature has increased. Younger, smaller mussels are able to tolerate higher temperatures than larger, older mussels. If temperature increases slowly, over a period of days or weeks for example, mussels are often able to acclimate to a higher temperature than if the change occurred over a shorter period on the order of hours. In addition, there also appears to be a genetic component to the lethal temperature, some mussel stocks being able to tolerate higher and more rapid changes in temperature than others.

- Water Column Stratification

In areas where water movements are not great enough to keep the water column completely mixed, a condition known as water column stratification may exist. This occurs when the surface water layer is less dense than the bottom water layer. The factors that cause this density difference are usually related to processes that create a difference in temperature or salinity between the two water masses. In the case of temperature differences, the warming effect of water by the sun is due mainly to infra-red radiation which only penetrates the water column to a depth of about one meter. As a result, only the upper surface layer of the ocean is heated by the sun and, if wind or tide induced currents are weak, the surface water will retain most of the heat and become less dense than the bottom water. Between the upper and bottom water layer is a zone of temperature transition, called the thermocline (Figure 11). The larger the temperature difference between surface and bottom waters, the stronger the stratification and the greater will be the amount of mixing energy required to mix the two layers.

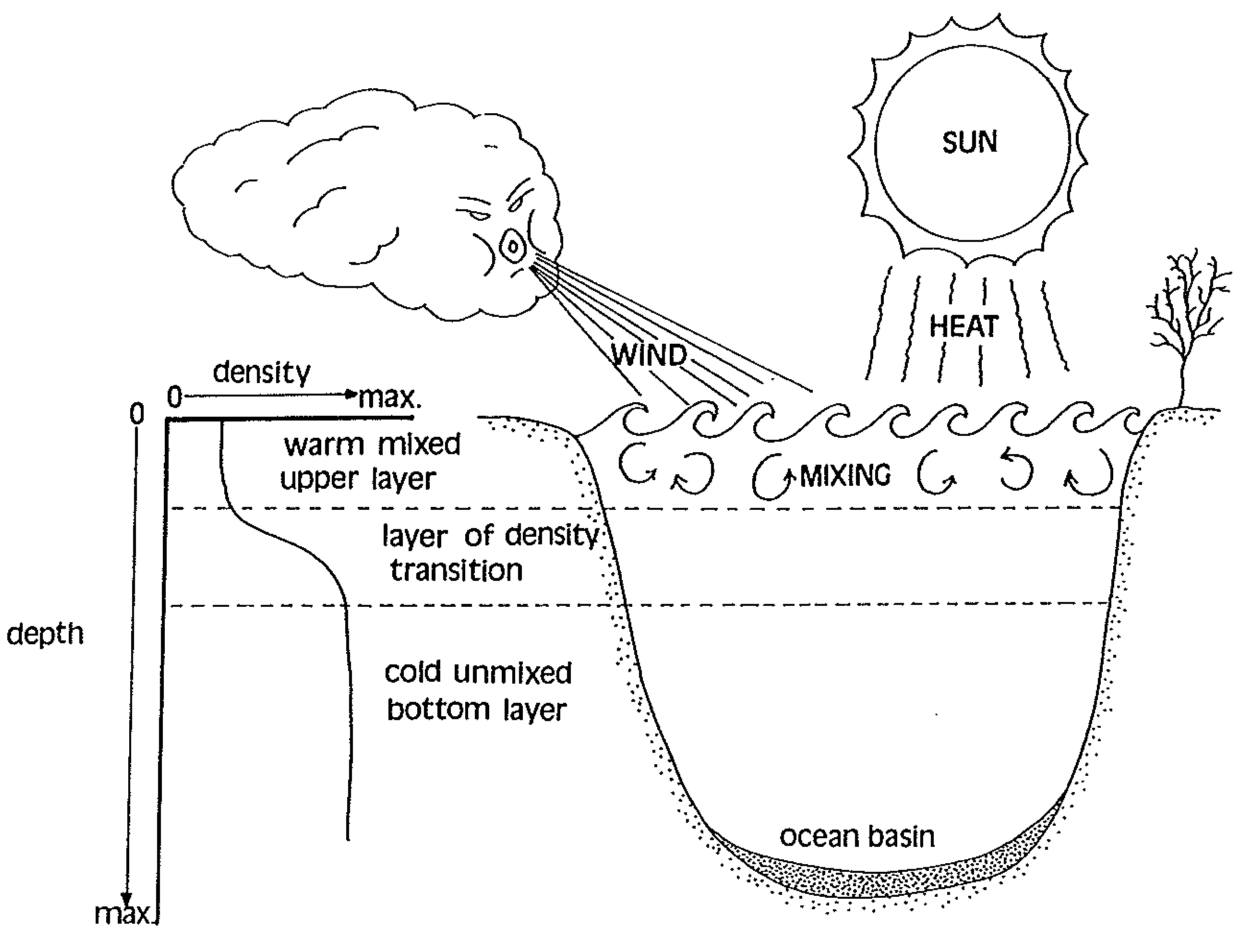

Figure 11. Water column stratification in a coastal bay.

In many coastal marine systems, particularly protected bays and lagoons, a thermocline begins to develop in early spring as the days get longer. As spring and summer progress the surface water layer accumulates more and more heat with a consequent increase in the temperature difference between surface and bottom waters. The larger this density difference becomes, the harder it is to break down the stratification. In many instances, this stratification is so strong that only the most severe storm conditions will cause it to break down. With the approach of fall, however, as the days begin to shorten, the surface waters begin to cool and sink to the bottom. Eventually the water column becomes completely mixed and the thermocline disappears. This phenomenon is often referred to as the fall overturn.

Another factor that can cause a water column to stratify is a density difference brought on by differences in salinity. Ocean water, with its high salt content, is considerably more dense than fresh water. In situations where ocean water and fresh water meet, such as in an estuary, a stratified system is often present. As is the case with temperature, the extent and persistence of salinity stratification depends on the degree of mixing between the two water masses. If tidal or wind induced currents are strong, the two water masses may mix completely where they meet and, although there will be a salinity gradient as one moves from river to ocean, there may be no distinct layering of water masses within the water column (a vertically homogenous estuary). If, however, density differences are great relative to mixing forces, a stratified system will persist with freshwater overlying saltwater (a vertically stratified estuary). The intermediate water layer, having a transition from low to high salinity in this instance, is called a halocline if due solely to differences in salinity, or a pycnocline if due to both salinity and temperature differences working together. The latter is often the case in estuaries where cold, high salinity ocean water meets warm, low salinity river water. In this case the less dense river water tends to "slide" over the top of the more dense oceanic water. Figure 12 illustrates a cross-section through a "typical" temperate estuary showing how the stratification is structured as one moves towards the sea. Note the "tidal wedge" shape. This wedge moves up and down the estuary as the tide ebbs and floods.

What are the implications of water column stratification for mussels that are growing attached to long lines suspended within the water column? With respect to direct effects, if the mussels are maintained within the surface layer of a stratified system, they will be subject to water temperatures somewhat higher and salinities somewhat lower than if the water column was completely mixed or if they were suspended in the bottom layer. Alternatively, if no stratification exists the temperature of the water will be less and the salinity will be higher. If they are being grown in a vertically homogenous estuary there will be little difference between top and bottom waters, but both temperature and salinity will vary according to the state of the tide.

There are also a number of indirect effects resulting from water column stratification that relate to phytoplankton blooms (and therefore food availability), oxygen concentrations, and the presence of indigestible inorganic materials. These factors will be discussed in subsequent sections.

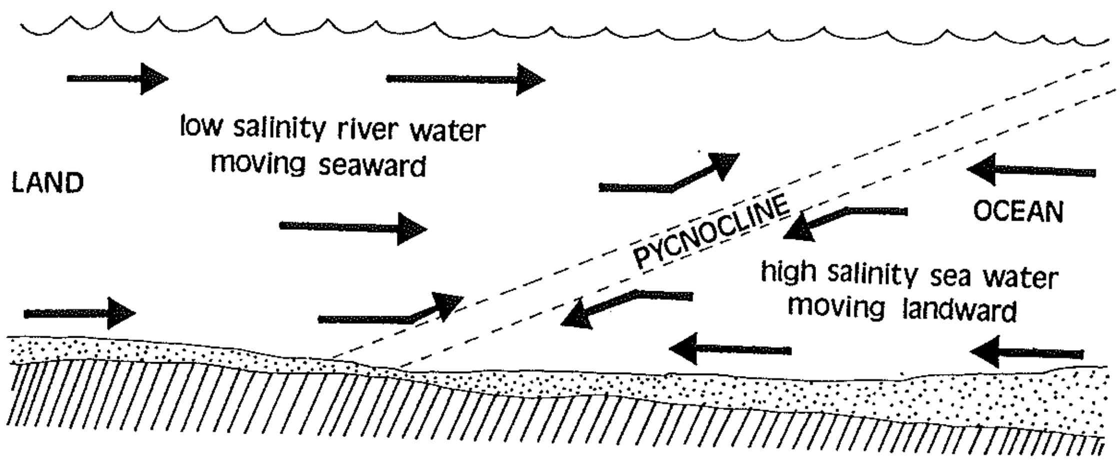

Figure 12. Water column stratification in a vertically stratified estuary.

- Suspended Particulate Matter

Suspended particulate matter (usually abbreviated SPM) is the material that exists as solids suspended in sea water. It is composed of two main materials, organic particles and inorganic particles. Suspended organic particles include living materials such as phytoplankton, zooplankton and bacteria, and non-living materials such as dead plants and animals that originate either within the water column, are resuspended from the sea bed, or are washed into the water column by rivers and streams. These materials are the major food items used by mussels.

Suspended inorganic materials consist of inorganic particles such as sands and clays. These enter the water column by being either resuspended from the bottom or washed in by rivers and streams. These materials, although filtered and collected by mussels, have no food value and if present in high concentrations for prolonged periods can seriously restrict growth rates.

As with the other environmental variables previously discussed, there are both direct and indirect affects associated with the level of SPM in seawater. The most important direct affect stems from the fact that mussels feed on the particles suspended in seawater and have limited ability to discriminate between organic and inorganic particles. If SPM levels are high and are composed mostly of organic particles, food availability will be high which leads to good growth. However, if the high SPM levels are due mainly to sand and clay particles, the mussel will expend energy in filtering water, but will collect little food material that can be used for growth.

An important indirect affect of high SPM levels on mussels is that these materials often absorb or scatter light making it unavailable to the phytoplankton that require it for photosynthesis and growth. Since phytoplankton are an important high quality food for mussels, anything that inhibits their growth will reduce the amount of food available to mussels.

Since high SPM levels usually result from resuspension of bottom sediments this condition tends to be characteristic of areas having strong water currents. In some cases high SPM levels may be the result of heavy erosion along shorelines or of erosion of soils within drainage basins that have rivers and streams entering the ocean. In some cases, dredging activities can temporarily elevate SPM levels.

Chemical Factors

- Salinity

The term salinity refers to the amount of salt dissolved in water and is usually expressed in parts per thousand (abbreviated ‰). Offshore seawater has an average salinity of about 34 ‰, which means that each liter of water contains about 3.4 grams of salt. Most river waters by contrast have salinities less than 1 ‰ and in coastal areas where river inputs are large, salinities can be quite low. How low they become depends primarily on the amount of river water entering the system and the rate at which the river water is mixed with seawater. In estuaries, where fresh and seawater meet, a gradient of decreasing salinity exists as one moves from sea to river. The salinity within a particular area of the estuary usually varies over time depending on the tidal stage and the volume of freshwater flow and can at times vary greatly over a single tidal cycle.

The ability to live at different salinities varies greatly among marine animals. Some cannot tolerate even small changes in salinity while others, such as *Mytilus*, have the ability to withstand rather large variations in salinity. *Mytilus* can be found growing in areas having salinities as low as 4 ‰ and as high as 35 ‰, but growth appears to be best at salinities between 18 to 31 ‰.

More important than the absolute salinity in influencing mussel growth, is the rate of change of salinity. Although *Mytilus* can tolerate a wide range of salinities, it must adjust or acclimate to sudden salinity changes. If the salinity change is large, on the order of 50%, such as may occur due to a sudden freshwater input from heavy rains, it may take days and even weeks before full acclimation occurs. The rate of acclimation appears to depend strongly on temperature, being faster at higher temperatures. This is important to keep in mind when transplanting mussels from one site to another.

The exact degree to which fluctuating, as opposed to constant, salinities affects mussel growth is difficult to study and not clearly understood. Under natural conditions, fluctuations in salinity are often accompanied by fluctuations in other important variables, such as temperature and food availability, and it is difficult to separate the effects of each variable.

- Micronutrients

The term micronutrients refers to a group of chemical substances that are necessary for the growth of plants and that are usually present in only very small amounts. The micronutrients most important in marine systems are nitrogen, phosphorous and silicon. Although the amounts of these materials present in seawater have little direct effect on mussels, they strongly influence the biological productivity of an area and therefore the amount of food available to mussels. Coastal waters that contain micronutrients in high amounts generally grow mussels at high rates.

The amount of micronutrients present in a particular area often varies greatly with season. Freshwater inputs by rivers and streams are often important sources of micronutrients. In areas where the drainage basin contains considerable agricultural activity, freshwater inputs often contain large amounts of nutrients that originate as runoff from commercial fertilizer and manures. Rivers and streams in drainage basins that are highly urbanized often contain nutrients that originate from garden and lawn fertilizers and domestic sewage. Forested watersheds, on the other hand, usually contain low amounts of nutrients, particularly if soil development is poor as it usually is in coniferous forests. However, forestry practices that create erosion of woodland soils often result in increased nutrient loss that eventually finds its way to rivers and oceans.

In coastal areas where there are no river inputs, micronutrient concentrations are often low unless in an area where deep nutrient-rich offshore water rises to the surface. These are known as upwelling areas and often result in some of the most productive marine systems.

Where neither river inputs or upwelling areas exist, the rate at which plants grow is determined primarily by the rate at which nutrients are recycled within the water column. In this case the main input of micronutrients is from plants and animals that have died and are undergoing decomposition during which the nutrients they contain are released into the water column. In some instances, even though the amounts of micronutrients are low, plant growth may be high if the nutrients are rapidly recycled in the system.

In some instances too high a level of micronutrients can be more harmful than too little. If nutrient levels are high and other conditions are favorable for photosynthesis, a strong algal bloom may develop. Although this will create lots of food for mussels, at the end of the bloom when the algae begin to die and decompose, the dissolved oxygen content of seawater may decrease to lethal levels. This process is called <u>eutrophication</u> and is becoming a serious problem in many coastal areas as a result of our increasing use of the ocean as a receptacle for our waste products.

- Dissolved Oxygen

Like animals and plants that live on land, most marine organisms require oxygen to live. However, unlike the large amount of oxygen present in air, seawater contains a relatively small amount of oxygen and, under some conditions, this dissolved oxygen can become depleted to the point where most organisms can no longer survive.

The actual amount of oxygen dissolved in seawater depends on the water temperature (cold water can hold more oxygen than warm water) and the rate at which oxygen is supplied and removed. The major input of oxygen is by diffusion from air into the water at the air-sea interface. In some cases the production of oxygen by plants during photosynthesis may be an important input. The major loss of oxygen is through respiration by plants and animals.

Under some conditions the rate of input may be less than the rate of removal and the dissolved oxygen becomes depleted to the point where many animals can no longer survive. This is particularly true in stratified systems because the major inputs of oxygen, from the atmosphere and photosynthesis, occur in the upper water layer and the presence of a thermocline or halocline prevents this oxygen from being transported into the bottom waters. Within the bottom waters, however, there may be considerable oxygen consumption by animals that are feeding on organic materials sinking from the upper water layer. If this organic input is large, such as at the end of a phytoplankton bloom or perhaps as a result of an accidental input of domestic sewage into a nearby river system, the dissolved oxygen may be completely used up resulting in anaerobic conditions within the bottom water layer. Aside from there being no oxygen present for respiration, an anaerobic environment often contains toxic materials such as ammonia and hydrogen sulfide which result from the incomplete oxidation of organic matter. This is an extremely harsh environment and is generally characterized by little life of any kind.

Mytilus grows best when oxygen concentrations are high, generally greater than 50% saturation*, but can withstand brief periods of low oxygen levels. Mussels usually respond to low oxygen levels by tightly closing their shell valves and waiting for conditions to improve. Unlike the case with salinity, they never adjust or acclimate to the point where they resume normal metabolic activity if the oxygen content remains low.

Biological Factors

- Marine Food Webs and the Food of Mussels

The food web of a biological system refers to the pathways along which food materials are passed from the plants that make the food to the animals and other organisms that consume it. The two general types of organisms common to all food webs are the producers, consisting mainly of plants that capture the energy of sunlight and transform it into energy-rich organic materials, and the consumers which feed on the materials made by the producers. The latter include not only animals, but also bacteria and fungi.

Consumers are in turn often grouped into one of the following four categories: (1) herbivores - organisms that feed solely on living plants; (2) carnivores - animals that feed solely on other living animals; (3) detritivores - organisms that feed on non-living organic materials that

---

*Because oxygen uptake in mussels is a diffusion process, the oxygen level that may be considered limiting depends more on the partial pressure of oxygen than its absolute concentration. The partial pressure of oxygen is a function of percent saturation, which is in turn a function of temperature and absolute oxygen concentration. Generally speaking, water of less than 50% oxygen saturation is thought to have a negative effect on aquatic organisms that obtain their oxygen by diffusion across a gill surface.

originate mainly from the death of plants and animals, and; (4) omnivores - organisms that feed on both living and dead materials.

Mussels are relatively non-selective in their feeding and can be considered omnivores. They will ingest small plants such as phytoplankton, some of the smaller zooplankton, and non-living organic particles that originate from the death and breakdown of phyto- and zooplankton and other plants and animals whose decomposition products are suspended in the water column.

The overall productivity of a coastal marine system in terms of the number of animals, including mussels, that it can support depends largely on the amount of food material present. With respect to mussels, the two factors of major importance are the quantity and quality of material.

The quantity of food materials is determined by a number of factors, the most important of which is the rate at which it is produced by plants. This in turn depends on the availability of micronutrients and solar energy, the two most important factors controlling the rate at which plants grow. Another factor of equal importance for sessile animals like mussels is the rate at which food materials are supplied. Because mussels often filter water rapidly, they can quickly deplete the food materials contained within the water immediately surrounding them. For good growth, this water must be quickly replenished to provide more food materials, which means that good water circulation is essential for good growth.

The quality of the food present is just as important as the quantity. Some organic materials, such as those that originate from seagrasses and salt marshes, are low in nitrogen and other nutrients and may not provide all the essential materials necessary for good growth. Other materials, such as phytoplankton and zooplankton, are relatively rich in nutrients and provide the best food for good growth. Our understanding of how to evaluate food quality in nature is poor at present and is one of the major problems faced by aquatic ecologists in trying to understand the relationship between food availability and animal growth.

It is important to note that the food materials present at a particular growing site may not have actually originated at that site. For example, large plants like salt marsh grasses, seagrasses and rackweeds upon death often become broken down into small fragments and particles that become suspended within the water column and transported over large distances by water currents. In addition, in some coastal areas organic materials that originate in terrestrial systems such as forests and agricultural lands may be carried to the ocean after being washed into streams and rivers. This diversity of potential food sources makes it difficult to evaluate the most important food source at any one particular site without doing a detailed study of the kinds and sources of organic particles present in the seawater.

- Phytoplankton Blooms

In many coastal areas phytoplankton appears to be the most important food of mussels. Very often seasonal variations in the condition indices of mussels can be correlated to the seasonal variation in amount of phytoplankton. This variation is often very pronounced and characterized by spring and fall peaks commonly referred to as the spring and fall "phytoplankton blooms."

The factors that control the beginning, extent and end of a phytoplankton bloom, although not completely understood, are related to events that affect the availability of light and nutrients, the two major factors that control the rate at which phytoplankton photosynthesize and grow. The availability of light depends mainly on the clarity of the water and the time of year, which determines the daylength and therefore how long light is available. Water clarity depends on the amount of various substances in the water that scatter or absorb light. For example, if the water contains high amounts of sediments, such as often occurs during storms when mixing is strong and the sea bed is disturbed, the sediment particles will scatter light preventing it from penetrating deeply into the water column. Another substance that tends to decrease the amount of light in the water column are the strongly light absorbing darkly stained materials often entering the sea from rivers and streams that drain coniferous watersheds. An equally important factor related to the amount of light available for photosynthesis is the depth to which surface waters are mixed. If the water column contains large amounts of substances that absorb or scatter light, and mixing is deep, the phytoplankton will be spending some time near the bottom of the water column where light conditions are poor and this will slow their growth rate. If, however, the water column is stratified, the phytoplankton will only be mixed to the thermocline and thus restricted to the surface water where light conditions are better for photosynthesis.

If light was the only factor controlling the rate of phytoplankton growth, understanding the causes of blooms would be much easier. However, phytoplankton, like all plants, also require nutrients to grow, particularly nitrogen, phosphorous and silica. The amount of nutrients available at a particular time varies greatly and is controlled by a number of processes, the most important of which involve the rate and amount of mixing of the water column, the amount of nutrients brought into an area by freshwater inputs and offshore water, and the rate of decomposition of plants and animals within the water column.

Generally, phytoplankton blooms occur when light and nutrient conditions are at an optimum for rapid photosynthesis and growth. This often occurs in the spring of the year as the daylength gets longer. In addition, if the water column becomes stratified as the surface waters heat, the phytoplankton will be restricted to the well-lighted surface waters, and there will be an abundance of nutrients available from the decomposition products that have accumulated during the winter. The result is a spring bloom, the extent of which will depend on the amount of nutrients in the surface layer. However, since there is a continual sinking of plants and animals, along with the nutrients they contain, the amount of nutrients within the surface water will eventually decrease. If the water column remains stratified, as it often does during the summer, there will be no way for the nutrients to be returned to the surface waters and phytoplankton growth will eventually become nutrient limited ending the bloom. This explains why phytoplankton levels are often very low during mid-summer.

With the approach of fall the surface waters begin to cool and sink towards the bottom to be replaced by rising bottom water. Since this bottom water contains an abundance of nutrients as a result of the accumulation and decomposition of sinking plants and animals over the summer, this leads to another spurt of phytoplankton growth, or the fall bloom. Eventually, however, as the days become shorter and cooler and stratification breaks down, the phytoplankton become mixed deeper into the water column and light becomes a limiting factor for photosynthesis and the fall bloom ends.

- Predators and Fouling Organisms

Mussels form only one part of a marine community. There are numerous other organisms present that interact with mussels in various ways. In some instances these interactions are beneficial to the mussels, such as when they play a part in the food sources of mussels, but in other instances the interactions produce a negative affect on the growth and survival of mussels. In the latter case, there are organisms that eat mussels (the predators), parasites that result in diseases that weaken or kill mussels, and various organisms that compete with mussels for either food or space, and often both.

Among the important predators of mussels are seaducks, starfish and crabs. There are about five species of sea ducks that feed on mussels; the common goldeye, the white-winged, surf and black scoters, and the eider ducks. Although all of these can be important predators, especially during migration periods, the eider duck is a particular problem in certain areas of the Atlantic Maritimes where it forms breeding or overwintering colonies. Whereas the other species of sea ducks feed on shellfish other than mussels, such as clams, the eider duck feeds solely on mussels. Although eiders prefer smaller mussels, on the order of a few centimeters, they will eat large mussels of harvestable size. Aside from the loss of mussels that they actually consume, their feeding behavior can result in large numbers of mussels being stripped from longlines. During feeding they dive underwater and remove clumps of mussels and return to the surface. At the surface they shake the clumps to dislodge and swallow a few mussels and the remaining mussels sink to the bottom. This behavior can result in high losses of mussels from longlines, particularly if byssal thread attachment is not well developed. In areas where eider duck predation is an extremely severe problem, such as British Columbia and some parts of Great Britian, various techniques have been devised for keeping eiders away from cultured mussels. They range from shooting the ducks to placing images of natural predators at culture sites. For the most part these attempts have not been successful and the problem is far from being solved.

Starfish can also be a major predator of cultured mussels. Starfish are well adapted for preying on a variety of molluscs. Their undersurface contains a system of small "tube feet" that attach to the shell of the mollusc and gradually pull the valves apart. Once opened, the starfish everts its stomach into and around the meat of the mussel which then becomes digested and absorbed by the starfish. Fortunately, because adult starfish cannot swim they cannot easily attack mussels grown on long lines suspended well above the bottom. However, like mussels, their life cycle involves a planktonic larval stage that can settle on the mussel lines. If this occurs and they settle at about the same time as the mussel spat, they can develop and grow along with the mussels, causing severe predation. There is little one can do to avoid this other than trying to collect mussel spat only when starfish larvae are not present. Once present on mussel lines the only known method of control is to remove the starfish by hand.

Crabs can also be a problem in some areas. Although many crabs can swim short distances above the sea bed, they do not appear to be much of a problem as long as the longlines are well above the bottom.

Although not predators, fouling organisms, which include a diversity of plants and animals that attach to and grow on mussel lines, can create problems under some conditions. Aside from the fact that many of these organisms feed on the same kind of food as mussels and therefore compete with them, if their number is large they can actually smother the mussels by reducing water circulation within the mussel mass. In addition, severe fouling can greatly increase the weight of a mussel line, causing floats to sink as well as increasing the labor required to clean mussels for marketing. As is the case with starfish, there is little that can be done to control the settlement and development of fouling organisms on mussel lines. Exposing the lines to air for short periods of time will kill some fouling organisms, but this a laborious procedure.

- Diseases of Mussels (By G. Johnson and S. McGladdery)

Diseases can affect cultivated organisms in two ways. They can either kill them directly, or they can reduce their condition to a level where growth and reproduction are retarded. In the latter case, poor condition can lead to mortality due to less severe pathogens or to environmental stresses such as higher than average water temperatures, large variations in salinity, minor pollutants, etc.

Mussels are old in terms of evolution and they have adapted a life style of acceptance. Their only response to insult is to close their shell and cease eating. Consequently, they tolerate disease more than they fight it. In fact, their ability to fight infectious disease appears to be quite limited. Cultured mussels do not demonstrate a lot of disease problems and the reasons for that are not well known.

Mussel leases in Atlantic Canada have undergone some very extensive monitoring and little in the way of primary diseases have been found. A ciliate parasite, as yet unidentified, has been seen intermittently in the cells of the digestive gland of mussels stressed by high temperatures. A similar parasite has been seen in the west coast and along the Eastern seaboard of the United States. The importance of this is not known, but it does not appear to be a primary infectious pathogen causing heavy losses.

There is an important disease on the west coast of Canada and the United States called, among other things, "Heomocyte Disease." Not much is known about it other than it is highly infectious and ultimately fatal (usually by 14 months of age) and occurs in conjunction with wild mussel and cultured mussel die offs. It is readily diagnosed histologically and in thousands of tissue sections of *Mytilus* in Eastern Canada, it has never been seen.

A number of parasites having an unpredictable potential for causing disease have been found in mussels. One of these, a digenean fluke, inhabits and destroys the gonads, castrating the mussel. This parasite has never been found in Atlantic Canada, however, it provides an example of the dangers of indiscriminate unmonitored introduction of new stock.

Most mussel stocks contain some pathogens, but where they have developed some degree of resistance to these, they show no sign of disease and, therefore, appear healthy. Introduction of such apparently "healthy" animals has the potential to completely destroy any local bivalves which may have no resistance to the "new" pathogens. In addition, local pathogens may affect the introduced bivalves. Disease control for infectious disease currently involves avoidance by restricting imports or tolerance until mussels evolve to develop their own resistance. Since the mussel industry uses an open form of culture it is more susceptible to disease spread than aquaculture systems where quarantine is a feasible preposition. And because of the inherent difficulty in controlling a disease organism once established in an open culture system, the most practical form of disease control is prevention of exposure to known or even potentially pathogenic organisms.

Longlines hold mussels away from many of the parasites and bacteria that may negatively influence their well being. Mussel culture, in terms of an intensive industry, is still very new and perhaps the problems have not yet arrived. Imports of new mussels have been limited, reducing the risks of a new (to that population) disease problem. Mussels also go to market within 24 months avoiding undue exposure to chronic diseases or abnormal long-term environmental stresses.

The rapidly developed (by an industry standard) testing and monitoring procedures have minimized the risk of human disease, but the domoic episode on Eastern Prince Edward Island in 1987 demonstrates the susceptibility of culturing mussels as it is currently done. Disease control in noninfectious disease can only be done by monitoring the consumer product.

- Shellfish Toxins

A number of marine organisms, mainly microscopic algae and bacteria, produce substances that are toxic to humans and, if present in large quantities, can be concentrated by mussels during feeding making them unfit for human consumption. The most common toxin is that causing paralytic shellfish poison, but there are others that growers should be aware of.

Paralytic shellfish poisoning, or PSP as it is commonly called, results from eating shellfish that have accumulated toxins produced by certain microscopic algae, most commonly dinoflagellates of the *Gonyaulax* group. On the east coast of North America, *Gonyaulax excavata* is the most common PSP-producing dinoflagellate.

Dinoflagellates are small (about .05 mm across) planktonic organisms capable of photosynthesis. Under the proper environmental conditions photosynthesis and growth can be very rapid and result in a bloom often referred to as a "red tide"*. In the case of dinoflagellates

---

*The term "red tide" is now commonly used to describe many different types of phytoplankton blooms, including those produced by non-toxic organisms.

factors other than light and nutrients appear to be important in stimulating blooms. We are far from understanding what these other factors are but the blooms often occur during higher-than-average water temperatures and nutrient concentrations, particular types of weather patterns and water movements, the presence of specific organic growth factors, proximity to land and usually a lowered salinity. Depending on the length of time favorable conditions persist, the bloom may last for days or weeks, and sometimes even months. When conditions are no longer favorable, the dinoflagellates encyst into a dormant form and sink to the bottom where they remain in the sediments until the proper conditions for growth reappear. Encysted dinoflagellates have been reported to be more toxic than the active planktonic forms and it is possible for shellfish to become contaminated if the cysts are resuspended into the water column and become ingested.

It is not known why some dinoflagellates produce toxins while others do not, but those that do produce very potent toxins. Although shellfish feeding on toxic dinoflagellates do not appear to be affected by the toxins, the toxins accumulate in the digestive gland and if eaten by vertebrate animals they produce a disease that inhibits the transmission of nerve impulses and eventually leads to muscle paralysis and sometimes even death.

Although most filter-feeding molluscs will accumulate PSP toxins if they are present, mussels have been known to accumulate them very rapidly. It is for this reason mussels are often used as the key indicator organism in PSP monitoring programmes. Fortunately, mussels also loose the toxin more quickly than other molluscs once the PSP-producing organisms disappear.

In some areas, PSP blooms have a fairly predictable seasonal occurrence, but because of our poor understanding of the environmental conditions that create the blooms, there is presently not much that can be done to control them. What is particularly disturbing is that PSP blooms seem to be occurring more frequently and in areas that have been previously thought to be free of toxins.

Monitoring of molluscs for the presence of PSP toxins is traditionally carried out using the mouse bioassay technique. The soft body parts of the mollusc are treated to extract the toxins and the extracted material is injected into a number of test mice. If toxins are present the time it takes to kill the mice, along with a number of other observations, is noted and the results are expressed in amount of toxin per weight of mussel. This procedure is not one that mussel growers themselves would want to perform since it requires very strict adherence to specific details and requires considerable experience for proper interpretation of the results.

In some parts of the world, particularly northern Europe, Diarrhetic Shellfish Poisoning (DSP) is becoming a major problem for mussel growers. The toxin causing DSP is, like PSP, produced by a dinoflagellate and, although less severe than the PSP toxin, it results in nausea, vomiting and diarrhea. Although there have been no reported cases of DSP in Canadian waters, the dinoflagellates producing the toxin are known to occur here and growers should be aware that it is a potential problem.

The surprising and unexpected events that led to the temporary closure of shellfish harvesting in Atlantic Canada in the late fall of 1987 was a result of mussels from the northeastern part of Prince Edward Island having become contaminated with domoic acid. This was the first time domoic acid had been implicated as a toxin in shellfish and a great deal of effort has since been devoted to identifying the source of domoic acid and the conditions under which mussels become contaminated. What is particularly surprising about domoic acid is that it appears to be produced by a diatom (*Nitzschia pungens*) as opposed to a dinoflagellate. Diatoms, including *N. pungens*, are common components of the marine phytoplankton and have never before been known to be a cause of shellfish toxicity. It is still unknown as to why this organism suddenly became a problem and whether or not this outbreak was a rare, localized event or one that will reoccur and perhaps spread to other growing areas.

- Bacterial Contamination of Growing Sites

A number of important and potentially fatal diseases, such as cholera, dysentry and typhoid fever, can be transmitted by ingesting water, or plants and animals that have been grown in water, that contains pathogenic microorganisms originating in human fecal material. In an attempt to prevent this, mussel growers are required to have their growing sites periodically monitored for the presence of these microorganisms.

The monitoring procedure most commonly used involves testing water, or mussel, samples for the presence of "coliform" bacteria. Although coliform bacteria are harmless in themselves, (they are present in the intestines of all warm blooded animals, including waterfowl and farm livestock) their presence indicates that fecal contamination of some sort is present.

There are a number of different tests for determining the presence of coliforms, but the most common involves incubating a sample in a specially prepared culture medium and determining the number of bacteria present. Although there are test kits on the market that allow almost anyone, with a little instruction, to test for coliforms on their own, it is best to leave this to public health officials who have a great deal of experience in test procedures and the interpretation of results.

PART III

WATER QUALITY MONITORING OF GROWING SITES

A. Introduction

Understanding the environmental factors that control the settlement, growth and mortality of mussels is only one part of being a good mussel aquaculturalist. It is also important that a grower understand which of the many factors are most important at their particular growing site and how these factors vary with season and from year to year. Anyone who has grown mussels has observed that there is considerable variation in the growth of mussels on both a seasonal and yearly basis as well as between different growing sites. Unless the reasons for this variability are understood, it is difficult to take corrective action if problems arise. And unless there is some sort of data base that documents past and present conditions, it is difficult to determine what change has taken place to cause the problem. Put simply, it is difficult to solve a problem unless one understands the reasons why the problem exists.

Monitoring water quality can be quite simple and not very demanding of time or equipment if one is interested in only the most basic environmental variables, such as temperature and salinity. However, if a grower wants to develop a data base that is more informative, particularly with regard to information on variations in growth rates, condition indices and food availability, considerably more effort is required and, unless an aquaculture operation is quite large, the expense of laboratory equipment and the time required for sample collection and analyses is usually prohibitive. It is for this reason that the present monitoring programme has been developed. Although growers in the programme are still required to record data and collect samples, the more tedious and expensive time-consuming analyses are carried out by ACER and the results reported to the grower.

The following discussion provides a brief overview of the monitoring programme in terms of the variables included and the techniques employed. The technical details associated with data and sample collection in the field are presented in another manual*. Table II presents a list of the variables included in the monitoring programme.

B. Physical Factors

The physical factors included in the monitoring programme include temperature, stability of stratification and Secchi disc depth.

---

*Field and Laboratory Techniques Guide for Mussel Growers' Water Quality Monitoring Programme. By M. Brylinsky and G.R. Daborn, Acadia Centre for Estuarine Research. August, 1987.

Table II. Variables Included in the Monitoring Programme for Mussel Growers

Physical Variables
- Temperature
- Stability of Stratification
- Secchi Disc Depth

Chemical Variables
- Salinity
- Dissolved Oxygen

Biological Variables
- Suspended Particulate Matter
- Chlorophyll
- Phytoplankton
- Zooplankton
- Total Particulate Organic Carbon
- Spatfall
- Mussel Data
  - Shell Length and Width
  - Dry Meat Weight
  - % Gonad Weight (Fecundity)
  - % Glycogen Content
  - Condition Index
  - Shell Growth Rate

Temperature - Temperature is measured with a Yellow Springs Instrument Salinity - Conductivity - Temperature meter (YSI S-C-T), which measures water temperature as a function of its ability to conduct an electrical current. Growers are asked to measure water temperature at one meter intervals from the surface to the bottom and thereby obtain a temperature "profile" of their site.

Stability of Stratification - How strongly stratified a water body is depends on the density difference between the top and bottom waters as well as the depth of each of these layers. Calculation of the stability of stratification involves analyzing temperature and salinity profiles to calculate the average density of each water mass (expressed as σ_t values) and then calculating the amount of work that would be required to mix the two water masses to the point where there is no longer any density difference. The value is reported as gram-centimeters per square centimeter, a unit of measurement for work commonly used by physicists and oceanographers.

Secchi Disc Depth - A Secchi disc is a 30 centimeter diameter black and white disc that is used to measure the depth to which sunlight penetrates into the water column. It gets its name from the scientist who developed it. In use, the disc is slowly lowered into the water until it can no longer been seen and then raised again until it reappears. Readings are made of the depths at which it disappears and reappears and the average of the two readings is recorded as the Secchi disc depth.

This measurement provides information on two important factors. Depending on the amount of suspended particulate matter present, either living or non-living, light will be scattered as it travels through the water column. If there are large amounts of suspended particulates in the water, the Secchi disc reading will be low. This information, together with information on the amount of SPM (discussed later), gives a rough indication of the amount of food materials in the water. In addition, the Secchi disc depth provides information on the depth to which phytoplankton can be mixed and still photosynthesize. Generally, phytoplankton will have enough light for photosynthesis down to a depth of about three times the Secchi disc depth.

C. Chemical Factors

The chemical factors included for measurement in the monitoring programme are salinity and dissolved oxygen. Although information on micronutrients, particularly nitrogen, phosphorus and silica, would be useful in understanding the nature of a growing site, the techniques required to obtain this information are very laborious and therefore not included in the programme.

Salinity - Salinity is measured with the same YSI S-C-T meter used to measure temperature and, as with temperature, growers are asked to make measurements at one meter depth intervals from surface to bottom.

Dissolved Oxygen - Although there are electronic meters available for measuring the amount of dissolved oxygen present in seawater, they are expensive and require constant calibration to insure reliable results. Although more tedious, a chemical procedure, called the Winkler technique, is the procedure used in the monitoring programme. Measuring dissolved oxygen using this procedure is time consuming and we suggest that it only be done under conditions that suggest dissolved oxygen levels may be low (for example, if a site has been stratified for a long period of time).

The Winkler technique involves collecting water samples in bottles specially made for this procedure, and then introducing a series of chemical reagents that result in the production of iodine. The amount of iodine produced is proportional to the amount of oxygen present in the water sample. The iodine is then reacted with another chemical using a titration procedure, and from the amount of titrant required the amount of oxygen in the original water sample can be determined.

Although this technique seems complicated and tedious, particularly to one having little background in chemistry, it becomes quite routine and produces very reliable results after a little practice.

D. Biological Factors

Most of the procedures used in the monitoring programme are concerned with obtaining data on the biological factors at a growing site, and most of these procedures are concerned with determining the amount of food present in the water. This is by far the most important factor determining how well a mussel grows. Unfortunately, it is also one of the most difficult to monitor adequately because of the diversity of potential food sources (phytoplankton, zooplankton, detritus, etc.) and the corresponding diversity in food quality associated with the different food types. There is no single technique that will provide an adequate indication of food quantity and quality and, consequently, a number of techniques are employed.

Suspended Particulate Matter (SPM) - SPM refers to the total amount of particulate material in the water. It includes both inorganic materials (sands, clays, etc., that contain no food value) and organic materials (living organisms and their breakdown products). Together with other information, knowledge of the SPM levels can provide an indication of the total amount of food available to mussels. It is measured by filtering a water sample through a special pre-weighed filter. At ACER, the filter is dried and re-weighed, the difference in weights, expressed as milligrams per liter, is the SPM value.

Chlorophyll - Chlorophyll is the major plant pigment that phytoplankton use to capture the energy contained in sunlight. By determining the amount of chlorophyll present in seawater we can obtain an estimate of the amount of phytoplankton present. Chlorophyll is measured by filtering a water sample through a special glass filter. At ACER, the chlorophyll collected by the filter is removed using an acetone extraction procedure. The resulting solution is light to dark green in color depending on the amount of chlorophyll present on the filter. A special instrument, called a spectrophotometer, is then used to measure the intensity of the green color and from this information the amount of chlorophyll, expressed as milligrams per cubic meter, is calculated.

Phytoplankton - Analysis of the kinds and amounts of phytoplankton in a water sample requires examining water samples using a microscope and determining the species present as well as the numbers and sizes of each species. This is a very tedious and time consuming task and the monitoring programme does not attempt to do this on a routine basis. However, it is important to have the potential for determining the kinds of phytoplankton present at a site if the need arises. If samples are available for analysis, and a problem arises such as a PSP outbreak, or perhaps the presence of domoic acid, the samples could be analyzed to document the development of the problem and perhaps identify the particular phytoplankton species responsible. To ensure this potential, the monitoring programme includes collection of phytoplankton samples that can be analyzed if the need arises. The collection technique is very simple: a small water sample, about 20 milliliters, is collected in a vial and then preserved by adding a few drops of an iodine solution. Once sent to ACER, the samples are stored in the cold and in the dark where they can be retrieved for analysis if the need arises.

Zooplankton - Zooplankton, the microscopic animals found floating within the water column, are traditionally collected using a fine meshed net that is towed through the water. The net collects not only zooplankton, but a good deal of other suspended materials. Quantification of a zooplankton sample requires examining it under a microscope, counting the number of animals present, determining the size of the animals present, and then converting this information to weight per unit volume. Quantifying zooplankton in this manner is much too tedious for a large scale monitoring programme, but it is useful to know how much material suspended in the water column is within the larger size material collected by a zooplankton net. Growers are therefore asked to collect a zooplankton sample by hauling a net through the water column vertically from a depth of ten meters (this standardization allows us to calculate the amount of water sampled). The sample is preserved in formalin and sent to ACER where it is filtered and weighed, after drying, to determine the total weight of material collected by the zooplankton net. Since, as noted previously, the net collects material other than zooplankton, this technique actually measures the total amount of large particulate material present. Because this technique is very crude, the information obtained must be interpreted with care. Its major usefulness is that if very high values of large particulates are recorded, we can examine the filters microscopically to determine the source of the material. For example, if we find that the value for samples collected during the fall at a particular site are consistently high, and examination of the samples reveals the presence of eel grass fragments, or perhaps materials washed in from a nearby saltmarsh, this would give us some indication that the eel grass or saltmarsh communities are potentially important food sources for the mussels at that site.

Total Particulate Organic Carbon - One of the best indicators of the amount of food available to a mussel is the amount of particulate organic carbon (POC) in the water. A reasonable estimate of POC can be obtained by filtering a water sample onto a pre-weighed glass filter, drying and re-weighing the filter to determine the total amount of particulate matter (both organic and inorganic) in the water sample, combusting the filter at 475°C for 24 hours to burn off the organic carbon, and finally, re-weighing the filter once again. The difference in the weight of the filter before and after combustion provides an estimate of POC.

Spatfall - The times and densities of spatfall at spat collecting sites are important to growers who collect their own spat. This is easily monitored by placing a suitable substrate in the water. A variety of materials can be used. In the monitoring programme growers are asked to use a piece of the small plastic "scrubbies" commonly available at most grocery stores. These are suspended in the water from a line and after a short time period (one or two weeks) they are retrieved and placed in a container containing formalin as a preservative. At ACER the spat collectors are examined microscopically and the number of spat present per collector is determined.

Mussel Data - Mussel samples sent to ACER are subjected to a number of analyses. The size and weight of shells and the weight of meats as well as measures of fecundity and glycogen content are determined. In addition, a condition index is calculated for each sample.

The size of the shell, both length and width, are measured using a vernier caliper. The meats from the same mussels are removed and the gonads dissected out of each. The meat and gonads are weighed separately and together to provide a total meat weight and the percent of meat weight due to the gonads. The latter provides an estimate of fecundity (the amount of eggs or sperm relative to total weight) which gives some information on how close the mussels are to spawning.

Another set of mussels from the same sample is analyzed for glycogen content. Since glycogen is the storage product of mussels, the percent glycogen content gives an excellent indication of the general condition of the mussel. If low, it means that there are little food reserves available to overcome stressful conditions and there may be reason for concern if environmental conditions are unfavorable (for example, high water temperatures).

The marketability of mussels, in terms of their quality, is usually measured by calculating a condition index. There are a number of different ways to calculate condition indices. For the purposes of this monitoring programme we have chosen one of the simpler indices to calculate, the ratio of dry meat weight to shell weight times 100. This simply compares the weight of the meat to the weight of the shell. A large number means that the meats are large relative to the shell and that they are probably suitable for marketing.

The calculation of growth rates is made on the shells only. It is not really feasible to calculate the growth rates of meats over the long term since this varies greatly depending on whether or not the mussels have spawned. Meat weights generally decrease greatly after spawning. Shell growth rates are calculated by determining the average shell length of samples collected over time. The results are expressed as millimeters per time. Eventually we hope to calculate monthly as well as yearly growth rates for each site.

Because we analyze a number of mussels from each sample, we usually report results in terms of averages. However, in calculating the averages, we also obtain information on the amount of variation in each measurement and this is reported as the <u>standard deviation</u>. In general, if the value of the standard deviation is larger than the average, it indicates that there is considerable variation among the mussels analyzed. If the standard deviation is small relative to the average, it means that the mussels analyzed are pretty much alike.

APPENDIX A

GLOSSARY OF TERMS

anaerobic (p. 27): without oxygen.

anterior (p. 4): the front, or head region, of an organism.

bilateral symmetry (p. 4): having two sides, each of which is a mirror image of the other.

body sinus (p. 13): a hollow space or cavity in the body of an organism.

carnivores (p. 29): organisms that feed on animals.

ciliary-mucous-filter-feeder (p. 10): an organism that captures food material by entrapping it in mucous strands that are moved to the mouth and into the digestive tract by the movement of small hairs called cilia.

consumers (p. 29): organisms that rely on organic substances synthesized by producers for their food source.

crystalline style (p. 11): a rod-shaped structure located in the gut of some molluscs onto which mucous food strands are coiled and thereby drawn into the digestive tract.

detritivores (p. 29): organisms that feed on dead plants and animals.

dinoflagellate (p. 34): a small planktonic organism capable of photosynthesis, some types of which produce toxic substances responsible for paralytic shellfish poisoning.

dorsal (p. 4): the upper or backside of an organism.

eutrophication (p. 28): literally means "much feeding"; term used to describe aquatic systems characterized by very high levels of productivity which often lead to anaerobic conditions.

food web (p. 29): the interrelationships between organisms of a biological community in terms of their food sources.

gametes (p. 14): the reproductive cells (eggs and sperm) produced by an organism.

gametogenesis (p. 14): the process of making gametes.

gonads (p. 14): the organ that produces gametes.

habitat (p. 2): the place where an organism lives.

halocline (p. 25): the area of the water column in a stratified body of water in which the density changes most rapidly as a result of changes in salinity.

herbivores (p. 29): organisms that feed on plants.

intertidal zone (p. 2): the area along the shoreline of the ocean that lies between the low and high tide marks.

labial palps (p. 8): a pair of fleshy projections surrounding the mouth of some bivalves; used mainly to pass food materials trapped on mucous strands from the gills into the mouth.

mantle (p. 7): the outer covering of the soft body parts of a mollusc; in mussels the mantle lies immediately below the shell.

mantle cavity (p. 4): the space between the mantle and visceral mass that contains the gills and through which water is pumped during feeding and ventilation.

metamorphosis (p. 17): a change in form or shape as when the planktonic mussel larvae change into the sessile adult form.

micronutrient (p. 27): a metabolic substance that is essential for the growth of an organism but is present in only very small amounts and which therefore may at times be limiting.

nacreous layer (p. 6): the irridescent, pearly inner layer of the shell of molluscs.

nephridium (p. 13): a very simple kidney.

omnivores (p. 30): organisms that feed on both living and non-living organic substances.

osphradium (p. 14): a small organ, located within the mantle cavity of some molluscs and thought to function in sensing the quality of inhalent water.

pediveliger (p. 17): one of the later developmental stages in the planktonic larval stages of bivalve molluscs; characterized by the development of the foot in preparation for settlement.

periostracum (p. 6): the outer dark horny layer of the shell of molluscs.

phytoplankton (p 16): the microscopic animals of the plankton community.

plankton (p. 4): aquatic organisms that occur free-floating in the open water having limited ability for movement against water currents.

posterior (p. 4): the rear or tail region of an organism.

producers (p. 29): organisms that have the ability to synthesize organic substances from inorganic substances using an external source of energy, usually sunlight.

pseudofeces (p. 10): in bivalves, materials that are expelled from the mantle cavity and which have not passed through the digestive tract.

pycnocline (p. 25): the area of the water column in a stratified water body in which the density changes most rapidly, usually as a result of changes in both temperature and salinity.

resuspension (p. 23): the movement of sediments from the sea bottom into the overlying water column.

sessile (p. 2): term used to characterize organisms that do not normally move about but remain attached to a substrate.

spat (p. 17): the larvae of bivalves, particularly at the stage just prior to and after settling.

statocyst (p. 14): an organ used to sense gravity.

stratification (p. 19): the layering of a water body as a result of differences in water density with depth.

thermocline (p. 24): the area of the water column in a stratified water body in which the density changes most rapidly as a result of changes in temperature.

trochophore larvae (p. 16): an early stage in the larval development of some invertebrates; in mussels the stage that develops shortly after gametes are spawned and characterized by a band of cilia around the equator.

turbidity (p. 23): the opaqueness of a water body caused, most commonly, by the presence of suspended particulate materials that scatter the light entering the water.

umbo (p. 5): the knob or "bump" beside the hinge on each half of a bivalve shell.

veliger larvae (p. 17): one of the planktonic larval stages of a mussel, characterized by a velum used in feeding and locomotion.

velum (p. 17): the ciliated lobe of a veliger larvae used in feeding and locomotion.

ventral (p. 4): the lower surface or belly of an organism.

visceral mass (p. 8): the soft body parts of a mollusc, exclusive of the mantle and gills, that contains the digestive tract and other internal organs.

zooplankton (p. 16): the microscopic animals of the plankton community.