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MANUAL FOR DESIGN AND OPERATION OF AN OYSTER SEED HATCHERY FOR THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA*

by

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PREFACE

The past decade has witnessed an extremely rapid development in the "science" of mariculture, especially the design and operation of modern oyster seed hatcheries. The Department of Marine Culture at the Virginia Institute of Marine Science over the past seven years has developed an oyster hatchery system through research and cooperative efforts with the fisheries industry. This system, including the biological protocol to be described in detail on the following pages, has been tested successfully and is a modification of that system used at Chesapeake Sea Farms, Inc. in Ridge, Maryland.

It is important to stress this system represents a totally integrated concept — an interdependent system, which, if taken apart and used as separate steps, will yield poor results, especially when modifying the oyster larval food diet. The detailed instructions are not meant to bore the expert, but are included to make sure the results can be reproduced.

The utilization of any brand name does not imply the endorsement by the authors or the Virginia Institute of Marine Science or the Office of Sea Grant Programs of NOAA or any other associated organizations. The particular designations of these materials and equipment are given because of their known reliability and non-toxic nature.

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We are especially indebted for the work done over the past years in the experimental cultures of oyster larvae by Ms. Susan Blaylock and to Ms. Laura Creekman for her typing of the different copies of this manuscript and aid in the technical interpretations of many of the aspects of this manual.

We are especially grateful for the initial guidance and continuing support of Dr. John L. Wood, Associate Director, which made possible the undertaking of this mariculture project.

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Finally we would like to recognize and thank Ms. Monica M. Dupuy for her extensive help in the initial organization, review, and editing of this manual.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I. INTRODUCTION

The husbandry of marine organisms has been practiced for many years in one form or another. In fact, the history of artificial oyster culture dates back to the small scale efforts during the Roman times in Lago Lucrino near Naples. As man has been more and more obliged to utilize the resources of the sea, he has been more and more concerned with the maximization of his yields in terms of time, labor, and money. More recent efforts in mariculture on a vast scale have been conducted principally in France, Holland, Japan, and the United States.

However, most of the efforts until the early 1950s have been based on the hunting of juvenile forms, which have then been grown under partially controlled conditions. Until this time natural sets of oysters had been sufficient to sustain the oyster industry in the United States at a reasonable level. However, disease, natural catastrophes, and increased pollution of the estuaries have drastically reduced the recruitment of juvenile oysters. The lack of a consistent production of juvenile oysters or spat on a natural basis had reduced the present oyster industry to a barely viable enterprise and has thus given impetus to the development of hatcheries to supplement natural seed production. Another major justification for the development and operation of oyster seed hatcheries has been the necessity to develop oyster stocks that exhibit resistance to disease, rapid growth, and good conformation. However, although much work has been done and a multitude of accomplishments noted, it must be observed that the "state of the art" remains to this day at the level of agriculture fifty years ago.

Past attempts to design and operate hatcheries have been based upon erroneous scientific information, lack of attention to the technological details necessary to a hatchery operation, and the underestimation of the cost of such a system. (At present a minimum of $500,000 is required to build and operate a hatchery system for the initial 18 months.) Thus, there have been numerous failures by people with fantasies of quick and easy profits. The failures of most of these "Ma and Pa" operations and supposedly the well-financed and carefully conceived operations have substantially discouraged the necessary investment by larger corporations.

From our observations and from the problems that are now besetting operational hatcheries, the success then of an oyster seed hatchery directly depends on accomplishing the following:

- the consistent and efficient production of seed oysters on a year-round basis;
- the production of genetically superior oysters having good growth, good shape, and resistance to disease;
- the production of cultch-free oysters or seed oysters on cultch; and
- the constant availability of seed as a supplement to natural seed production.

Since so much depends on the reliable and consistent production of oyster seed, the technology must be comparable to a brewery operation. The technological protocol, which includes attention to minute details and absolute control over the organism to be cultured, must be eventually achieved. Failure to operate such a hatchery system under strict control will result in production failure and eventual collapse of the hatchery system, regardless of the capital investment.

The operation of any hatchery system depends as well on the ability of management. The fanciful notion any person could operate such a system "cook book style" must be laid to rest. What is needed is a person with training and experience in the culture of bivalve mollusks. Lack of experience will certainly result in catastrophes which could otherwise have been avoided if a basic understanding of the biology of these marine organisms had been part of the manager's qualifications.

In summary, a successful hatchery operation must have a workable technology, sufficient capital funding and qualified management.

Historical Accomplishments

The development of hatchery methods for commercial production of the American oyster began with the report by Brooks (1879) that oyster eggs could be developed into the larval stage in the laboratory. Though many workers had attempted in the past to rear larvae in the laboratory, it was

not until 1920 when Wells demonstrated successful rearing and setting of oyster larvae in the Great South Bay area of Long Island, New York, that further development of life history studies and refined techniques for hatchery operations were able to be developed.

Cole (1937) first demonstrated that pure cultures of naked phytoflagellates could be used to produce growth of *Ostrea edulis* larvae under laboratory conditions. Subsequently, under the direction of Dr. Victory L. Loosanoff, Harry Davis (Davis, 1950, 1953) working at the U.S. Bureau of Commercial Fisheries Biological Laboratory at Milford, Connecticut, using a modified technique of Bruce, Knight and Parke (1940), successfully reared American oyster larvae on cultured phytoplankton. Many other investigators (Walne, 1956, 1963, 1965, 1966; Davis and Guillard, 1958; and Ukeles and Sweeney, 1969) have shown the best single foods to rear the larvae of *Ostrea edulis* and *Crassostrea virginica* to metamorphosis were *Pyramimonas grossi* and the chrysophytes, *Isochrysis galbana,* and *Monochrysis lutheri.* In addition, Davis and Guillard (1958) concluded the combination of *Isochrysis galbana* and *Monochrysis lutheri* with *Platymonas* sp. and *Dunaliella terctiolecta* provided better growth of oyster larvae than did any of these foods singly. Mackie (1969) demonstrated larvae of *Crassostrea virginica* from Delaware Bay littoral waters, selected phytoplankters 1 to 30 μ in size. She further stated that as the oyster larvae grew in size from straight-hinge to "eyed" larvae, the size of the phytoplankton they selected also increased. She cautioned, however, there appeared to be qualitative selection with respect to phytoplankters of comparable size, and, in each size category, several species of algae were utilized by the oyster larvae.

In contrast to the usage of cultural algal species singly or in combination, the Wells-Glancy method utilized bay water centrifuged to remove the animals and larger algal cells. The remaining small algal cells were allowed to grow in a greenhouse for 24 hours and then fed to the larvae. In a similar method, (Hidu *et al.,* 1969) the bay water was strained through a fine mesh cloth to remove the larger animal and algal cells and then fed to the larvae. However, the difference between the Wells- Glancy method and Hidu method was the latter method required the changing of bay water in larval culture tanks daily instead of every two to three days as in the former method. Though these two methods had been used extensively and successfully by commercial hatcheries and some research operations during certain periods of the year, they were inadequate as a method to obtain a consistent food supply for a year-round hatchery operation.

The Virginia Institute of Marine Science, in 1969, embarked on the crash program to obtain algal larval food species, which would result in the rapid growth and setting of oyster larvae to adequately support the genetic or selective breeding program for the oyster *Crassostrea virginica.* The major thrust of this program was to obtain oyster stocks resistant to the disease caused by *Minchinia nelsoni* (MSX). As a result of those efforts, three new algal species were isolated and, when tested in combination, provided growth of larvae of the oysters *Crassostrea virginica* and *Crassostrea gigas* to achieve setting in 9 to 11 days (Dupuy, 1973; Dupuy *et al.,* 1974; Dupuy, 1975; Windsor and Dupuy, 1976; Windsor, 1977). The culture of three species, *Pyramimonas virginica* nom. prov. (Va-17), *Pseudoisochrysis paradoxa* nom. prov. (Va-12), and *Chlorella* sp. nom. prov. (Va-52), have become the basis for the successful operation of the hatchery system to be described.

Operating Hatcheries According to Culture Methods

The United States has the greatest number of operational bivalve hatcheries in the world. As of July, 1975, there were sixteen privately owned hatcheries in operation. We do not know, however, how many of these hatcheries have actually been economically successful or how many would still be classified as research and development. In addition to these commercial units, six to seven state or federal units have been producing bivalve seed on a pilot scale.

Though three general methods (the Hidu, Glancy and unialgal methods) for the production of food to feed bivalve larvae have been reported, only the Glancy method and unialgal culture method are presently being used. Of those two methods, the unialgal culture method is being used almost exclusively. Only the Blue-Point Hatchery in Sayville, Long Island, New York uses the Glancy method. The remaining commercial bivalve hatcheries culture unialgal mass cultures of *Monochrysis lutheri, Isochrysis galbana, Dunaliella* sp., *Phaeodactylum tricornutum, Cyclotella nana,*

Pseudoisochrysis paradoxa, Pyramimonas virginica, Nannochloris oculata, Chlorella sp., *Chaetoceros calcitrans,* and *Skeletonema costatum* to feed the bivalve larvae.

The various methods to culture bivalve larvae are quite similar, with the exception of the algal combinations used for feeding the bivalve larvae to the point of setting. Furthermore, the density of bivalve larvae in the culture tanks may vary according to practices of the industrial hatchery, the species of bivalve, and the locality where they are cultured (Loosanoff and Davis, 1963).

The methods used for setting oyster larvae on cultch generally fall into three categories: 1) utilization of whole washed clam, oyster, or scallop shell 2) utilization of ground pieces of clam, oyster or scallop shell, and 3) utilization of artificial cultch (plastic sheets, plastic netting, or marble slabs). The sizes or size range of the ground pieces of clam, oyster, or scallop shell will vary according to whether the hatchery is producing cultchless or non-cultchless seed. Most of the west coast hatcheries primarily produce seed that is set on natural cultch. East coast hatcheries for the greater part of their production produce cultchless seed. The two methods used are 1) to set oyster larvae (one per particle) on very small particles of oyster shell which are so small as to be undetectible once the oyster larvae have grown to seed size, and 2) to set oyster larvae on plastic sheets or marble slabs followed by removal at various sizes to obtain cultchless seed. The size at which the set oysters are removed from setting surfaces is a function of the silt and organic load present to obtain oyster seed at a minimal labor cost. Oysters which are one to two millimeters in size and which are removed to be grown on an horizontal surface require constant cleaning in an area where the sea water contains heavy detrital, silt and kaolin particle loads. The holding of oyster spat on plastic surfaces to a larger size decreases the mortality rate and the labor cost from the reduction in time required to keep these small oyster spat clean.

Comparative Advantages of the Virginia Institute of Marine Science Hatchery Method and other Hatchery Methods

There are three major differences between the Virginia Institute of Marine Science Hatchery method and other methods presently being utilized by other hatcheries. First, the Institute method utilizes a combination of three species of algae, which consistently yields the setting of larvae in 9 to 11 days. In addition, yields of over 12 percent can be expected from the fertilized egg to the set oyster on a year-round basis. Second, the over-all design of specialized tanks and flumes allows for a regulated system which produces 3/4 inch oyster spat in 110 to 120 days. And third, the hatchery produces cultch-free spat of relatively uniform shape in areas where heavy silt and organic loads are present in the salt water with a minimun of labor and cost.

The design of the setting and holding system allows the hatchery to hold large quantities of newly set oysters with a minimum of labor cost. Unlike other hatcheries which keep their set on shell or pieces of shell on a horizontal plane in tanks, the VIMS system utilizes the Mylar sheets oriented in the horizontal plane for setting but then grows the set vertically. This vertical arrangement is particularly advantageous in areas where the incoming water which is distributed to the grow-out system contains heavy silt and organic detrital loads. It reduces losses due to effects from sediment materials. Each spat tank can hold 130,000 oyster spat without danger of smothering the small, 1 mm oysters. In addition, the cleaning of these spat tanks is simplified, since tanks can be emptied and flushed of the feces and pseudofeces without moving or distrubing the oyster spat. The oysters on this Mylar sheet will also grow faster, since a surface is supplied on which shell deposition can spread.

Once the oyster spat have grown to the 3/8 to 1/2 inch size, they can be easily removed from the Mylar sheets. The spat come off the sheets as individuals with a minimum of damage and are transferred to the horizontal grow-out flumes (30,000 spat per flume) where the oysters are easily cleaned because of their larger size. These cultch-free spat remain in the flumes until they reach seed-market size. Each of the six holding baskets within the flume holds 5,000 seed oysters.

Other advantages inherent to the VIMS hatchery grow-out system are: 1) control of predators, 2) control of pilferage and 3) the ability to keep these seed oysters relatively clean both in the grow-out spat tanks (containing the Mylar sheets) and in the flumes. The ability to keep seed oysters relatively

clean enhances the rate of growth and the uniformity of shape (Dupuy, unpublished).

The following chapters describe a system for producing seed oysters that not only has been tested, but also has been in full operation at VIMS and at a commercial hatchery. It is not the intent of the authors of this manual to attempt to provide the final chapter and verse in a field of aquaculture which is continually developing. Instead we offer the basics of a reasonably well-tested system, which, like beginning agriculture fifty years ago, adds another chapter to a fledgling industry. We feel that potential oyster-hatchery aquaculturists should *not,* at least until this system is made operational and mastered, yield to the temptation of improving the design. The very detailed description of this methodology and system has been purposefully included in order to help preclude errors which can easily produce catastrophes in a complex system such as the one prescribed here. The specificity of the types of products and equipment, though not implying endorsement of any specific product by the use of brand names, gives some assurance to the beginning entrepreneur or culturist as to those parts of the whole system described here which have been tried and are regarded as reliable and workable.

Finally, it must be emphasized that the hatchery design and methodology offered here undoubtedly should and will be improved in the future. Certainly many aspects of nutrition and engineering must be improved in order to maximize the efficiency of such a system.

CHAPTER II. ENVIRONMENTAL CONSIDERATIONS

Water Quality Aspects

Of the multiple factors which must be considered to achieve a viable hatchery system, the selection of a site for the hatchery becomes one of the most important — one which will control the technological success or failure of such a system. Failure to adhere strictly to adequate water-quality criteria, which will be described subsequently, will result in additional biological problems that will negate the technology which has been laboriously and successfully developed and tested for this hatchery system.

Salinity

One of the most important parameters that must be considered to insure continuous operation of an oyster hatchery is the seasonal salinity range of the area. We consider a range of 11 to 17 ^{o}/oo to be optimal for the Chesapeake Bay region and south when trying to produce *Crassostrea virginica* seed oysters. One must also consider the optimal salinity range for the three algal species utilized as food for the larvae. The lower limit for these marine algal species is 11 ^{o}/oo salinity. It is important that the salinity level not be allowed to drop below 11 ^{o}/oo if the hatchery system is to operate continuously, even under environmental stress conditions such as heavy rains, and snow, during the various seasons.

An important aspect, which affects the salinity fluctuations during adverse weather conditions, is the total watershed area draining into the location of the proposed hatchery site. If the watershed area is relatively small, then the effect of runoff will be minimal. A large watershed area will not only increase the total fresh water input into the basin during a storm where the hatchery is to be located, but also will prolong any period of stress to the estuarine system and therefore to the hatchery system.

An additional consideration affecting the salinity of the proposed hatchery site is the circulation flushing rate of the area. The relative stability of the salinity regime (salt content) for any given small area is dependent on the interaction of the circulation and flushing rate during a tidal cycle. The circulation must be such that the hatchery receives its incoming water from an area where the water is well-mixed. In addition, the flushing rate in any constricted body of water should be rapid so that an adequate exchange of water will occur during any particular tidal cycle. This exchange insures not only an influx and renewal of nutrients for phytoplankton growth, but also provides for the dilution of potentially toxic materials.

Sediments and Organic Detritus

The quantity of the sediments and organic detritus must also be determined in the water column of the area being considered for the hatchery site. The primary concern here is the cost of operations of the filtration system resulting from the process of removing silt and detrital loads from the salt water in the hatchery. The filtration of 5,000 gallons of incoming water through 1 μ filters for both larval and algal cultures every other day can substantially increase the operational costs due to the rapid clogging of the filters. It is important that the hatchery be erected in an area where natural silt and detrital loads are at a minimum and when mixing of the water columns in estuaries with relatively hard bottoms will not greatly increase the silt and detrital loads under normal circumstances and even during heavy winds and storms. Areas that have deep waters will usually have a reduced silt and detrital load, since wind action will have a minimal effect on the stirring of the bottom sediments. It can be expected shallow areas will be more quickly affected by wind action and will be susceptible to an increase in the quantity of silt and detritus in the water column when stirred by the wind.

Pollution

Serious consideration must be given to possible pollution sources that may affect the operation

of the hatchery. Optimally the hatchery site should be in an area where pollution is non-existent or at a minimum. Point sources of pollution within the immediate area of the hatchery should be completely avoided, such as marinas, boat yards, refineries, sewage disposal plants, and other light and heavy industry. Heavy metals, such as copper from marinas and boat yards, are extremely toxic. Chlorine, utilized by sewage treatment plants to sterilize the effluent is also highly toxic if released in the immediate area. The presence of light or heavy industry adds to the pollution loading factor, in that effluents from these plants may include toxicants of unknown biological hazard. In general the hatchery should be located as far as possible from all point sources from which contaminants will emanate.

In addition to point sources, there are non-point sources of pollution, such as the agricultural input into any given body of water. This type pollution comes from the runoff from the watershed area. It is very important to survey the extent of farm acreage that will directly drain into the hatchery site area. Herbicides and pesticides used by agriculture are potentially toxic to oyster larvae and to the algal species of food which are grown by a hatchery. Therefore, an area should be chosen with a relatively restricted watershed and a minimal of drainage from agricultural sources.

Finally, consideration must be given to the future growth potential of the area, including population projections, recreational and industrial sectors. Selection of a hatchery site in an area where the pressures of industry, the recreational needs and population projections are minimal does not guarantee the location will remain in that condition. It is important that an area be chosen where these pressures will not be felt appreciably for at least ten years. General trends can be surmised if the future operators of a hatchery consult the county planning boards regarding the expected time schedule of expansion for population growth, sewage treatment plants, and potential light or heavy industrial growth.

Biological History of Area

The biological history of any given area serves as an excellent indication of the quality of the environment and what potential biological problems a hatchery can anticipate.

One of the primary indicators of good water quality in any given locality is the presence of and the setting of oyster larvae. Though a lack of oyster larvae in the water column and setting does not always portend poor water quality, the occurrence does add to the probability that water quality is acceptable *a priori.* If the area has had an history of setting but recent records show an absence of oyster sets, then the question must be asked — Why? Changes in physical factors can alter the patterns of setting in any given area. Dredging of channels can alter circulation patterns and, in some cases, increase flushing rates in any particular area to the point where oyster larvae are carried out before they can set. The changing of circulation patterns, which can be caused by a change in the bottom topography in a given area, can also increase sediment deposition, concurrently reducing the availabilty of clean cultch material to which oyster larvae can attach. Therefore, it is important that the future operators of an oyster hatchery obtain as much information as possible from state and local records (oyster growers and shuckers) concerning the past history of the area of the site. It must be remembered any hatchery depends on the utilization of filtered water for a growing medium in the algal and larval tanks.

Another good indication of good water quality of any area is whether there are adult oysters in the area. Not only is the presence of oyster populations noteworthy, but also their rate of growth and their condition (fatness) are important. These parameters are an indication of the seasonal availability of food in the water column. Since the hatchery depends on natural food (algae) in the water to condition its broodstock and to grow the seed oysters after setting to the 3/4 inch size, it is imperative that an area be chosen that exhibits good growth and condition in natural oyster populations. It is also important that information be obtained as to the seasonality of growth for the natural populations of the area being considered. This will allow the hatchery manager to plan for the scheduling of the number of spawnings and sets which will reach seed market size during a particular year. This apsect becomes essential when calculations of cash flow for the venture are required. Again, this information can usually be obtained from records compiled by state fishery management agencies or research institutions.

Of considerable interest to the prospective hatchery operator are the problems associated with oyster mortalities that can occur in the natural populations near the hatchery site. Though the general salinity regime recommended above will tend to keep mortalities from *Dermocystidium marina* and *Minchinia nelsoni* (the MSX disease agent in *Crassostrea virginica* on the east coast) to a minimum, location of a hatchery at the higher salinity levels (15 to 17 °/oo) will increase the chances that mortalities will occur in the broodstock population in the hatchery. Since the hatchery will require special broodstock populations of oysters, such as those yielding good quality eggs or genetically selected strains, it is important the chosen area demonstrates low mortality in natural populations, otherwise hatchery broodstock populations may suffer undesirable levels of mortality. Again, much of this information can be obtained from state or university records and from local oyster growers.

When planning the day-to-day operations of an hatchery system, another major factor to consider is the presence and intensity of setting of other marine organisms, especially fouling organisms. The major concerns are the fouling of salt water pipes, holding spat tanks, and oyster seed flumes. In most cases heavy sets of *Molgula* (sea squirt), bryozoans, and barnacles will increase the labor costs of an hatchery by virtue of the fact that all parts of the system must be kept very clean. In addition, the time required to keep the oyster spat clean will increase to a point where a major portion of the personnel must cope with problem sets of bryozoans on the Mylar sheets containing newly set oysters. This can be a serious problem, because in that competition for space and an increase in the adhesion of detritus not only decreases growth rate but increases mortality. Sets of the worm, *Polydora,* which burrows into the shell of the oyster, can cause higher than normal mortalities in the seed oysters kept in the holding flumes. Though this worm causes mud blisters on the inside of the shell of an adult oyster, newly-set oysters cannot wall off this invader rapidly enough. In many cases, the intensity of set of these worms in the holding flumes is a function of the cleanliness of the flumes. Flumes containing large amounts of mud will tend to enhance the successful setting and burrowing of this worm. In considering a potential hatchery location, the potential hatchery operator should again inquire as to the history of fouling from records held by the state or local academic institutions in the considered area. Observations of the intensity of fouling in the environment in any given area can be made by looking at the pilings of piers or other structures that are submerged. Collection of natural populations of oysters can also give some measure of the intensity of infestation by the burrowing worm *Polydora.* All suitable esturaine areas are faced with some degree of setting of unwanted organisms; however, the hatcheries should be erected where fouling is minimal.

The last major consideration of the biological history of an area is the seasonal occurrence of red-water blooms. Though red-water outbreaks occur regularly in many areas, there is no definite evidence to directly implicate these dinoflagellates with mortality of oyster larvae or seed. However, there is evidence that heavy blooms of these organisms will inhibit the growth of both larvae and seed oysters (Dupuy and Rivkin, 1972). Further, mortalities have occurred indirectly from the settling out of these blooms to the bottom of the body of water where the hatchery water suppy is drawn or in hatchery tanks and flumes, with subsequent depletion of existing oxygen in the water column by the decomposition of the bloom-causing dinoflagellate cells. Red-water blooms of long duration also may cause serious filtration problems in the hatchery, particularly by the quick clogging of the filters, resulting in increased costs of operation. In some cases sufficient dinoglagellate cells will pass through the filters and reduce growth of the larvae in the larval culture tanks (Dupuy and Rivkin, 1972). Therefore it would be preferable to select a site without a history of red-water blooms.

Physical Characteristics of Area

After selecting an area with acceptable biological and water quality characteristics, consideration must be given to the physical characteristics of the specific site in relation to the actual construction of the hatchery and its pumping system. The foundation of the hatchery building must be sufficiently high so as not to be affected by high tides, storm tides, or hurricanes. Not only will insurance rates be relatively high for a hatchery that is located too close to sea level, but the

chances of losing the facility and/or its equipment and products will also be increased. Most localities have records indicating maximum high water attained for a given storm. These should be consulted and utilized. Additional allowance must be given to the added height of wind-driven waves. If at all possible, the foundation site for the hatchery building should have good bearing capability and drainage. The need for pilings to assure a good foundation will increase the cost of the building by 30 percent. The enormous weight per square foot of floor space from the water in the tanks and flumes requires very solid footing.

The main pumps, which supply 140,000 gallons per hour for this particular hatchery design, require a minimal intake depth of 6 feet at mean low water in order to pump safely on a continuous basis. The intake area should preferably have a hard bottom to reduce the amount of silt and detritus which will be sucked into the hatchery. The water-use permit requires that the intake be screened to stop fish and other invertebrates from being sucked in. However, a small screen at the intake pipe alone will usually clog from detritus, algae and shrimp very rapidly with the large volumes being pumped, resulting in damage to the pump. In addition, the pump intake should be surrounded on three sides with a bulkhead which, in addition to providing an adequate surface bearing for the heavy pumps, will provide a frame for a 1/2 inch wire mesh across the front of the screened intake lines.

Because of permit requirements and serious shore bottom erosion problems, the slope of the fast land from the hatchery building to the shore line should be such that the rate of flow of the hatchery effluent will not exceed 0.2 feet per second. It is advisable that the effluent canal or other means of effluent conveyance be placed so that the exit is sufficiently distant from the intake to prevent recycling. Preferably it should be placed on the opposite side of a point of land. This would assure that the effluent will not be immediately recycled into the hatchery. One possible arrangement for the intake and discharge system is given in Figure 3.

The distance from the pump intake to the hatchery building should be as short as possible. For every added foot of pipe from the main pump to the hatchery building, the rate of flow will proportionally decrease from frictional losses. This will mean fewer gallons delivered to the hatchery building at the same cost. In addition a minimal velocity of 7 feet per second inside the major pipes is required to assure a minimum of fouling.

Consideration of Permits

It requires at least one year to obtain the necessary federal, state, and county permits to build and operate a seed oyster hatchery in the United States. Regulations require that the owner of the property submit the permits for consideration to the various regulatory agencies. Therefore, land must be purchased, or a contract to lease the property must be established, in order to commence the permit process since the permit must be in the name of the property owner. If financing is to be arranged through loans directly from banks or through federal loan guarantees from the banks, the permits must be completed and approved before funds will be released by the lenders.

The following diagrams 1 and 2 will give the process for obtaining the federal, state, county and local permits. As indicated by the diagrams, the jurisdictions involved in applying for a permit to alter the shoreline are often interrelated and interdependent. In the case of intake and outfall pipes, the agencies involved in Virginia would be the Corps of Engineers (navigable waters), the Virginia Marine Resources Commission (state bottoms), the State Water Control Board (water quality), and, if wetlands are present, the local Wetlands Board and/or Virginia Marine Resources Commission. The permit process described in the diagram is suitable for the state of Virginia only. However, permit processes are very similar in other states with minor changes in the pathway or direction required to obtain permits. For example, in the state of Maryland the permit from the Environmental Protection Agency is obtained automatically when the Department of Natural Resources permit has been issued. Both the state and federal agencies in this case have similar requirements with an agreement that, if the state requirements are fulfilled, then federal agencies will agree to issue a permit.

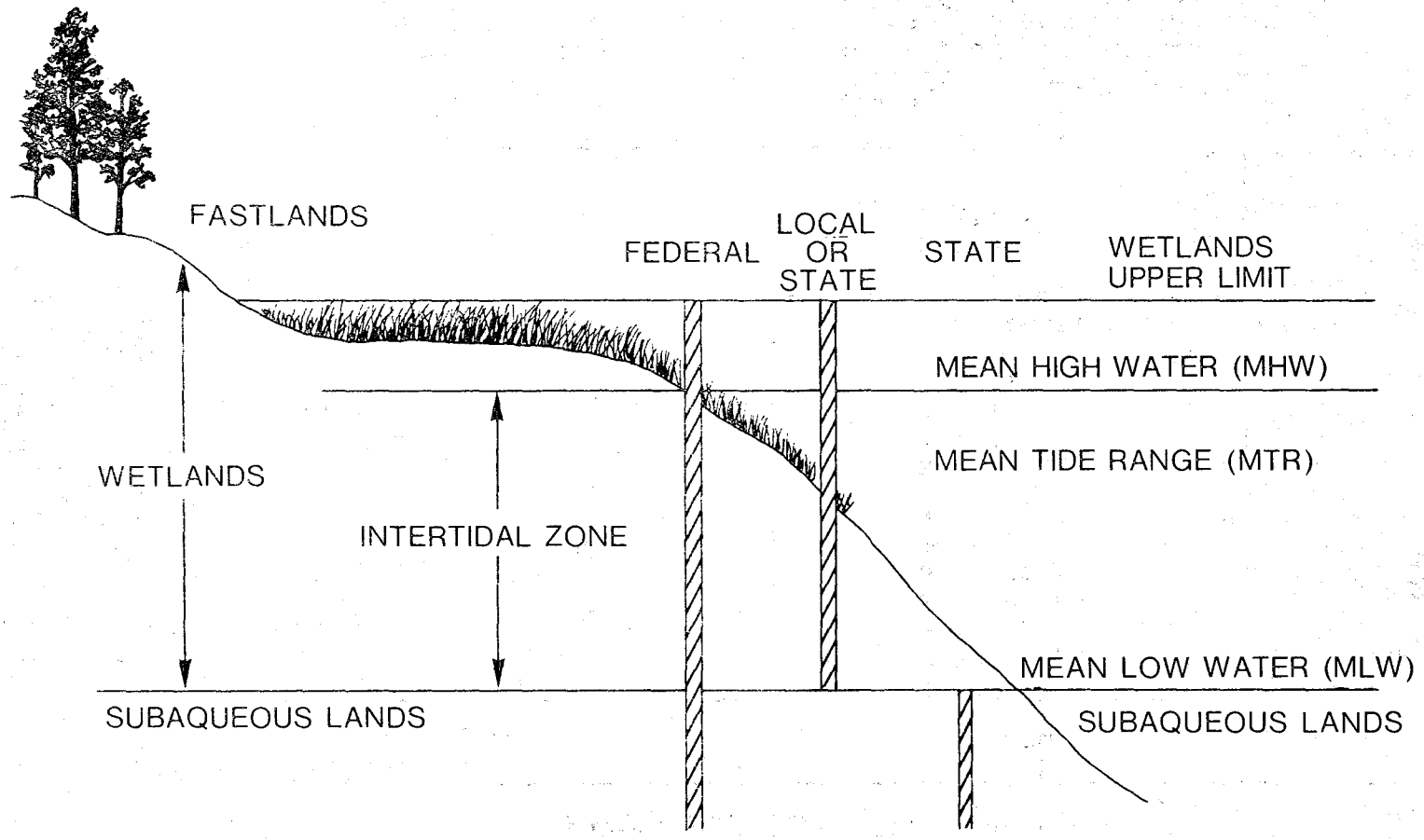


Figure 1. Shoreline Permit Jurisdictions.

FIGURE 2

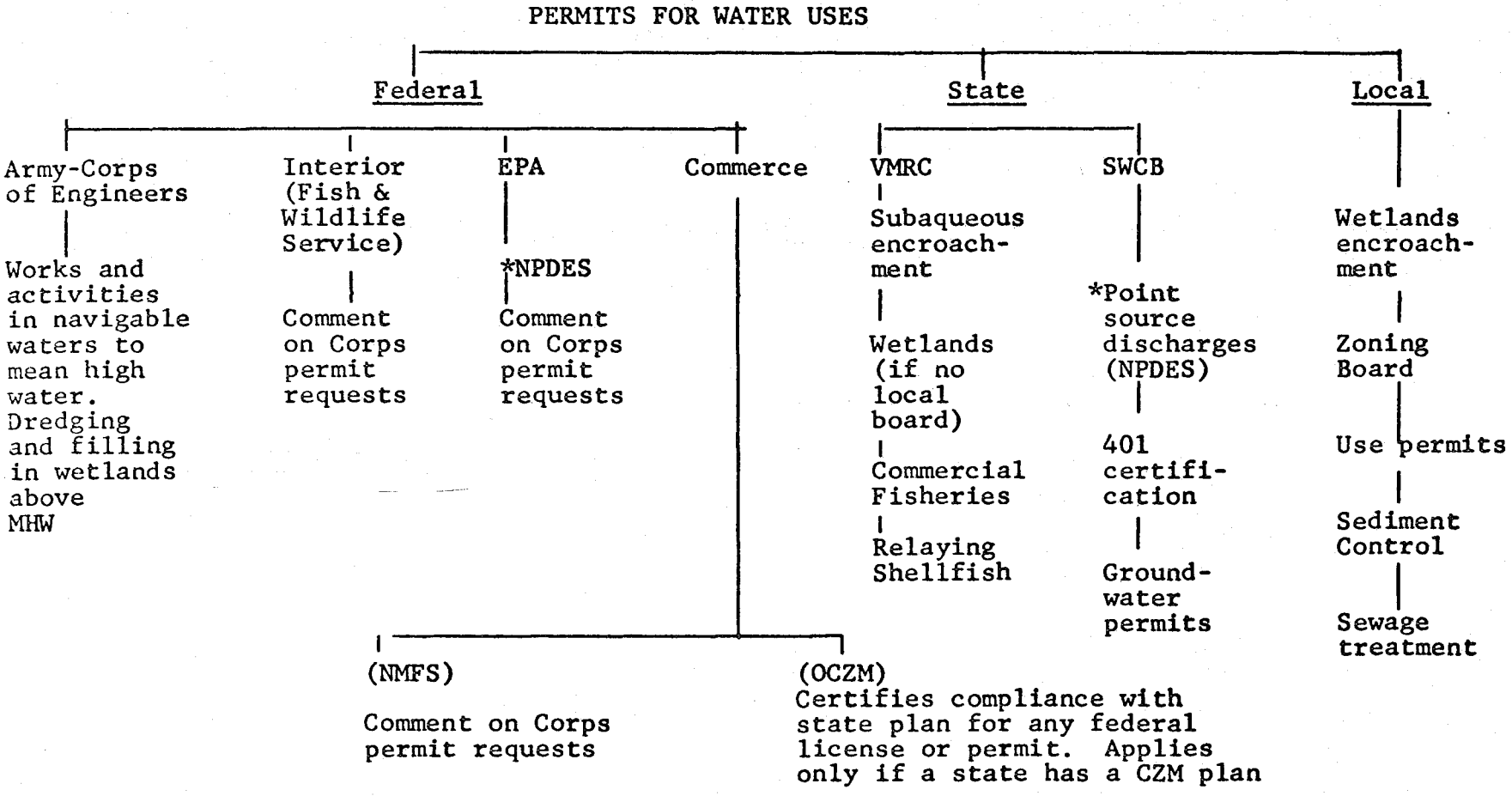


Figure 2. Permits For Water Use.

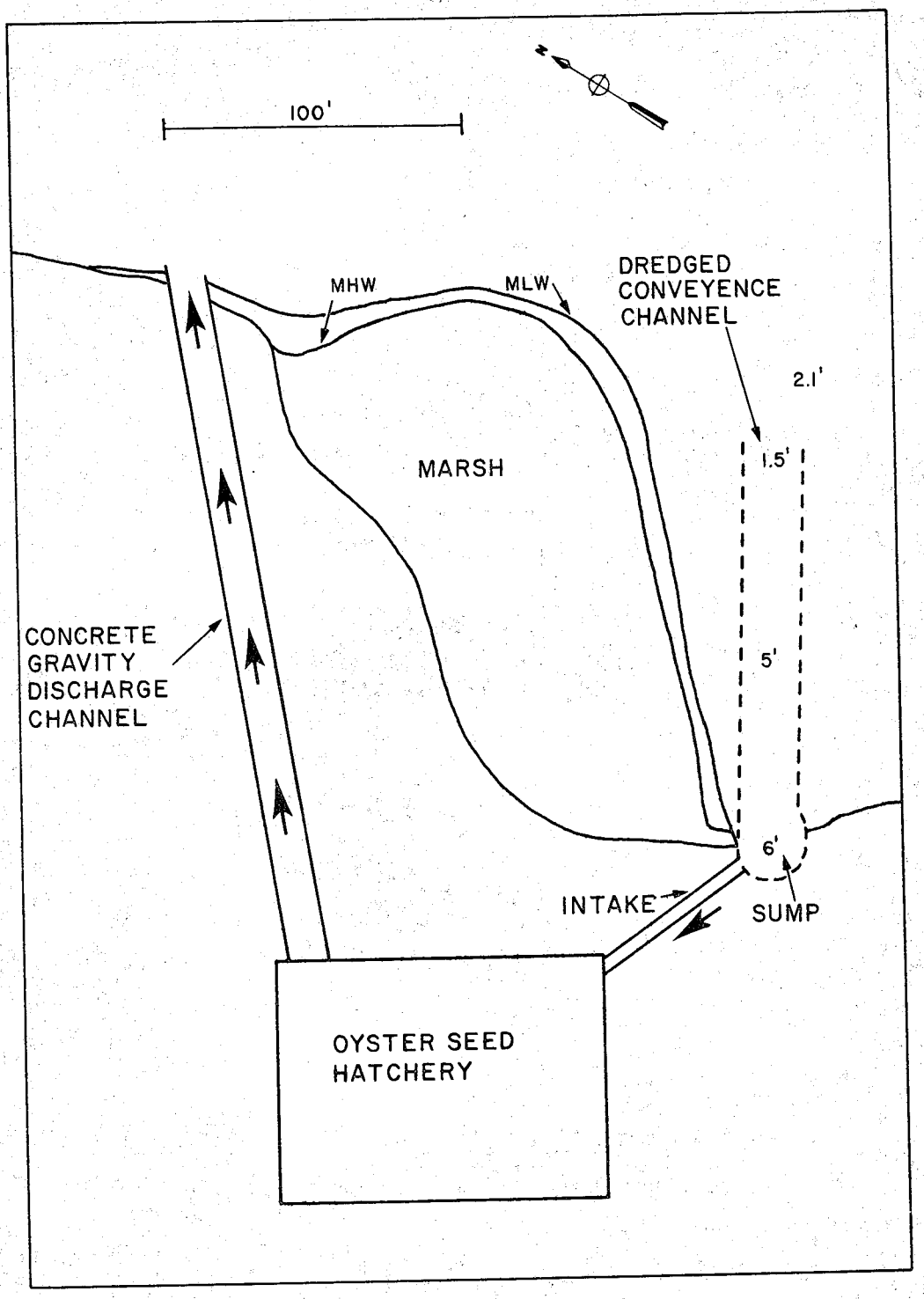


Figure 3. Description of General Oyster Seed Hatchery Site for Intake and Effluent Considerations.

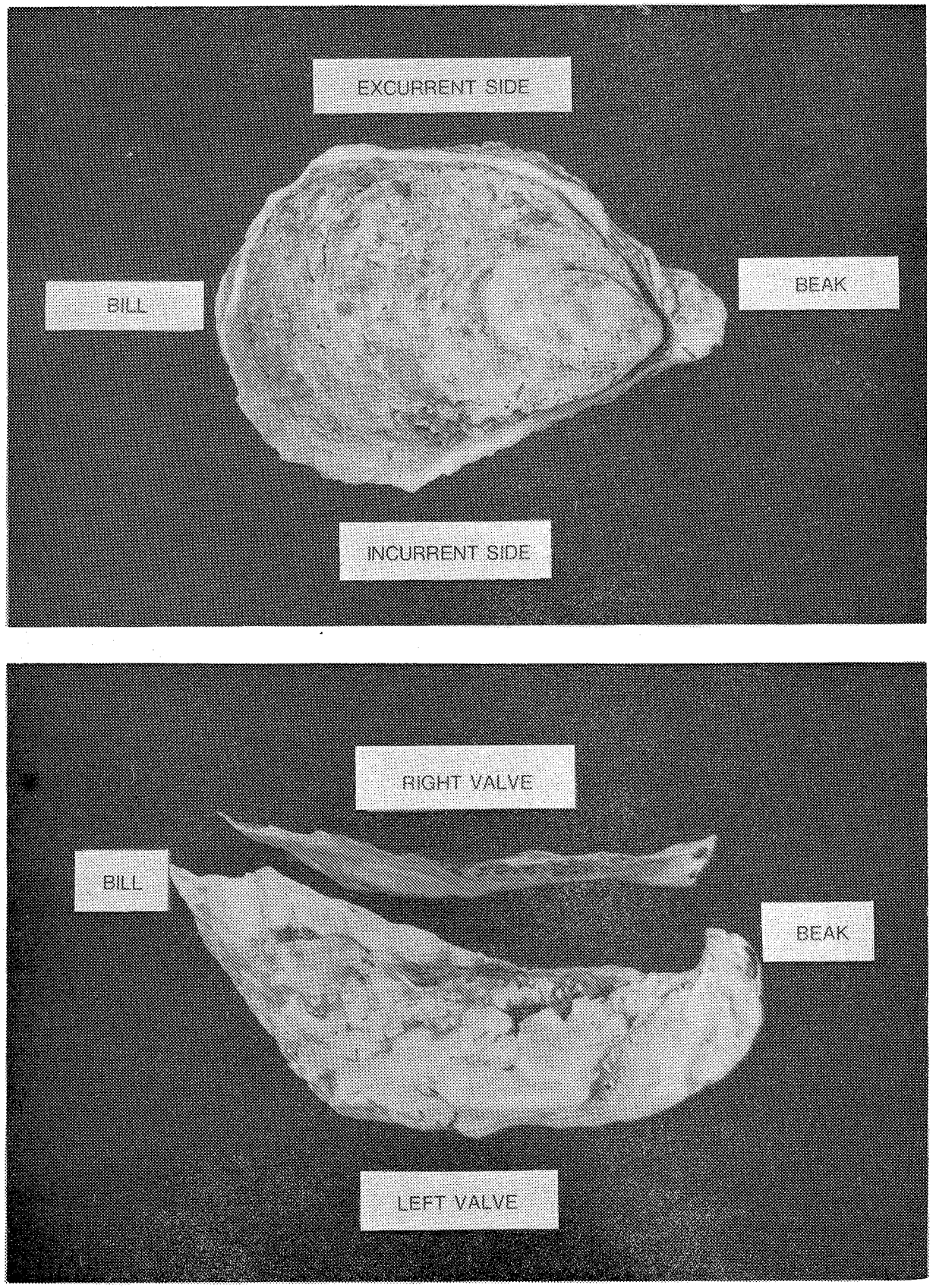


Figure 4. Top and Side View of the Valves of the American Oyster.

CHAPTER III. THE BIOLOGY OF THE PRODUCTION OF GAMETES AND SPAWNING OF ADULT OYSTERS

General Description of the Anatomy of the American Oyster*

Probably no bivalve or shellfish is better and more widely known scientifically than the oyster. The left valve, on which the oyster sets and rests, is cup- or saucer-shaped and the right valve is usually flat. [See Figure 4]. The hinge, which consists of the beaks from the right and left valves, together with the horny ligament, which allows the shell to open and close, as the adductor muscle relaxes or contracts, is known as the anterior or the front of the oyster. The area of the bill or the edge of the shell allows water to enter and exit. The tentacles on the mantle and the mantle curtain inside the bill control the water flow and allow water and food to enter and be filtered out by the gills, while also permitting water and wastes to exit [see Figure 5].

The enclosed main body of the animal, which is confined within mantle and shell, must now be generally described. The removal of the right shell-valve and mantle exposes the oyster lying on the left mantle and shell valve. Figures 4 and 5 describe the general anatomical features of the opened oyster. The oyster is divided functionally and anatomically into two parts, an incurrent side and an excurrent side. An increase in water temperature will usually increase the water flow rate through the oyster. Normally an American oyster pumps about 2 1/2 gallons per hour when the temperature of sea water is around 72 F.

Reproduction of the American oyster, in contrast to many other animals, is fairly simple. The reproductive area, the gonad, is located under the mantle of the oyster and consists of branching tubes and follicles on each side of the body. With the beginning of the production of eggs and sperm, the reproductive area becomes much thicker. When the eggs and sperm are ripe, they are passed along a series of larger tubes or canals which have minute hairs or cilia beating in one direction towards the outside. The eggs and sperm are passed to two openings, one from each side of the oyster, which are located in the exit chamber inside the gill chamber [see Figure 5].

The simplicity of the reproductive organs and the absence of any complex architecture in their ducts make possible a relatively easy change from one sex to another. The lining of the follicles can either produce eggs or sperm. It has been well documented that young oysters are primarily male (60-70%) and with the passage of time finally become primarily female. However, our experience has shown that the American oyster can change from a female back to a male quite rapidly after the eggs have been discharged. The process of changing from a functional male to a functional female is slower because the formation of egg yolk is required for early development of the oyster larvae. In summary, the sequence of events would be that a greater percentage of young oysters first become sexually mature as males, then change relatively slowly to functional females with age. The fact that functional females may revert to functional males must also be kept in mind. After the first year of sexual maturity, an oyster many begin any season as either a functional female or male.

The speed with which the various sexual stages (functional female or male) follow one another in the American oyster depends primarily on the quality and quantity of food available to the system. Observation has shown a greater preponderance of females where the diet is more than adequate. A greater quantity of nutritional components is required to produce an egg compared to sperm.

The male and female oyster reproductive areas (gonads) are located on both sides (right and left) under the mantle. The sex of the oyster can be determined only if the oysters spawn by, a biopsy of: the gonad or by opening and sacrificing the oyster. Samples of the gonadal tissues must be examined under the microscope in order to determine whether the oyster is female or male.

*Reference — Galtsoff, Paul S., 1964, The American Oyster. Fisheries Bulletin, Volume 64, p. 1-480

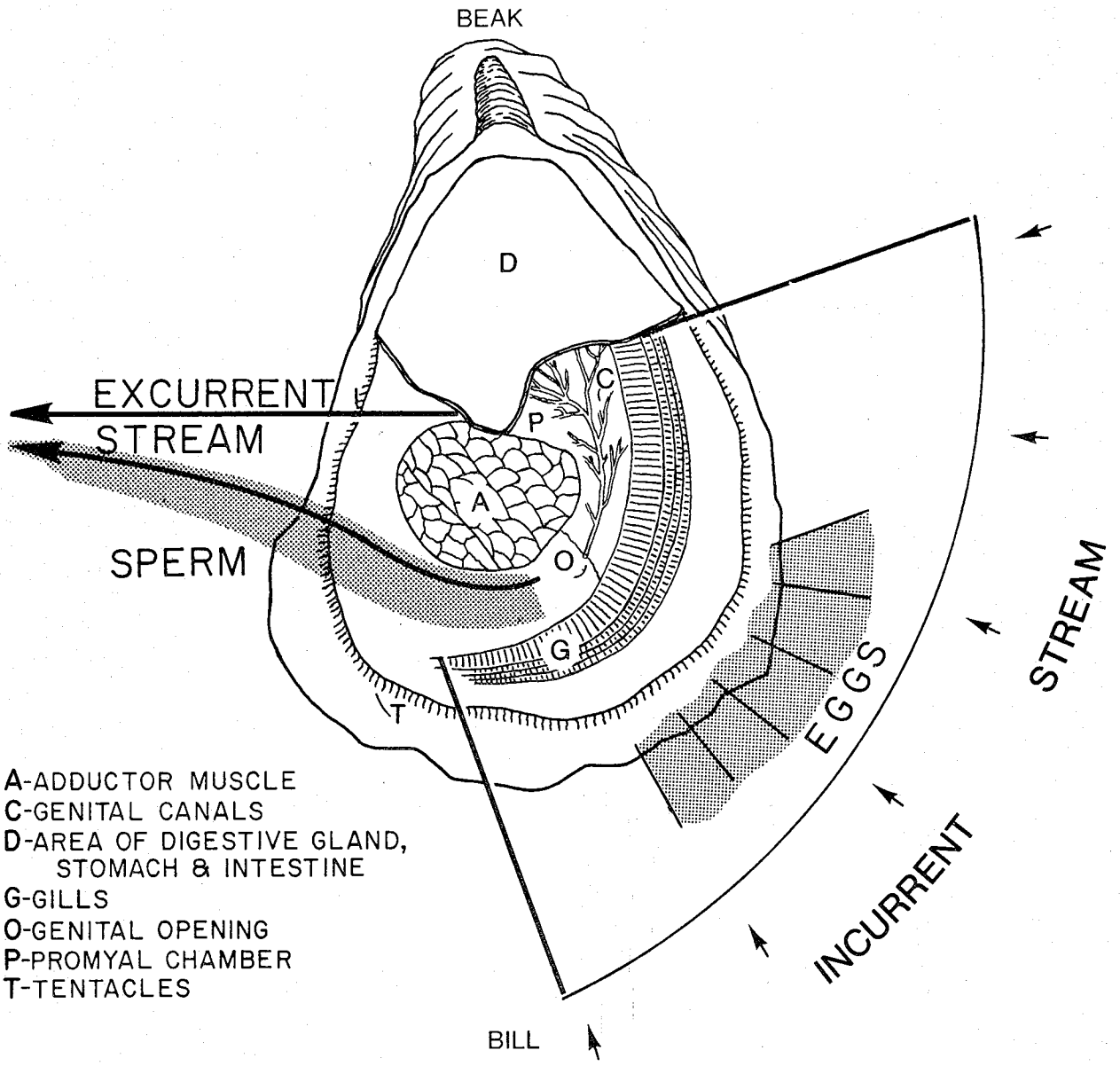


Figure 5. General Internal Anatomy of the American Oyster (Portion of Right Valve Removed).

Spawning, the release of sperm and eggs into water, occurs when the reproductive organs are ripe. This ripening occurs in nature and under controlled conditions when the temperature in the Chesapeake Bay area reaches and remains at about 19-22 C for three to five weeks. The male and female spawning processes are distinctly separate in appearance. As previously described, the sperm or eggs are both discharged into the promyal chamber or upper gill chamber.

The process by which the sperm and eggs are discharged is completely different. If the oyster is functioning as a male, then the sperm are discharged by the contraction of muscles in the walls of the genital ducts. The sperm are then carried away with the outgoing water current, appearing as a dense white stream emerging from between the shells or valves which quickly disperse in the water [see Figure 4].

The spawning process is more complex in the oyster functioning as a female. Unlike the male, the female rhythmically ejects the eggs through the incurrent side. The process is controlled by the mantle curtain which can close or open like a zipper. The tentacles which are part of the mantle curtain are kept closed except for a small opening while the eggs are collected in the outside gill cavity. The eggs pass through the gill chamber. The adductor muscle is relaxed at this point. When the eggs have accumulated in the outside gill chamber, the main adductor muscle contracts forcefully ejecting the eggs to the outside on the incurrent side with the excurrent side still closed. These contractions occur at relatively timed intervals [see Figure 5].

Conditioning of Oysters to Produce Gametes

Natural populations of oysters produce gametes (eggs and sperm) according to the seasonal changes in water temperature. In Chesapeake Bay a period of "hibernation" occurs generally from mid-December to mid-March. During this period the oyster pumps very little sea water and therefore does not feed very much, with heart rate slowing down to one or two beats per minute, since the activity of this animal is almost solely dependent on temperature. The other body functions are also at a minimum. When the water temperatures rise above 7 C the oyster's activity increases. From approximately mid-March, as the water temperature slowly rises, the oyster starts to feed and fatten. When the water temperature reaches 15 to 17 C in Chesapeake Bay the production of sex products begins (Dupuy, *et al.*, 1974). In Chesapeake Bay water temperatures from 19 to 22 C signals the optimum conditions for sperm and egg production. From the beginning of May until the oysters are ready to spawn at the end of June the water temperature slowly rises to 25 C. Spawning usually occurs at about this temperature in nature. During the months of July, August, and September the oyster will spawn intermittently, since not all oysters spawn at the same time and since those that have spawned will produce more eggs and sperm. Two major spawnings occur in Chesapeake Bay, one at the end of June and one in September. During the latter part of September to mid-November the oysters are feeding and fattening. As the temperature slowly drops, sex products remaining are re-absorbed. By December the sex of the oyster cannot be determined. The condition of the sex-producing areas at this time is what we term "neutral". This cycle is repeated year after year.

Artificial Conditioning

The protocol of the hatchery strives to control and manipulate the temperature regime of oysters so ripe oysters will be available for spawning at any time. This stage is known as broodstock conditioning. The initial requirement is the selection of the broodstock oysters.

First, it is an absolute requirement the oysters chosen for conditioning should be as fat as possible when the hatchery starts to operate. They should be harvested from populations in areas where they are known to be in good condition. This information can be obtained usually from local marine laboratories or the state fishery management agency. Additional information may be obtained from oystermen, oyster growers, or oyster shucking houses. These local people are usually well aware of the condition of the various oyster populations in different localities.

Second, the oysters chosen for conditioning should be selected from an area where the salinity regimes are similar (8 $^{o}/oo$ spread). Oysters that are not from areas of similar salinity conditions must be acclimated before they can be conditioned.

Third, the oysters acquired should be selected for shape and appearance of rapid growth. The production of new growth is characterized by the presence of a relatively thin shell or the appearance of a relatively large growth ring. Local watermen can point out loci of faster growing oysters.

It is important 30% of the population in the hatchery broodstock be young (1 1/2 to 2 years old or 1 1/2 to 2 1/2 inches in length) to assure a supply of males. If at all possible, the oysters to be used should be a mixture from different localities to assure a vigorous and a varied gene pool.

Last, a minimum of 600 oysters should be acquired for the hatchery system subsequently described. Of these 10 should be opened to ascertain if all sex products from the previous season's spawning have been reabsorbed. A new group should be acquired, if sex products are still present, since those oysters will not condition properly. Each conditioning flume will hold about 150 oysters optimally.

For clarity the procedures for causing the oysters to ripen on a year-round basis will be divided into four time sections: winter, spring, summer and fall. These time sections for the conditioning of oysters have been chosen to facilitate the spat production scheduling depending on when the hatchery begins operation. They are also generally applicable to other areas in the United States, however, the temperature regime will vary according to the geographical populations. Ideally the best time to start such operations is either mid-November (fall) or mid-April (spring), when oysters are generally in the best condition.

Winter

Oysters acquired during this period are normally in good condition. In Chesapeake Bay the ambient average temperature of the water has been running about 10 C. About the 15th of November 150 oysters should be placed in the first DupMo Mark II flume on Nestier trays in the conditioning area. These oysters should be thoroughly cleansed of mud and other marine life. To insure adequate nutrition for the production of eggs and sperm, a supplementary addition of cornstarch should be metered to the conditioning flumes as described below. This supplement increases the supply of materials for the oyster to produce its main reserve called glycogen (Haven, 1965; Creekman, 1977).

The directions are as follows:

Supplementary Feeding Utilizing Cornstarch

final concentration on flumes = 2.0 ppm or 2.0 mg/1
flow rate on flume = 10 liters/oyster/hour
150 oysters per flume = 1500 liters/hour or 25 liters/min
2.0 mg/1 starch X 1500 1/hr = 3000 mg or 3.0 g/hr
3.0 g starch/hr X 24 hrs/day = 72 g/day/flume

Unit of 10 flumes:

(1) Weigh out 720 grams of cornstarch
(2) Add starch to 5 liters cold water; stir until the starch is completely dissolved
(3) Stirring vigorously, add the above concentrated starch solution to 10 liters boiling water and remove from heat to avoid burning the starch on the bottom
(4) Dilute the 15 liter solution to 456 liters (120 gal) with water; keep this solution aerated with airstone bubblers
(5) To each flume monitor 27 ml/min of the starch solution to obtain a 2.0 ppm concentration

The temperature of the incoming salt water should be increased at the rate of 3° every two days until 19 C is reached. The flume should receive 10 liters of heated water per oyster per hour or 1500 liters per hour. After three more days the temperature should be increased to 23 C. This temperature should be held for five weeks after 23 C has been attained. At the end of five weeks the temperature should be readjusted to 19 C for the remainder of the time before spawning is attempted. Several oysters should be opened and sampled to determine if sex products are being produced. The lowering of the temperature is primarily to insure ripened oysters will not spawn accidentally, since male oysters can spawn in four weeks and female in four to six weeks at 22 C.

The flume and these 150 oysters should be cleaned frequently to remove mud and silt. The cleaning schedule will depend on the amount of silt and organic detritus which settle in the hatchery.

After these oysters have reached the spawning stage, they should be spawned within six weeks or the quality of the eggs will deteriorate.

These 150 oysters should be ready for spawning during the first half of January and should supply sufficient eggs and sperm to fill the 18-250 gallon larval tanks with approximately 5 million straight-hinge larvae per 250 gallon tank for at least three spawnings. Each female (4-5 inches) usually spawns approximately 15 to 20 million eggs on the average when fully ripe. It is important to note that not all of these oysters will be ripe at this point, nor will they all spawn at the same time, even if they are ripe.

Three weeks after the first group of 150 oysters have started conditioning, and every three weeks thereafter, another group of 150 oysters should be placed in separate DupMo Mark II flumes with the identical procedures being followed. This will assure a supply of spawnable oysters through mid-June at the rate of two spawnings per month.

The oysters from this winter group should be returned as they are spawned to incoming ambient temperature water and treated in the following manner. A fifth flume should be set up to receive these spawned oysters. When this flume has accumulated 150 oysters, the temperature of the heated water should be reduced from 19 C to ambient temperature at the rate of 1 C every two days. When these oysters have been acclimated they should be transferred to flumes which receive only ambient temperature water and held for later re-use.

Spring

During the first week in April, 300 oysters should be chosen as before and the procedure described in the previous section repeated. However, the second group of 150 oysters should be started *two* weeks after the first group of 150 oysters. These oysters will insure spawnable stocks through July 1. This interim group makes possible the natural conditioning of oysters for the summer section.

Summer

During the third week in May, 750 oysters should be carefully chosen from natural populations as previously described. These oysters should already be partly conditioned by the natural rise of temperature of the natural waters flowing over the oyster beds. Again, they should be divided equally in five DupMo Mark II flumes placed in the cooling flume section and fed ambient water at the rate of 10 liters per hour per oyster. When the natural water temperature reaches 23 C, the cooling water system should be started and the temperature kept at 23 C until the second week in June. These oysters at this point should be ready to spawn. The temperature of the cooling water should then be lowered to 19 C for the duration of the time of use to mid-September.

Fall

The conditioning of oysters for spawning during mid-October, November, and the beginning of December should begin in the second week of July. Oysters spawned earlier in the hatchery during the latter part of June and the first two weeks of July should be chosen carefully from those individual broodstock oysters which have shown, in the daily log records, their larvae exhibit good growth or vigor and a high percentage yield of setting from each spawned group. This will assure in the future individual oysters used for broodstock are capable of producing good quality gametes. It is important oysters chosen for use during this conditioning period be completely spawned out, since the greater quantity of eggs and sperm that remain in the oysters a longer period will be required for these individuals to reabsorb. From our experience oysters must be put through a cold period in order that they will start to condition properly. We have observed that most oysters can spawn twice in a season with little difficulty or loss of vigor; however, a period of rest, or cooler temperatures, is required to produce optimum quantities of eggs and sperm for hatchery use

beyond the first two spawnings. From these observations approximately 450 oysters must be artifically cooled down to 10 C and remain at that temperature for a period of six weeks. This allows the reabsorption of old eggs and sperm and the buildup of food reserves.

These oysters in the flumes should receive incoming water whose temperature is reduced by 2 C every two days for the first eight days and 3 C every three days thereafter until 10 C is attained. Thereafter, this population should be kept at 10 C for four weeks. At this time the increase in temperature should follow the protocol described in the winter section.

Summary

Approximately 2,200 adult oysters will be required during the period of one year to insure a constant supply of spawnable stock for a hatchery of the design specified herein. These spawning stocks must be chosen for both quantity and quality of egg and sperm production. As previously mentioned, oysters that are one to one and a half years old, (or one and a half to two and a half inches in length) must be chosen primarily to insure adequate sperm production. About 30% of the population should be in this age and size group. The remaining 70% of the total broodstocks should be oysters three to five inches long and between two and three years old in order to obtain sufficient quantities of eggs. In addition to the consideration of age and size, the oysters should be chosen where there is continuous observable growth. The prime objective of the hatchery in utilizing these broodstocks is to produce sex products which will yield not only sufficient quantities of sex products, but eggs and sperm of high quality. The production of low quality eggs by older oysters and oysters which do not exhibit growth ultimately results in poorer yields of setting larvae.

Parasites Affecting Production of Gametes

Parasitic infestations of adult oysters become a serious problem when the production of gametes (eggs and sperm) is of prime importance as required in a hatchery.

Populations infested with the parasitic Pea Crab (*Pinnotheres ostreum*) are not usually in satisfactory condition for the production of gametes or sex products. The damage to gills and the robbing of food from the gills of the oyster by this crab interferes with the feeding process. This creates a nutritional deficiency in the oysters and inhibits production of sperm and especially eggs. If the distribution of these parasitic crabs is unknown in the population being considered for spawning stock, then 50 to 100 oysters should be opened to determine the percentage of infestation. The appearance of percentage infestation even as low as ten percent should warn the hatchery manager to look elsewhere for spawning stock.

The trematode *Bucephalus* is another parasite affecting the ability of the oyster to produce gametes; in fact it essentially causes the castration of the oyster. Visual observation of the meat of an oyster reveals the yellowish to orange spots which indicate *Bucephalus* infection. If the infestation is sufficiently severe, then the mantle and the underlying gonad may reveal yellow-orange looking spheres and lumpy undulations. The number of oysters infested by this trematode may vary. Again, the opening of 50 to 100 oysters to determine the percentage of infestation of this parasitic trematode in oysters from a given locality will tell the hatchery manager whether oysters for spawning stocks should be put into the conditioning system. Normally *Bucephalus* infestation is not general, but occurs at point foci in any given locality.

General diseases will always affect the capability of the oyster to produce gametes or sex products of sufficient quantity or quality. Even if the disease is of low intensity and the effect is chronic, then gametes will be produced in small quantities and of doubtful quality. Of greater concern is the long-range effect of the disease-producing organisms after the oysters have been put into the conditioning system of the hatchery. This disease process is usually accelerated because of the increased temperature resulting in mass mortalities of broodstock and total upset of the spawning schedule. Of special concern are oysters from those geographical areas beginning with Delaware Bay on the north and running south along the coast to include the Gulf Coast region. Two major diseases, *Dermocystidium* ("Dermo") and *Minchinia* (MSX), are of great concern here, since they may affect entire oyster populations, particularly oysters which are two years and older. Mass

mortalities of hatchery broodstock can occur since elevated temperatures increase the time of continuous incubation. In natural populations infestations of "Dermo" are curtailed temporarily by a seasonal drop in the temperature. Both of these diseases can be controlled or eliminated if the hatchery and the obtained broodstock are located at a site where estuarine water is below 15 ^{o}/oo salinity. *Dermocystidium* and *Minchinia* usually cannot survive these lower salinites.

Contaminants, Other Organisms, Physical Phenomena

As indicated above proper screening and preparation of the adult oysters to be spawned are extremely important. Contamination by other undesirable organisms or their larvae, transported with the oyster sex products during spawning and subsequent transferal to the static larval culture containers, can result in serious competition with the larvae for the food supplied to the larval cultures. In addition, various contaminating organisms may cause physical interference with the swimming and feeding of the oyster larvae. There are two primary sources of contamination which can occur from the spawning broodstock oysters. The first source is those organisms which live on the shell and within the detritus adhering to the surface and within the holes, crevices, and the space between the beaks of the oyster. In addition, some of the polychaete worms living within the shell in burrows or in the crevices have also been observed to spawn simultaneously with the oyster during the spawning period. The second source originates from within the gill cavity, where, just prior to removing the oysters from the conditioning tables, other organisms, e.g. protozoans, rotifers, copepods, or their fertilized eggs, have been trapped and may be expelled during spawning. This will result in the transportation of these contaminating organisms with the oyster eggs to the larval culture tanks. For this reason it is important oysters used each time for spawning be thoroughly scrubbed and cleaned the day before an attempted spawning. These cleaned oysters should be put into the black spawning flume with 1μ filtered water and allowed to depurate, i.e. clear the gill chamber of extraneous material and organisms [see Figure 6]. The black color is utilized as a background to facilitate the observation of spawning by the broodstock of sperm and eggs.

Another phenomenon which inhibits spawning is "gassed oysters", resulting from warm water not properly de-gassed in spawning flume. In order to assure normal conditions when the oysters are put into the spawning flume, the heated, 1μ filtered salt water must be de-gassed. When cold water is heated, the solubility or the ability of the gases to remain in solution decreases. The greater the difference in temperature from the ambient outside water temperature to the final temperature of the heated water, the more gas will evolve or come out of solution. This gas comes out of solution in the form of minute bubbles. When these micro-bubbles are taken into the oyster as it is pumping water the oyster will stop pumping normally and pseudofeces will float instead of settling to the bottom. At this point the oysters are in distress, which is manifested by the cessation of pumping. Spawning is inhibited. For this reason heated, filtered water must be aerated prior to entering the spawning table to the point where feces and pseudofeces will *not* tend to float upwards in the water column. The floatation of pseudofeces or feces indicates minute air bubbles are being trapped in the mucus and incorporated in the pseudofeces and feces [Figure 6]. In another smaller flume above the spawning flume, aeration stones provide larger bubbles thereby increasing surface area for the micro-bubbles to coalesce. The rate at which this heated filtered water is delivered or passed through the aeration flume will be a function of the amount of gas coming out of solution and the rate at which the aeration can remove this gas to stop the incorporation of minute bubbles in the mucus of the filtration feeding system of the oyster gills. This procedure is also used for the water which is heated for the conditioning flumes. The filtered water temperature should be 19 C during the cleansing period. The cleansing period should start late in the afternoon the day before spawning and continue until early in the morning. The spawning flume should receive about 60 liters per hour of this 1 μ filtered water during this period. Early in the morning these cleaned oysters should be temporarily removed from the flume and salt water should be emptied and the spawning flume cleaned with fresh water to remove feces and pseudofeces. As rapidly as possible the oysters should be put back into the spawning flume in new 1 μ filtered salt water at a temperature of 19 C.

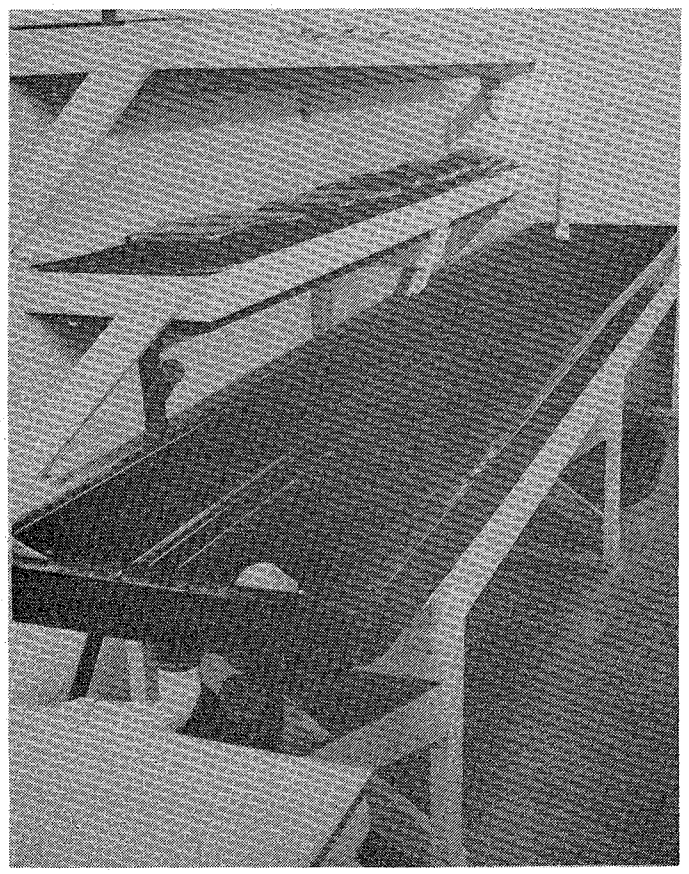


Figure 6. Spawning Flume Module.

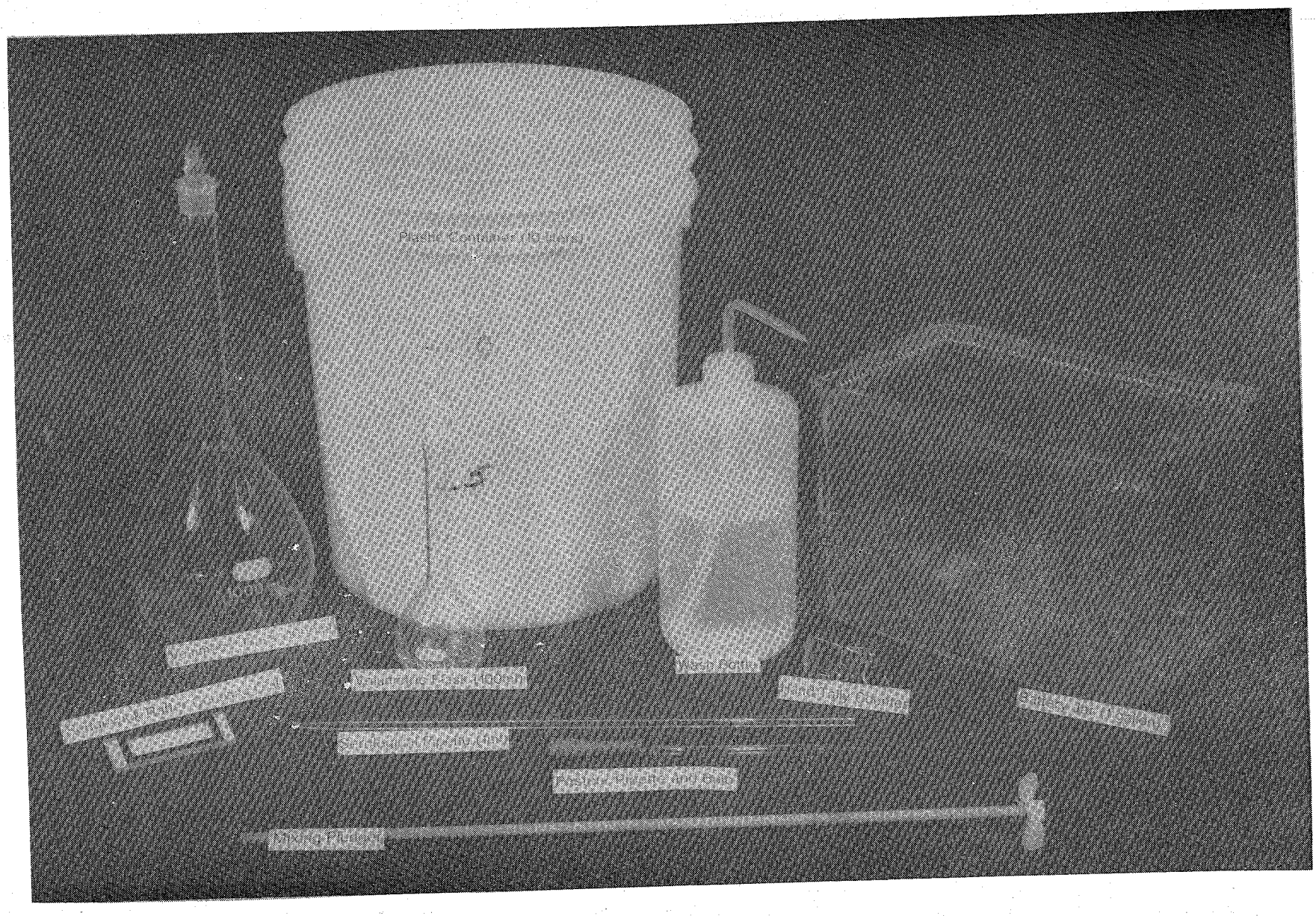


Figure 7. Equipment and Supplies Used For Oyster Larval Culture.

The 19 C 1 μ filtered water should be fed to the spawning flume at about 60 liters per hour until the oysters are open and pumping, while increasing the temperature over a 30 minute period to 30 C. At this point the male oysters may commence spawning spontaneously. There is, however, the remote possibility that females will also begin spawning. After the oysters have been pumping for 60 minutes in running 30 C filtered water, stripped oyster sex products should be used to induce spawning.

Several oysters of the group should be opened and examined to ascertain the sex and obtain sperm and eggs for the induction of spawning. The procedure is to make multiple shallow cuts of the mantle surface with a Pasteur pipette and then gently role and squeeze the Pasteur pipette over the surface. The Pasteur pipette is then used to aspirate the fluid and tissue, a drop of which is placed on a slide with a cover slip. Under low magnification (10 X objective and ocular) of a binocular compound microscope, determine if the oyster contains sperm, eggs, or glycogen. These will be seen as particles at low power of the scope. Rapid directional movement in the particles should indicate sperm. Particles having a random movement are not sperm but are only particulate matter displaying Brownian movement. Though active sperm are preferable for the induction of spawning, eggs can also be used for inducing spawning in males. The head of the sperm contains a hormone known as Diantlin, which activates the spawning reaction in females. Likewise, eggs contain an unknown substance which activates spawning reaction in males.

In order to obtain a sperm or egg suspension for placement in the spawning flume, the same procedure as described previously for the microscope sample should be used. The sex products can be stripped and added to a beaker containing 200 ml of 1 μ filtered water. The solution can then be transferred with a Pasteur pipette to the spawning flume. Utilizing this egg or sperm suspension obtained from the opened oysters, the tip of the filled Pasteur pipette should be immersed and placed above the oyster — behind the bill of the right valve along the incurrent side. The contents of the pipette should be released slowly along the edge of the bill. This stimulation should be given to all the oysters individually. If the oyster is pumping, the milky white solution can be observed entering the gill cavity. Most oysters, especially females, will react relatively soon to the sperm solution by clapping their valves. At given intervals of 10-15 minutes more sperm or egg suspension should be added over a 1 hour period or until the oysters start to spawn (c.f. pg. 42-45). The induction of spawning may occur rapidly (within 30 minutes) or at times may take as long as three hours.

Once the oyster starts to spawn, it should be transferred to a 1 gallon battery jar containing 1 μ filtered water at 27 C [Figure 7]. Twenty battery jars should be available for spawning purposes with at least ten containing 1 μ filtered water at 27 C. Only one oyster should be put into each jar with the males and females being kept separate. Each individual oyster should be allowed to finish its spawning in the jar. If the female is a heavy spawner and the water becomes relatively cloudy, she should be transferred to another jar to complete spawning. The spawning oysters in the battery jars should be carefully watched so each is removed as spawning ceases. If this is not done, the oysters will start to filter out their own eggs or sperm from the water in the battery jars.

The filtered water containing eggs from each battery jar should then be poured through a #75 mesh (100 μ pore opening) stainless steel sieve into a single plastic polypropalene container calibrated to 5 to 10 liters [see Table 1 and Figure 7]. If the female oysters are heavy spawners then the eggs from the battery jar containing the eggs of three females should be emptied into one ten liter plastic container. At least four of these plastic containers should be available at each spawning. Once the eggs are in the containers, they should be aerated to insure sufficient availability of oxygen from air bubbles produced by an aquarium airstone.

The eggs in each plastic container should be fertilized within 45 minutes after being spawned from a pooled sperm suspension obtained from the individual battery jars in which the males were spawned. Add 50 ml of a pooled sperm suspension, obtained from as many males as possible, to each plastic container containing egg suspension. Thirty minutes later a 2 ml sample of the fertilized eggs should be examined microscopically to ascertain approximately what percentage of eggs have been fertilized. This can be determined by noting if most of the eggs have a polar body. If 10% or greater of these eggs do not exhibit this polar body, then more sperm suspension should be added

(10-15 ml). Another sample 15 minutes later should be examined to determine if most of the eggs have been fertilized. When it has been determined fertilization is complete, the 1 μ filtered water should be added up to the 10 l mark so that a count can be made of the total number of eggs. This is necessary in order to properly allocate 25 million fertilized eggs into each of the five 250 gal larval tanks.

The counting procedure is as follows: When the volume in the container has been brought up to the 10 l mark, the fertilized eggs are uniformly suspended with a mixing plunger. In mixing, avoid creating a vortex (water cyclone) which will cause as error in the count. After 10-15 seconds of rapid mixing a 1 ml sample is taken using a serological (blowout) pipette. The sample should be drawn quickly as the eggs have a tendency to settle out of suspension. The sample is then transferred to a Sedgewick-Rafter cell and filtered sea water is added to achieve even distribution of liquid within the cell. Place this cell on a compound mono objective microscope with a 10 X objective and 10 X ocular. Using a hand tally counter, count and record all the fertilized eggs [Figure 7]. After the slide has been completely counted, the number of fertilized eggs per ml will be multiplied by the dilution factor in order to estimate the number of fertilized eggs in the total 10 liters. For example, if a 1 ml sample is withdrawn from a 10 liter container, then the count on the hand tally is multiplied by a factor of 10,000 (10,000 ml in 10 liters).

If the suspension of fertilized eggs is very dense, further dilution may be desired. An example of this would be to remove a 1 ml sample from the 10 liter container as before. Then, instead of placing it directly on the slide, place it in a 100 ml volumetric flask and dilute to volume. Pipette a 1 ml sample from this diluted suspension into the Sedgewick-Rafter cell and count as before. The estimated total number of fertilized eggs in the original 10 liters is then calculated as follows: number recorded on hand tally X 100 (100 ml volumetric) X 10,000 (10 liter container).

The expected production of eggs by a 3 1/2 to 5 inch female averages about 25 million eggs. Some large females (5 inches) have been known to produce up to 100 million eggs per female. If the female has partly spawned or is not fully ripe, then spawning of as low as 10 to 15 million eggs per female can be expected. Normally, ten fully ripe females (4 to 5 inches) will produce well over 200 million eggs. A fully ripe male oyster will produce sufficient sperm to fertilize this number of eggs. It is preferable to utilize a mixture of sperm from at least three males. If a smaller number of eggs is desired, use the gametes from three broodstock females for spawning. The use of three or more pooled female gametes and three or more pooled male gametes decreases the chances of failure of the brood to achieve adequate setting in terms of production of oyster spat. We know that the quality of eggs produced varies sufficiently between females and within the egg production of each female, so that the chances of high mortalities during the larval period are increased if less than three females are used.

Abnormal fertilization can occur if too many sperm are added at one time. This may result in several sperm penetrating the membrane of a single egg. This phenomenum is called polyspermy and causes irregular division or cleavage of the egg. Several other factors can cause abnormal development of the zygote through the trochophore stage to the straight-hinge larval stage. A delay of one hour or more in adding sperm to newly-spawned eggs will increase the percentage of abnormal larvae. The longer the delay the greater percentage of abnormal larvae. Finally, the age of the eggs (the amount of time they have resided within the female oyster after becoming mature) may also affect the incidence of abnormal larvae. The longer they have been retained the more abnormal larvae there will be. If microscopic examination under high magnification (45 X objective and 10 X ocular) reveals the detachment of the egg membrane in a good percentage of eggs, then the eggs spawned by the female are too old. The result will be a low percentage yield of straight-hinge larvae. When the eggs spawned by the female are tear-drop shaped when first spawned and then round up, the eggs are relatively new. The percentage of straight-hinge larvae resulting from fertilization of such new eggs should then be between 90-100%.

CHAPTER IV. CULTURE OF OYSTER LARVAE

Description of Larvae

The typical sequence of events in the development and growth of the larvae from the straight-hinge to the "eyed" or pediveliger stage is described in Figure 8 and 9. For a more detailed description of the anatomy of the larvae, the reader is referred to The American Oyster by Paul S. Galtsoff (1964).

Relevant Comments on: Equipment and Its Care

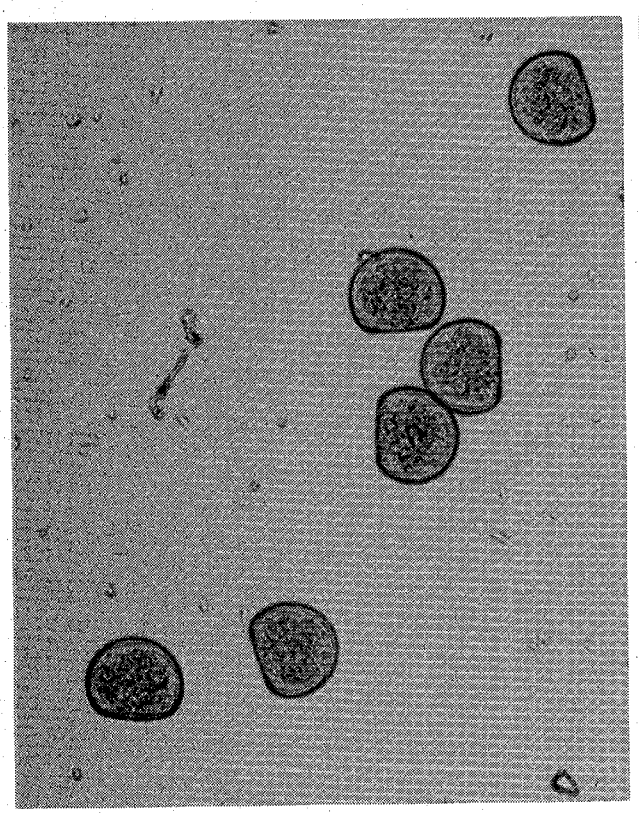
Several comments are required before describing the general daily schedule. First, it is very important that the pipes which carry the 1 μ filtered water from the water filtration area to the larval culture and setting room be kept very clean. These pipes should be flushed with fresh water at the end of each day. It is mandatory that the pipes then be filled with fresh water to kill protozoa or other organisms that may have gotten past the last filtration stage. At the beginning of each day these pipes should be emptied and flushed with fresh 1 μ filtered estuarine water prior to filling the larval tanks or the setting tables. The larval tanks should be cleaned with a brush and fresh water with a final rinse of 1 μ filtered water. Last, the 1 inch PVC valves should be removed once a week, taken apart, and thoroughly cleaned. These precautionary steps reduce the possibility of contamination of the larval cultures.

A necessary part of equipment for the larval culture procedures described herein are the stainless steel and Nitex mesh sieves. It is recommended that 8 #400 mesh (50 μ) sieves be available. The 50 μ designation commonly used in the lab refers to the calculated diagonal measurement of the square openings in the mesh, but the catalog description for the #400 mesh refers to the side measurement of the square opening, i.e. 32 μ (c.f. Table 1, page 65). In this manual other size sieves will be described by the mesh size and the important diagonal measurement. It is important in the use of these sieves to immerse them in hot fresh water in between use to cleanse them and prevent cross contamination between tanks.

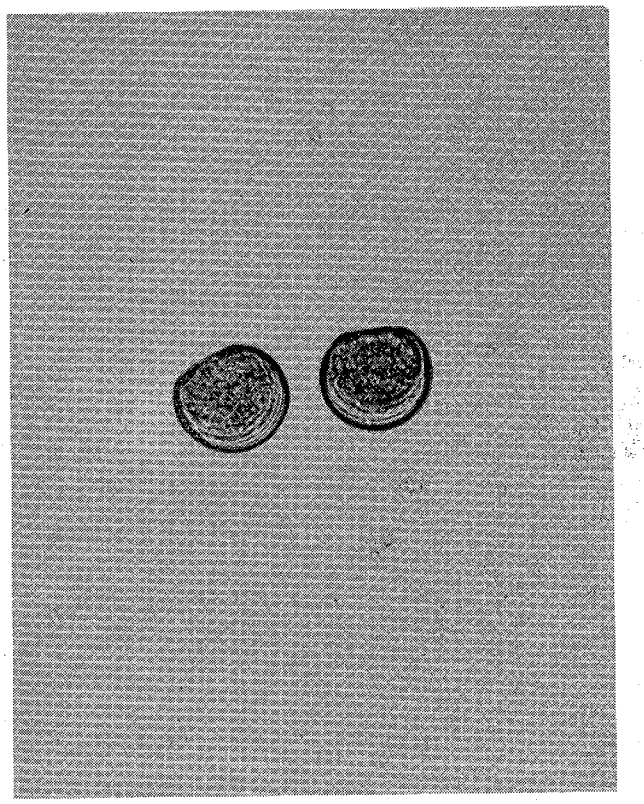
Methodology — Development to Straight-Hinge Stage

Using the density calculations as previously described, the fertilized eggs should be put into the 250 gal larval tanks to give a concentration of 25 eggs per ml of 1 μ filtered bay water or 25 million fertilized eggs per larval culture container [Figure 10]. The temperature of the filtered water should be 27 C. Straight-hinge larvae should be observed during the 12 to 16 hours after the eggs have been fertilized. A representative sample of the contents of the larval tank may be taken by making one sweep across the surface of the tank at a depth of 4 to 5 inches with a #400 mesh (50 μ) stainless steel sieve. The contents are transferred to a clean Sedgewick-Rafter cell for microscopic examination, c.f. page 52.

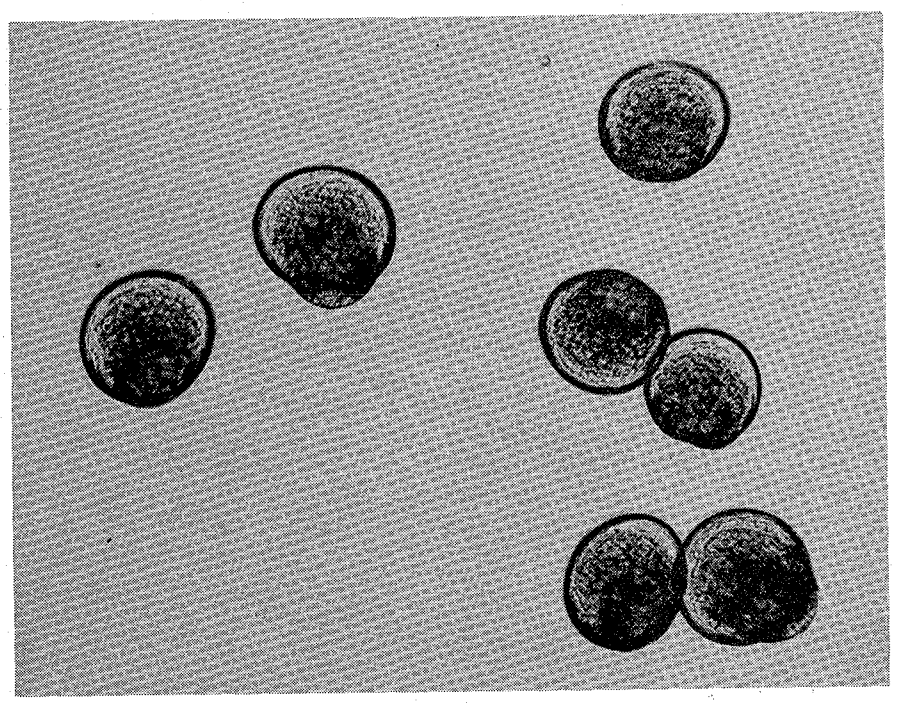
After 20 hours have elapsed from fertilization, the straight-hinge or veliger larvae are collected by filtering the larvae out on a #400 mesh (50 μ) screen. The filtering out of the straight-hinge larvae should be at a rate not exceeding 45 minutes per 250 gal larval tank. For each larval tank have a separate plastic container with 5 and 10 liter calibrations ready to receive the larvae. As the larvae from each tank are collected on the screen they should be periodically removed by flushing with squirts from a wash bottle containing filtered water into this plastic container. A complete transfer of the pink material (the oyster larvae) is accomplished by flushing the sides and finally the bottom of the tank with 1 μ filtered water at 27 C obtained from the one inch valve located over each 250 gal tank. A flexible plastic hose attached to this valve will make the process easier. When the washings have been filtered and all the straight-hinge larvae are in the plastic container, the volume is adjusted to the 10 liter mark with 1 μ filtered water at 27 C prior to the counting of the larvae described herein. Once the larval tanks have been emptied, they should be scrubbed with a brush and fresh water and then finally rinsed with 1 μ filtered water prior to refilling.



a. Straight-Hinge (Day 0)

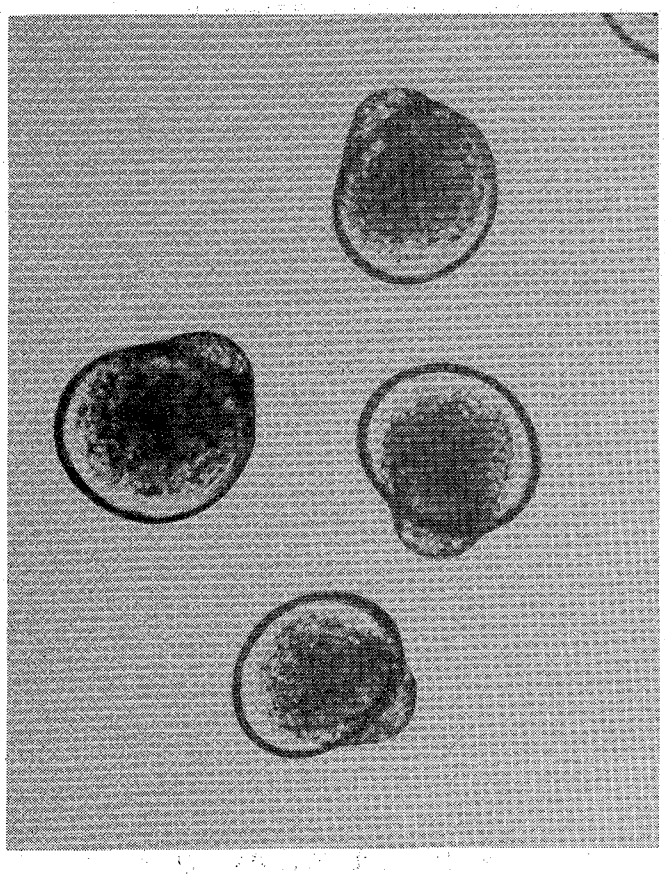


b. Early Umbo (Day 1)

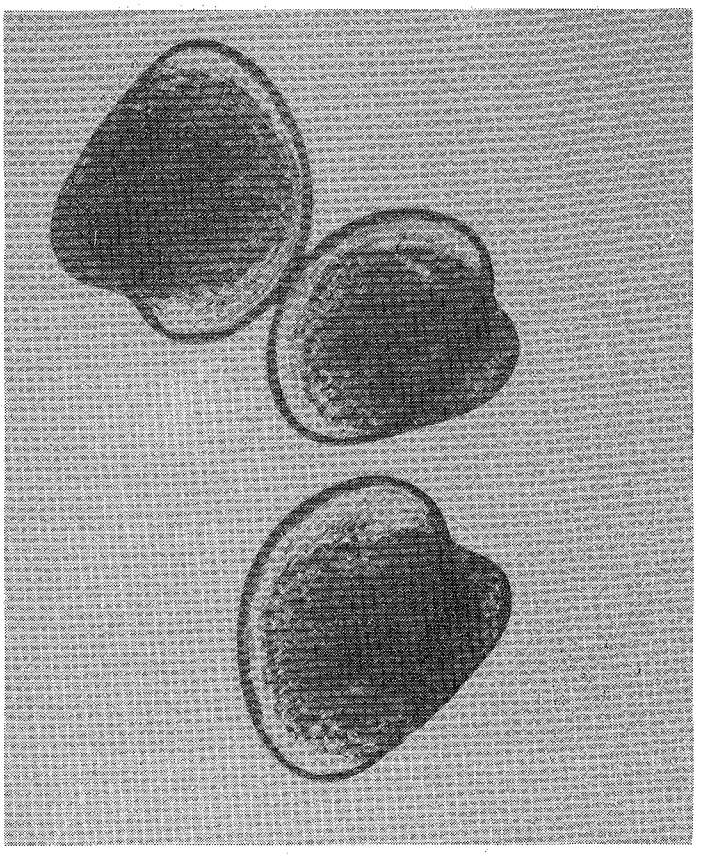


c. Umbo (Day 3)

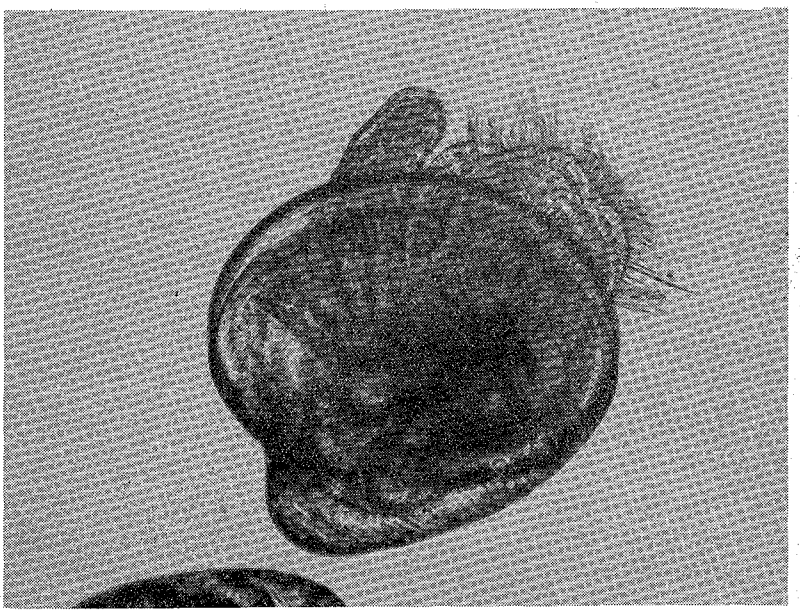
Figure 8. Photomicrographs of Oyster Larvae (40x) Straight-Hinge to Umbo Stages.



d. Late Umbo (Day 5)

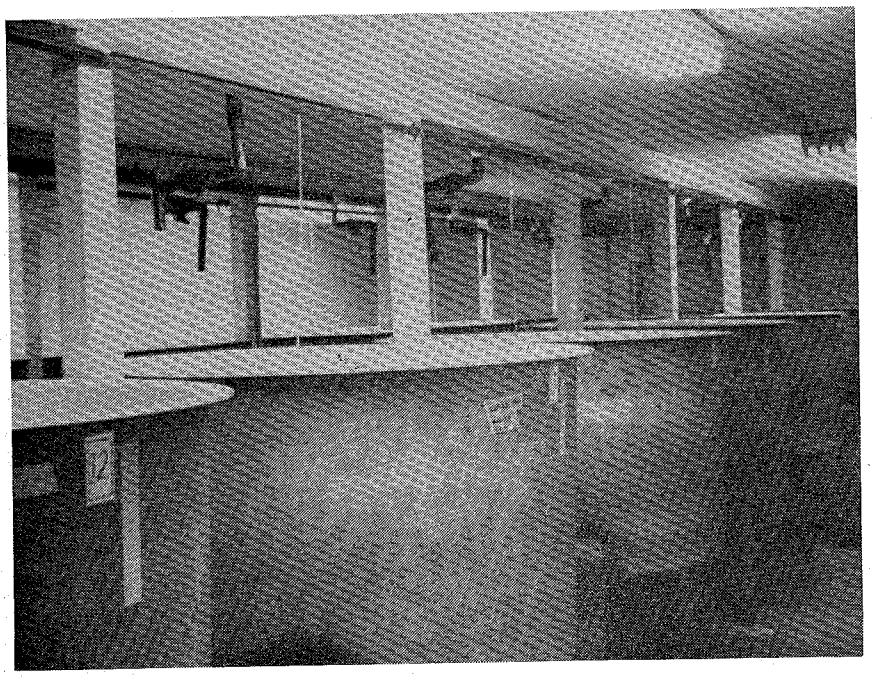


e. Mature (Day 7)

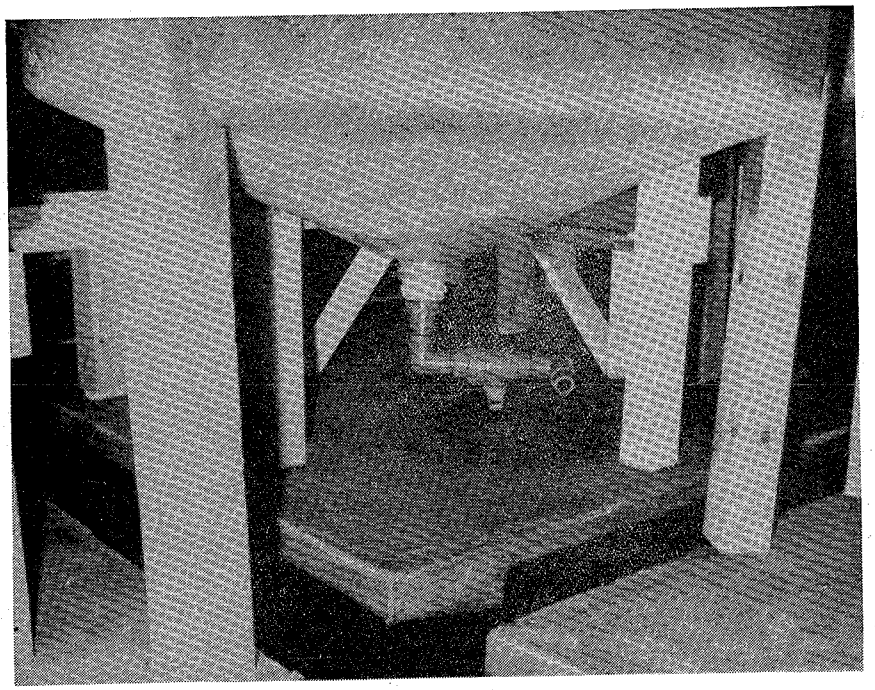


f. Pediveliger or "Eyed" (Day 9)

Figure 9. Photomicrographs of Oyster Larvae (40x) Late Umbo to Pediveliger Stages.



a. View showing top to tanks, air line for airstone and filtered water valve for each tank



b. View showing detail of the underneath of one tank with pipe and valve arrangement

Figure 10. Oyster Larval Culture Tanks, 250 gal (1000 liters).

The counting method for determining the number of straight-hinge larvae in the 10 liter container is similar to the fertilized egg count described on pages 53-54. The method is only slightly modified by the addition of several drops of 10% formalin in sea water to the Sedgewick-Rafter counting cell. The total count of larvae in the 10 liters is then calculated. Subsequently the straight-hinge larvae in the 10 liter container are then redistributed in the 250 gal tanks to give a final concentration of 5 larvae per ml or 5 million larvae per tank. This will give a total of 90 million larvae in the 18 larval culture containers. The rest of the straight-hinge larvae should be thrown out.

Normally a 90 to 100% success should be expected in the growth of straight-hinge larvae from the fertilized eggs. If the percentage yields are in the 50 to 80% range then: (1) the eggs were not totally fertilized, (2) the eggs were not fertilized within the 45 minutes, (3) the eggs that were spawned were old (greater than 1 1/2 months old) and (4) the eggs came from oysters in poor physiological condition as a result of preconditioning factors.

The presence of very active, straight-hinge larvae swimming in the upper levels of the water column (i.e. upper 8-10 inches) of the larval tank is usually an indication that the larvae are normal and vigorous. If these same larvae are seen to raft at the surface of the plastic container and very few settle to the bottom of the container, then there is further evidence that this batch of larvae are vigorous and normal. If the straight-hinge larvae are sluggish, there is either a problem with the quality of that particular batch of filtered water or the larvae are abnormal.

The initial microscopic examination of the larvae prior to filtration (c.f. page 59) should note the presence of larvae with malformed larval valves, absence of one valve, and abnormal swimming action. A slow circular movement of the larvae seen under the microscope indicates abnormal development of the velum or swimming apparatus. If 10% of the larvae show a crooked hinge line (i.e. hinge not straight as it should be but with undulations), or the surfaces of the valves are irregular, then the larvae must be considered as being of marginal quality. Serious consideration should be given to discarding the entire batch and beginning again. The larvae can be kept and fed for 2 days and then thrown out if these abnormalities do not disappear. If greater than 30% of the larvae have these abnormalities, then the batch should be terminated immediately and a new one started. At this point the discarded larvae do not represent any appreciable loss. However, continuation of such a batch would be expected to result in a poor yield of setting oysters and the added investment in time and money required to bring them to the setting stage would be lost.

Methodology — Development to Beginning of "Eyed" Stage

As previously described, each of the 18 larval culture tanks should contain approximately 5 million straight-hinge larvae on the day after fertilization. An airstone should be placed in the center of the bottom of each tank and vigorous aeration provided. The larvae in each of the tanks should be checked each day for growth and general condition, that is, approximate percent mortality, activity, and possible contaminants such as rotifers, protozoa and copepods. Again a #400 mesh (50 μ) screen should be swept across the surface of the tank to about a 6 inch depth to collect a sample of the swimming larvae. In addition, a 1 liter sample from the bottom tank valve should be passed through the same sieve. This will give a representative sample to check the condition of the oyster larvae. A Pasteur pipette can then be used to aspirate some larvae from the screen for microscopic evaluation. The remainder of the larvae on the screen should be put back into the tank by rinsing the screen with a wash bottle containing 1 μ filtered water. Once the screen has been flushed it should be immersed in hot fresh water to prevent possible cross-contamination between larval tanks. A complete written, daily record of larval growth and condition should be maintained for each tank.

Two times each week, e.g. Monday and Thursday or Tuesday and Friday, the larval culture tanks should be drained, placing the appropriate mesh size stainless steel sieve under the bottom valve of the larval tank to collect the larvae [see Table 1]. The mesh size chosen should be small enough to filter out the majority of the larvae, and for this reason a sample should be taken prior to emptying to note size distribution. As the tank is being emptied through the stainless steel mesh sieve, the flow should be stopped periodically to transfer the larvae into the 10 liter plastic container

being used for that tank and labeled as such. The larvae are flushed off the screen into 5 liters of filtered water contained in the plastic container. After the tank has been cleaned, refill it with 1 u filtered water at 27 C. The larvae are then transferred back into the tank and aeration renewed [Figure 10]. Emptying of the larval tanks should occupy no less than 45 minutes, because damage to the oyster larvae may occur at a faster flow rate. The 18 larval tanks may be split into two groups of tanks for changing the filtered water twice a week if it is more convenient. This procedure of checking the larval condition daily and renewing the water twice per week should be continued until all larvae have been removed and finally put into the setting modules.

Table 1

Screen Sizes for Larval Handling

Tyler Series Equivalent[1]			Nitex Bolting[2]		
stainless steel (9" screen)	square opening (u)	diagonal opening (u)	36" X 42" cloth	square opening (u)	diagonal opening (u)
No. 400	37	50	HC3-44	44	62
No. 200	75	100	HC3-73	73	103
No. 140	105	150	HC3-85	85	120
No. 100	149	200	HC3-110	110	155
No. 80	180	250	HC3-127	127	179
No. 60	250	355	HC3-163	163	230
			HC3-183	183	258
			HC3-202	202	285
			HC3-253	253	357

[1]Fisher Scientific Co.
Corporate Headquarters
711 Forbes Ave.
Pittsburgh, Pa. 15219

Tel: (412) 562-8000

[2]Nitex Bolting
Tetko Inc.
420 Saw Mill River Rd.
Elmsford, N.Y. 10523

Tel: (914) 592-5010

We have noted previously that each 250 gal larval tank should contain 5 million straight-hinge larvae at the beginning of the larval cycle. One can expect each day a larval mortality rate of somewhat less than 2%. If the mortality is significantly higher something is abnormal. The renewal of filtered water twice a week, along with the utilization of larger mesh size stainless steel screens as the larvae increase in size will remove the dead and non-growing larvae.

When the oyster larvae reach a size of 180 μ (Day 5), the number of larvae per tank must be reduced [see Figure 11]. It is *absolutely* necessary to reduce the number of larvae to between 3 to 4 million larvae per 250 gal tank, since these larger oyster larvae require greater space per individual for optimal feeding and growth (Windsor, 1977). The culling procedure to reduce these numbers to 3 to 4 million per larval tank can be achieved by first straining out all the larvae from each tank on a # 200 mesh (100 μ) sieve separately. The larvae are then resuspended in 5 liters of 1 μ filtered water in a plastic container. The resuspended larvae are then poured through a # 140 mesh (150 μ) sieve into another plastic container. The larvae on the # 140 mesh sieve are resuspended in a plastic bucket with the 1 μ filtered water, brought up to a total volume of 10 liters aerated and kept until a larval count is made. In order to achieve the necessary reduction in numbers, this procedure requires that a count be made of the larvae on Day 5 for each 250 gal larval tank. The procedure for counting these larger larvae is the same as that for the straight-hinge larvae, except that the 1 ml sample taken from the 10 liter plastic container should be put directly on the counting slide. Failure to do so will result in a large counting error.

If there are more than 3 million oyster larvae in the 10 liters, then the larvae that were less than 150 μ (those which went through the 150 μ sieve) can be discarded. If there are less than 3 million, then the larvae which went through the 150 μ screen must be retained until all the larval tanks have been culled and counted. This procedure must be followed for each larval tank. If the larval count of greater than 150 μ larvae is greater than 4 million, a proportion of the 10 liters must be removed. This is relatively easy since the count is recorded as the number of larvae per ml. To insure that there will be sufficient larvae greater than 150 μ to fill all the larval tanks the excess from each tank should be retained until all tanks have been processed. The larvae greater that 150 μ should be returned to the larval tanks as quickly as possible.

The time required to bring the larvae to the "eyed" (285 μ) stage will vary from 9 to 11 days. Figure 11 illustrates the daily growth increment and size distribution of *Crassostrea virginica* larvae for a range of salinity of 11 ^{o}/oo to 20 ^{o}/oo at 27 C. Figure 12 illustrates the daily growth increments and size distribution of *Crassostra gigas* larvae at a salinity of 20 ^{o}/oo at 27 C. If, during these 9 to 11 days the daily checks of larval growth indicates a lag in the growth of the larvae lasting as long as one day, special attention should be focused on the possibility that there may be a water quality problem. If larval growth should lag a second day, then the water in the larval tanks should be immediately replaced with fresh processed estuarine water. The possibility of the presence of contaminants (rotifers, etc.) should be eliminated before consideration of any possible water quality problem. Up to the present, both at our laboratory and in a commercial hatchery with which we have been involved, there has never been demonstrated a problem with bacterial contamination of such magnitude to cause mortality or lack of growth with the oyster larvae. We believe that this happy situation will prevail if our rigid protocol is faithfully followed. Many of the bacterial problems are due to a faulty filtration system and attention to lack of cleanliness with the total filtered water system. For this reason we have not found antibiotics to be useful and do not recommend their utilization.

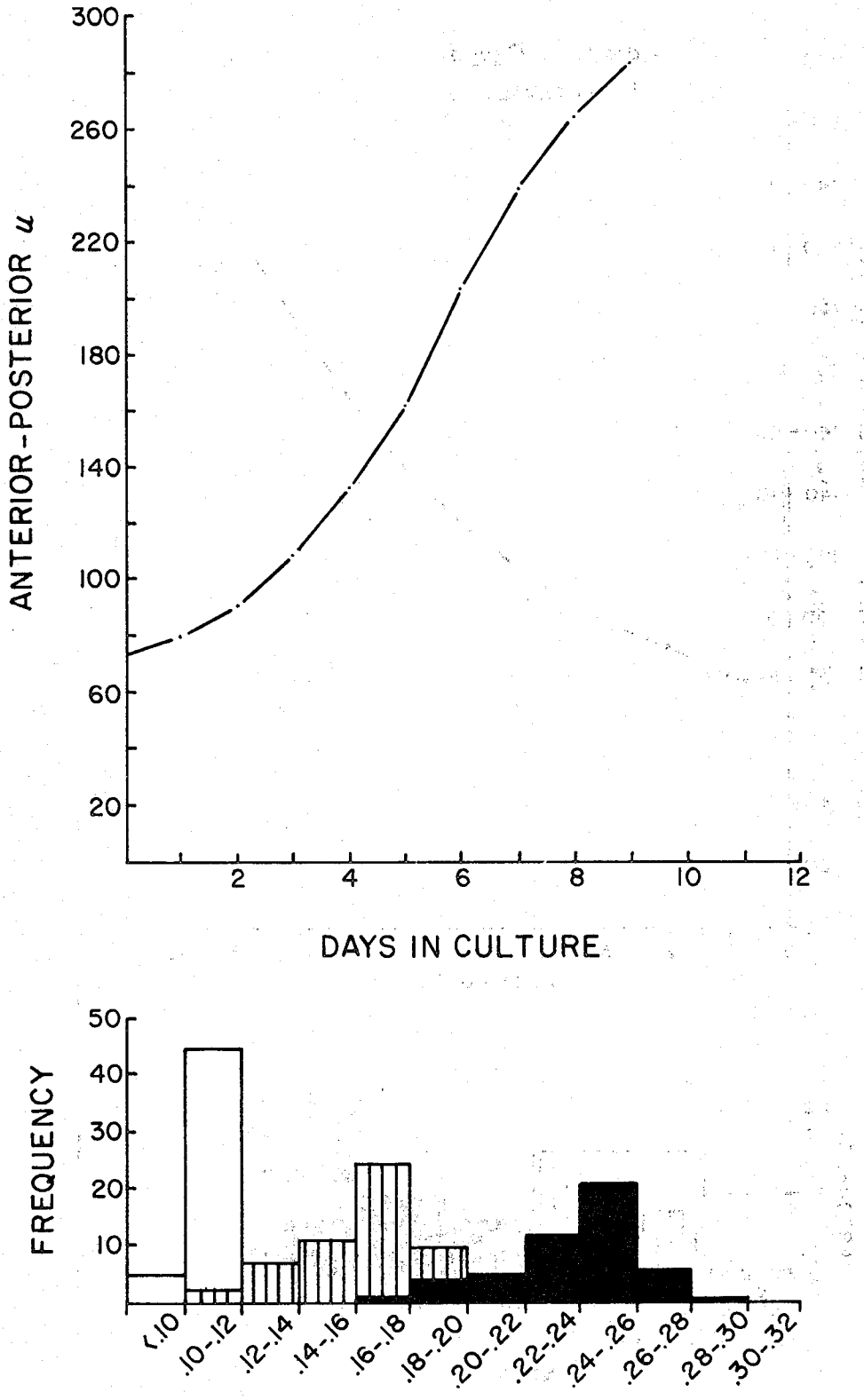


Figure 11. Growth Rate of Oyster Larvae and Size Distribution of Larval Population of *Crassostrea virginica* on Days 3, 5 and 7.

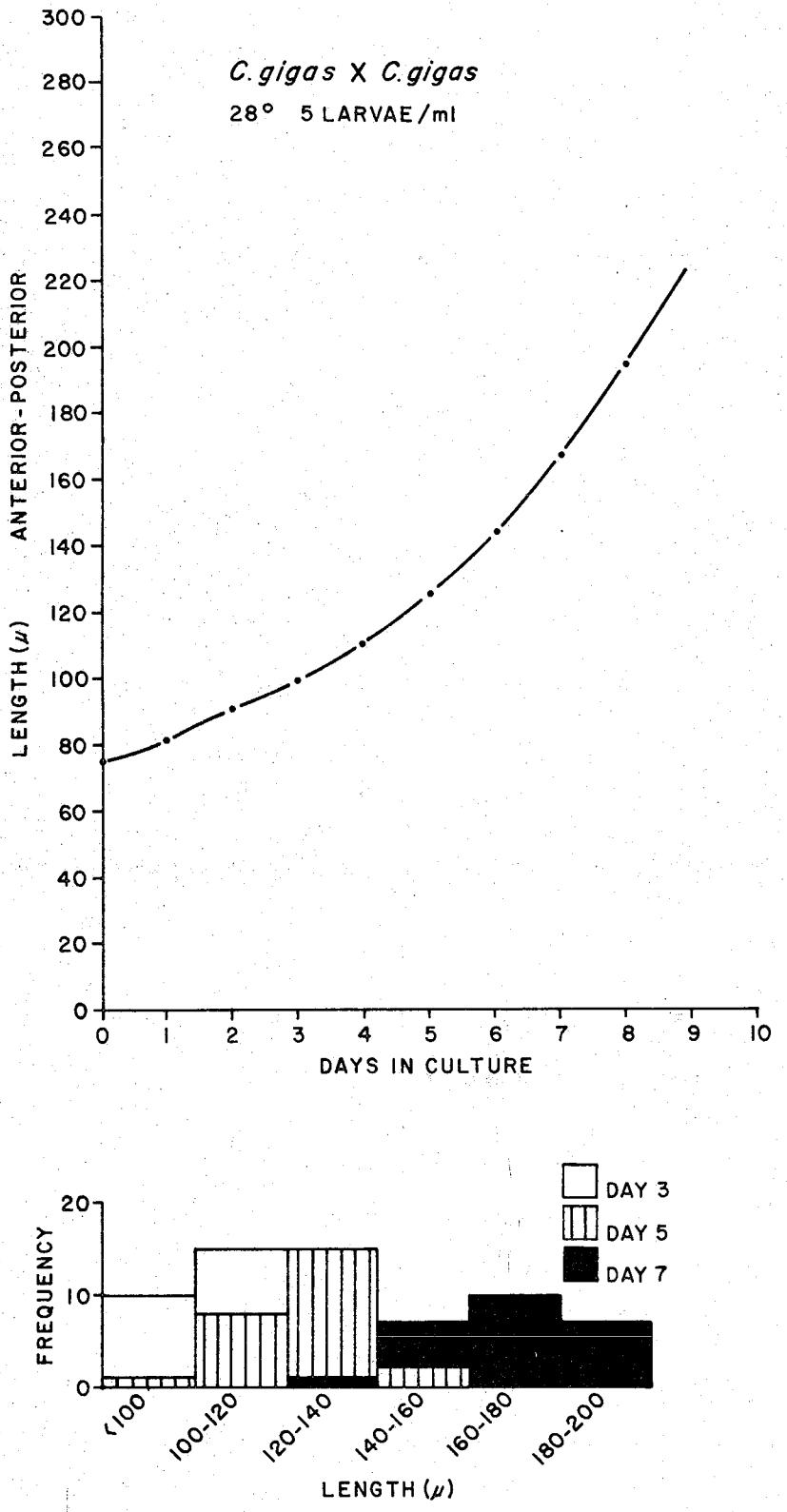


Figure 12. Growth Rate of Oyster Larvae and Size Distribution of Larval Population of *Crassostrea gigas* on Days 3, 5 and 7.

Feeding Protocol

For purposes of maintaining an appropriate schedule for feeding, the larval period has been divided into two major growth phases. They are:

Stage I: larvae from straight-hinge to 120 μ

Stage II: larvae from 120 μ to 290 μ ("eyed")

The feeding protocol during the larval period is based on the relationship of the size of the larvae to the size of the individual cells of the three algal species used as larval food. In addition, the quantities fed at any one time are based on the nutritional requirements of the larvae being increased as the size of these larvae increases. The advantage in utilizing these particular species of algae is their relatively high content of protein and fat (lipids) when compared to utilization of previously used food combinations. The feeding protocol also reflects the volume of the individual cells of the three species. The calculations of the quantity (in liters) of the different species is based on the following formula:

$$F = \frac{Vf}{Vo \times Cs}$$

where F = liters of one species of algae to be fed per 250 gal larval tank
Vf = theoretical volume required of one algal species per 250 gal larval tank
Vo = known volume of a single algal cell of one species
Cs = number of cells per ml of the batch algal culture

The Vf and Vo values for the three species of algae used are as follows:

	Pyramimonas virginica (Va-17)	*Pseudoisochrysis paradoxa* (Va-12)	*Chlorella* sp. (Va-52)
Vf at Stage I	920 $\mu^3 \times 10^9$	920 $\mu^3 \times 10^9$	920 $\mu^3 \times 10^9$
Vf at Stage II	1840 $\mu^3 \times 10^9$	1840 $\mu^3 \times 10^9$	1840 $\mu^3 \times 10^9$
Vo	33.50 μ^3	47.70 μ^3	4.85 μ^3

To determine what volume of algae culture of the three species should be fed each day for each larval tank, several tables have been prepared. The tables are presented according to the stage of growth of the larvae, i.e. stages I and II. Every other day or more frequently as needed, the hatchery manager should obtain the algal counts of the 250 gal mass algal cultures from the person in charge of algal production unit. These counts can then be used to establish the volume of algal food in liters to be fed to *each* oyster larval tank according to the protocol and larval stage [Tables 2,3,4,5,6, and 7].

In order to remove the larger pieces of detritus, the algal culture should be screened through a 62 μ sieve prior to adding to the larval tanks. It is also necessary that the larvae in each tank be fed at the same time each day, preferably in the late afternoon.

Table 2

Protocol

Liters of *Pyramimonas virginica* (Va-17) to be fed per larval tank per day during Stage I of the Protocol.

Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed
1.0	27.6	4.0	6.8	7.0	4.0
1.1	24.8	4.1	6.8	7.1	4.0
1.2	22.8	4.2	6.4	7.2	4.0
1.3	21.2	4.3	6.4	7.3	3.6
1.4	19.6	4.4	6.4	7.4	3.6
1.5	19.2	4.5	6.0	7.5	3.6
1.6	17.2	4.6	6.0	7.6	3.6
1.7	16.0	4.7	6.0	7.7	3.6
1.8	15.2	4.8	5.6	7.8	3.6
1.9	14.4	4.9	5.6	7.9	3.6
2.0	13.6	5.0	5.6	8.0	3.6
2.1	13.2	5.1	5.2	8.1	3.2
2.2	12.4	5.2	5.2	8.2	3.2
2.3	12.0	5.3	5.2	8.3	3.2
2.4	11.6	5.4	5.2	8.4	3.2
2.5	10.8	5.5	4.8	8.5	3.2
2.6	10.4	5.6	4.8	8.6	3.2
2.7	10.0	5.7	4.8	8.7	3.2
2.8	10.0	5.8	4.8	8.8	3.2
2.9	9.6	5.9	4.8	8.9	3.2
3.0	9.2	6.0	4.4	9.0	3.2
3.1	8.8	6.1	4.4	9.1	3.2
3.2	8.4	6.2	4.4	9.2	2.8
3.3	8.4	6.3	4.4	9.3	2.8
3.4	8.0	6.4	4.4	9.4	2.8
3.5	8.0	6.5	4.4	9.5	2.8
3.6	7.6	6.6	4.0	9.6	2.8
3.7	7.6	6.7	4.0	9.7	2.8
3.8	7.2	6.8	4.0	9.8	2.8
3.9	7.2	6.9	4.0	9.9	2.8
				10.0	2.8

Table 3

Protocol

Liters of *Pyramimonas virginica* (Va-17) to be fed per larval tank per day during Stage II of the Protocol.

Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed
1.0	54.8	4.0	13.6	7.0	8.0
1.1	50.0	4.1	13.2	7.1	8.0
1.2	45.6	4.2	13.2	7.2	7.6
1.3	42.4	4.3	12.8	7.3	7.6
1.4	39.2	4.4	12.4	7.4	7.6
1.5	36.8	4.5	12.4	7.5	7.2
1.6	34.4	4.6	12.0	7.6	7.2
1.7	32.4	4.7	11.6	7.7	7.2
1.8	30.4	4.8	11.6	7.8	7.2
1.9	28.8	4.9	11.2	7.9	6.8
2.0	27.6	5.0	10.8	8.0	6.8
2.1	26.0	5.1	10.8	8.1	6.8
2.2	24.8	5.2	10.4	8.2	6.8
2.3	22.8	5.3	10.4	8.3	6.8
2.4	22.8	5.4	10.0	8.4	6.4
2.5	22.0	5.5	10.0	8.5	6.4
2.6	21.2	5.6	10.0	8.6	6.4
2.7	20.4	5.7	9.6	8.7	6.4
2.8	19.6	5.8	9.6	8.8	6.4
2.9	18.8	5.9	9.2	8.9	6.0
3.0	18.4	6.0	9.2	9.0	6.0
3.1	17.6	6.1	9.2	9.1	6.0
3.2	17.2	6.2	8.8	9.2	6.0
3.3	16.8	6.3	8.8	9.3	6.0
3.4	16.0	6.4	8.4	9.4	6.0
3.5	16.0	6.5	8.4	9.5	5.6
3.6	15.2	6.6	8.4	9.6	5.6
3.7	14.8	6.7	8.0	9.7	5.6
3.8	14.4	6.8	8.0	9.8	5.6
3.9	14.0	6.9	8.0	9.9	5.6
				10.0	5.6

Table 4

Protocol

Liters of *Pseudoisochrysis paradoxa* (Va-12) to be fed per larval tank per day during Stage I of the Protocol.

Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed
2.0	9.6	5.0	4.0	8.0	2.4
2.1	9.2	5.1	3.6	8.1	2.4
2.2	8.8	5.2	3.6	8.2	2.4
2.3	8.4	5.3	3.6	8.3	2.4
2.4	8.0	5.4	3.6	8.4	2.4
2.5	7.6	5.5	3.6	8.5	2.4
2.6	7.6	5.6	3.6	8.6	2.4
2.7	7.2	5.7	3.2	8.7	2.4
2.8	6.8	5.8	3.2	8.8	2.0
2.9	6.8	5.9	3.2	8.9	2.0
3.0	6.4	6.0	3.2	9.0	2.0
3.1	6.4	6.1	3.2	9.1	2.0
3.2	6.0	6.2	3.2	9.2	2.0
3.3	6.0	6.3	3.2	9.3	2.0
3.4	5.6	6.4	3.2	9.4	2.0
3.5	5.6	6.5	2.8	9.5	2.0
3.6	5.2	6.6	2.8	9.6	2.0
3.7	5.2	6.7	2.8	9.7	2.0
3.8	5.2	6.8	2.8	9.8	2.0
3.9	4.8	6.9	2.8	9.9	2.0
4.0	4.8	7.0	2.8	10.0	2.0
4.1	4.8	7.1	2.8		
4.2	4.4	7.2	2.8		
4.3	4.4	7.3	2.8		
4.4	4.4	7.4	2.8		
4.5	4.4	7.5	2.4		
4.6	4.0	7.6	2.4		
4.7	4.0	7.7	2.4		
4.8	4.0	7.8	2.4		
4.9	4.0	7.9	2.4		

Table 5

Protocol

Liters of *Pseudoisochrysis paradoxa* (Va-12) to be fed per larval tank per day during Stage II of the Protocol.

Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed
2.0	19.2	5.0	7.6	8.0	4.8
2.1	18.4	5.1	7.6	8.1	4.8
2.2	17.6	5.2	7.6	8.2	4.8
2.3	16.8	5.3	7.2	8.3	4.8
2.4	16.0	5.4	7.2	8.4	4.4
2.5	15.6	5.5	7.2	8.5	4.4
2.6	14.8	5.6	6.8	8.6	4.4
2.7	14.4	5.7	6.8	8.7	4.4
2.8	13.6	5.8	6.8	8.8	4.4
2.9	13.2	5.9	6.4	8.9	4.4
3.0	12.8	6.0	6.4	9.0	4.4
3.1	12.4	6.1	6.4	9.1	4.4
3.2	12.0	6.2	6.4	9.2	4.0
3.3	11.6	6.3	6.4	9.3	4.0
3.4	11.2	6.4	6.0	9.4	4.0
3.5	11.2	6.5	6.0	9.5	4.0
3.6	10.8	6.6	6.0	9.6	4.0
3.7	10.4	6.7	5.6	9.7	4.0
3.8	10.0	6.8	5.6	9.8	4.0
3.9	10.0	6.9	5.6	9.9	4.0
4.0	9.6	7.0	5.6	10.0	4.0
4.1	9.6	7.1	5.6		
4.2	9.2	7.2	5.2		
4.3	8.8	7.3	5.2		
4.4	8.8	7.4	5.2		
4.5	8.4	7.5	5.2		
4.6	8.4	7.6	5.2		
4.7	8.4	7.7	5.2		
4.8	8.0	7.8	4.8		
4.9	8.0	7.9	4.8		

Table 6

Protocol

Liters of *Chlorella* sp. (Va-52) to be fed per larval tank per day during Stage I of the Protocol.

Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed
30.0	6.4	60.0	3.2	90.0	2.0
31.0	6.0	61.0	3.2	91.0	2.0
32.0	6.0	62.0	3.2	92.0	2.0
33.0	5.6	63.0	3.2	93.0	2.0
34.0	5.6	64.0	2.8	94.0	2.0
35.0	5.6	65.0	2.8	95.0	2.0
36.0	5.2	66.0	2.8	96.0	2.0
37.0	5.2	67.0	2.8	97.0	2.0
38.0	4.8	68.0	2.8	98.0	2.0
39.0	4.8	69.0	2.8	99.0	2.0
40.0	4.8	70.0	2.8	100.0	2.0
41.0	4.8	71.0	2.8		
42.0	4.4	72.0	2.8		
43.0	4.4	73.0	2.4		
44.0	4.4	74.0	2.4		
45.0	4.4	75.0	2.4		
46.0	4.0	76.0	2.4		
47.0	4.0	77.0	2.4		
48.0	4.0	78.0	2.4		
49.0	4.0	79.0	2.4		
50.0	3.6	80.0	2.4		
51.0	3.6	81.0	2.4		
52.0	3.6	82.0	2.4		
53.0	3.6	83.0	2.4		
54.0	3.6	84.0	2.4		
55.0	3.6	85.0	2.4		
56.0	3.2	86.0	2.4		
57.0	3.2	87.0	2.0		
58.0	3.2	88.0	2.0		
59.0	3.2	89.0	2.0		

Table 7

Protocol

Liters of *Chlorella* sp. (Va-52) to be fed per larval tank per day during Stage II of the Protocol.

Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed	Cell Count in millions	Liters of Algae to be fed
30.0	12.8	60.0	6.4	90.0	4.4
31.0	12.4	61.0	6.4	91.0	4.0
32.0	12.0	62.0	6.0	92.0	4.0
33.0	11.6	63.0	6.0	93.0	4.0
34.0	11.2	64.0	6.0	94.0	4.0
35.0	10.8	65.0	6.0	95.0	4.0
36.0	10.4	66.0	5.6	96.0	4.0
37.0	10.4	67.0	5.6	97.0	4.0
38.0	10.0	68.0	5.6	98.0	4.0
39.0	9.6	69.0	5.6	99.0	4.0
40.0	9.6	70.0	5.6	100.0	3.6
41.0	9.2	71.0	5.2		
42.0	9.2	72.0	5.2		
43.0	8.8	73.0	5.2		
44.0	8.8	74.0	5.2		
45.0	8.4	75.0	5.2		
46.0	8.4	76.0	4.8		
47.0	8.0	77.0	4.8		
48.0	8.0	78.0	4.8		
49.0	8.0	79.0	4.8		
50.0	7.6	80.0	4.8		
51.0	7.6	81.0	4.8		
52.0	7.2	82.0	4.8		
53.0	7.2	83.0	4.4		
54.0	7.2	84.0	4.4		
55.0	6.8	85.0	4.4		
56.0	6.8	86.0	4.4		
57.0	6.8	87.0	4.4		
58.0	6.4	88.0	4.4		
59.0	6.4	89.0	4.4		

Methodology — "Eyed" Stage to Setting

Before describing the detailed methodology, there are several general aspects of the larvae at this stage which must be considered, particularly as they relate to setting module deisgn.

The "eyed" larvae, both prior to and during setting, are extremely sensitive to any chemical or metal contamination, being in a relatively small volume of filtered water and in a static culture condition. The demand for oxygen of this number of larvae at 27 C is relatively high for this small volume of water. After the "eyed" larvae have settled, "crawled" around and finally attached themselves, they go through a process called metamorphosis. During metamorphosis the oyster looses its swimming apparatus (velum). Its body structure is also being reorganized and its filtering apparatus, the gills and palps, are developing. At this stage the setting oyster is under great physiological stress. Any additional stress such as low oxygen or chronic chemical contamination of the processed water will reduce the percentage yield of successfully set oysters.

After much experimentation, both at the laboratory and in a commercial hatchery, a new setting tray module has been designed. This module consists of three parts: A rectangular container, four sheets of Mylar, and a retaining ring. It is very important that the rectangular tank (96" x 32" x 8") be constructed of fiberglass, with the inside surface having a smooth gel coat. It is the only material which has demonstrated a consistent lack of toxicity. At one end of the container should be incorporated a 1 inch drain with a 1 inch valve. The retaining ring should be constructed of Plexiglass and be positioned in the rectangular container so it can be lifted on a hinge system and hooked to the wall. The bottom of the edge of the retaining ring should have a silicone beading added to obtain the best possible seal between the inside and outside of the retaining ring. Nothing other than Dow Corning Silicone Rubber (Industrial Grade) should be used to make this gasket. Many of the other silicones contain antifungal agents which are extremely toxic to "eyed" larvae. The major purpose of this silicone gasket is to keep the "eyed" larvae on top of the Mylar sheet. If the retaining ring is not utilized, most of the larvae will migrate under the sheet where depletion of oxygen will be rapid, resulting in death of most of those larvae. This apparatus is depicted in Figure 13.

The pre-treatment of the Mylar sheets (27 1/2" x 22") consists of soaking the sheets in filtered water for at least 2 weeks when they are new. The filtered water should be changed every other day to insure that any of the finishing chemicals are removed. The complete setting module should also be leached by washing and soaking with filtered salt water for at least 2 weeks prior to use. This water should be renewed every other day to make certain that any finishing chemicals are removed.

The last important aspect of the setting module is the use of aeration to insure an adequate supply of oxygen and to increase the setting intensity. The movement or turbulence of the filtered water inside the retaining ring has been demonstrated to increase setting intensity both in the laboratory and in a commercial hatchery. The utilization of 2 aquarium airstones inside the retaining ring accomplishes the two major requirements for "eyed" larval oxygenation and turbulence.

The mean size of the larvae has now become about 230 μ, and some of the larvae will be "eyed" [Figures 9 and 11]. At this point the larval tanks should be taken down and the salt water with larvae filtered through a 150 μ sieve. Once these larvae have been resuspended in the 10 liter plastic containers, they should be filtered again through a 250 μ sieve. The larvae which pass through the 250 μ screen should be put back into the 250 gal larval tank with fresh 1 μ filtered water. Before the larvae are screened through the 250 μ sieve, it is important to thoroughly mix them in the bucket to insure the majority, which are less than 250 μ, pass through the screen. When a larger concentration is poured through the sieve at one time, many of the smaller larvae are trapped and remain with the larger oyster larvae to create problems. Once the larvae greater than 250 μ have been collected, they should be screened again through a 285 μ sieve to remove the "eyed" larvae. Those which pass through the screen should be put back in their respective tanks. The "eyed" larvae from all the larval tanks should then be pooled in one 10 liter bucket and counted.

This procedure should be repeated every day until few larvae remain. When it becomes evident from the counts that few larvae remain, less than 500,000 larvae per tank, then the larvae under 285 μ from each larval tank can be combined into fewer tanks, making absolutely certain that no larval

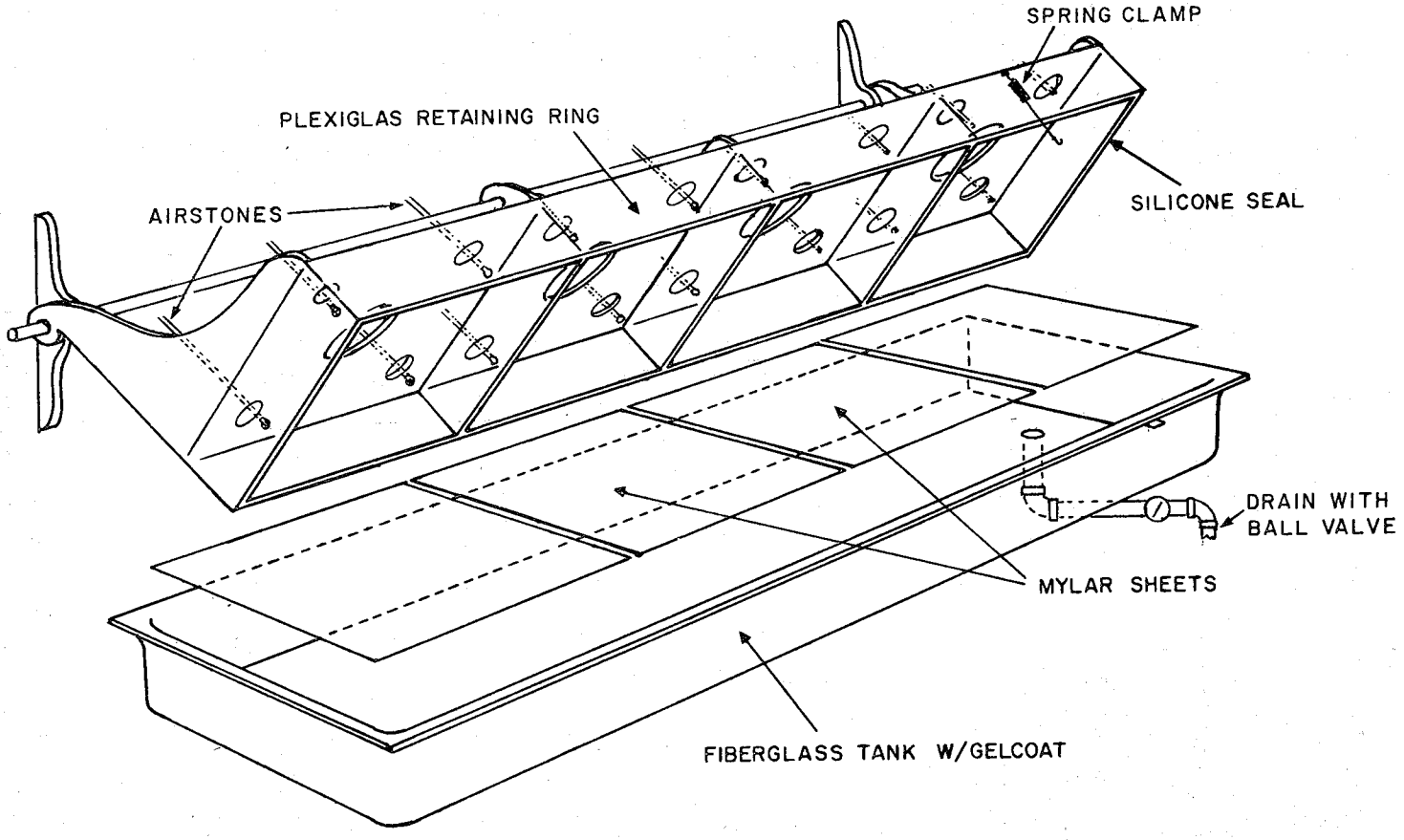


Figure 13. Setting Tray Module.

tank contains more than 3 million larvae.

The development of the "eye" begins when the oyster larvae reach 280 μ in size. One must note that at this point the dark spot is irregular. If the "eyed" larvae are put into the setting tanks at this point very little or no setting will occur. With the increase in the size of the larvae, the "eye" spot will fill out and be fully developed at about 290 μ. This developmental process usually occurs over a 24 hour period. There will be at this point a greater percent of "eyed" larvae. It is for this reason that the following schedule be adhered to.

Just before the "eyed" larvae are put into the setting module, the Mylar sheets should be thoroughly scrubbed and rinsed with fresh water. The 1 μ filtered water at 27 C should be put into the tank to a depth of six inches. The 4 sheets are then placed in the tank, matte side up [see Figure 13]. It will take anywhere from 4 to 6 minutes for the sheets to settle to the bottom. At this time the retaining ring is lowered into the tank, and the Mylar sheets should be positioned to line up with the retaining ring. A spring clamp is then locked into position to put pressure on the silicone gasket [see Figure 13]. The air is then turned on to the airstones at a pressure of 2 pounds per square inch.

Once the "eyed" larvae have been counted, they are ready to be transferred to the setting module. It is advantageous that the "eyed" larvae be passed through a 357 μ sieve as they are put into the setting module. This removes the clumped algal detritus and a good part of the colonial protozoans (*Vorticella*) which can interfere with larval movement and setting. Distribute the larvae as evenly as possible within the retaining rings in the setting modules. No more than 300,000 "eyed" larvae should be put into each retaining ring section, or a total of 1,200,000 "eyed" larvae per setting module of 4 Mylar sheets. Add 300 ml or 18 x 10^8 cells of *Pyramimonas virginica* (Va-17) to each of the 4 retaining ring areas.

The processed water in the setting module should be renewed at least 3 times per day. The normal timing is early in the morning, in the middle afternoon, and late evening. There is usually a 16 to 18 hour waiting period before heavy setting occurs, when the "eyed" larvae from a batch are first harvested. Thereafter setting continues constantly in the setting module. Each time the module is drained, the water should be passed through a 250 μ sieve to avoid loss of larvae. The larvae that are collected on this screen should be put back into the setting module. A sample of these larvae should also be checked under the microscope for contamination, mortality, and foot movement.

It is necessary to check for set each time the water is renewed to control the number of set oysters that will remain on the Mylar sheets. There should be no more than between 15 and 20 set oysters per square inch. The method for checking this is as follows: Once the sheets are removed from the setting module, they should be hung over the setting module and thoroughly flushed to remove "eyed" larvae that are not actually set. A wash bottle or a hose from a small pump in a fiberglass tank can be used to do this. Few larvae which have set will be removed even if a strong jet is used. Using a 10 x magnifying glass and a sheet of paper with a 1 inch square hole, the number of oysters which have set can then be counted. This method works adequately if setting is uniform. Unfortunately, estimating the number of set oysters by "eye-balling" the sheet usually gives erroneous impressions even after much experience. The optimum number of set oysters per Mylar sheet will depend upon the size of the oyster desired for removal from the sheet at the end of 3 or 4 weeks of growth in the spat tanks. The optimum size for adequate handling in the "grow-out" flumes is approximately 3/8 inch. In order to achieve this size the Mylar sheet should not hold more than between 7 to 10 thousand or 7 to 10 spat per square inch. The recommendation of 15 to 20 set oysters per square inch is to assure that there will be at least 7 to 10 set oysters per square inch at the end of 6 days. A greater number per square inch will result in the loss of oysters as a result of their growing over each other. Mortality after 6 days is usually less than 2 percent to a size of 3/8 inch.

Methodology — Spat Tanks

The number of set oysters that will successfully go through metamorphosis will vary between 50 and 70 percent. This process takes at least 5 days. These oysters at this time are vulnerable to predation by protozoans. For this reason the Mylar sheets are kept in 1 μ filtered water and fed for 6

days before exposure to raw ambient water.

After the sheets are removed from the setting module, 2 sheets are put back to back and mounted on a Plexiglass frame [Figure 14] to be put into the spat tank containing 1 μ filtered water this time at 25 C. Each spat tank is designed to hold 13 frames or 26 Mylar sheets. Approximately 6 liters of *Pyramimonas virginica* are added each day. These spat tanks containing filtered bay water and newly set oysters should also be aerated and contain an airstone in the middle and at the bottom between each frame. The filtered water should be renewed daily and be kept at 25 C.

At the end of 6 days the frames should be transferred to spat tanks receiving raw ambient water. If the temperature differential between the 25 C filtered water and the ambient raw water is greater than 3 C, then a section of 5 spat tanks should be utilized to allow acclimation to the ambient water temperature over a 2 day interval.

Through experience at our laboratory and in a commercial hatchery a minimum of 250 gal per hour of raw ambient estuarine water should be given to *each* spat tank. This is necessary to get optimal growth of a minimum of 100,000 oyster spat per spat tank.

The spat tank was designed so that the raw ambient water containing natural food would be available to all the Mylar surfaces. The water is introduced as an upwelling from four 1 inch PVC pipes which are located at the bottom of the tank. In each of these pipes there are fourteen 7/32 inch water jet openings. They are placed so that each frame straddles the pipes in between the water jets. There are a total of 4 pipes straddling each frame or a total of 56 jets per spat tank [Figures 15 and 16]. As the water is pushed upwards, it carries a good portion of feces, pseudofeces, detritus, and silt to the surface, where it then is carried over a dam into the exit pipe system [Figure 15]. The flow of 250 gal per hour per tank insures turbulence which also helps to keep the surface of the Mylar sheet cleaner. Increasing the flow of raw ambient water should be done cautiously, since increased turbulence can interfere with the feeding of the oyster spat.

The spat tanks should be cleaned at least once per week. The raw ambient water should be shut off and the spat tank drained by opening the 2 inch valve that controls the 5 spat tanks. When the spat tanks have been emptied, they should be sprayed with a fresh water hose to remove the silt from the surfaces of the Mylar sheets. When this is complete, the bottom of the tanks should be flushed to remove the accumulated slit on the bottom. Failure to clean the sheets and the bottom of the tank will result in slower growth of the oyster spat. In some areas where the natural silt load is considerably less than in Chesapeake Bay, the cleaning frequency can be reduced.

The timing of removal of the oyster spat from the Mylar sheets is dependent on their rate of growth. When the oyster spat start growing together and when the bills of these oysters start growing away from the surface of the Mylar sheet, they should be removed. There are two advantages to this procedure. First, calcium carbonate is deposited without the organic matrix at the point where the bills meet, resulting in easy separation of single oysters. The second advantage is that the forcing of the bill of the oyster to grow up allows the oyster to start growing immediately after removal from the Mylar sheet, instead of first thickening the bill to allow spreading and growth from a very thin left valve.

To remove the oysters from the Mylar sheet, slide and bend the sheet back and forth over the sharp edges of a piece of sheet metal under water. The attached oysters should be on the side opposite the surface in contact with the piece of sheet metal. This will force the oysters to loosen from the sheet, resulting in single oysters dropping to the bottom of the tank into a basket. The oyster spat are then collected for grading through a series of screens to separate these oysters into 2 convenient size categories, those less than 3/8 inch and those greater that 3/8 inch.

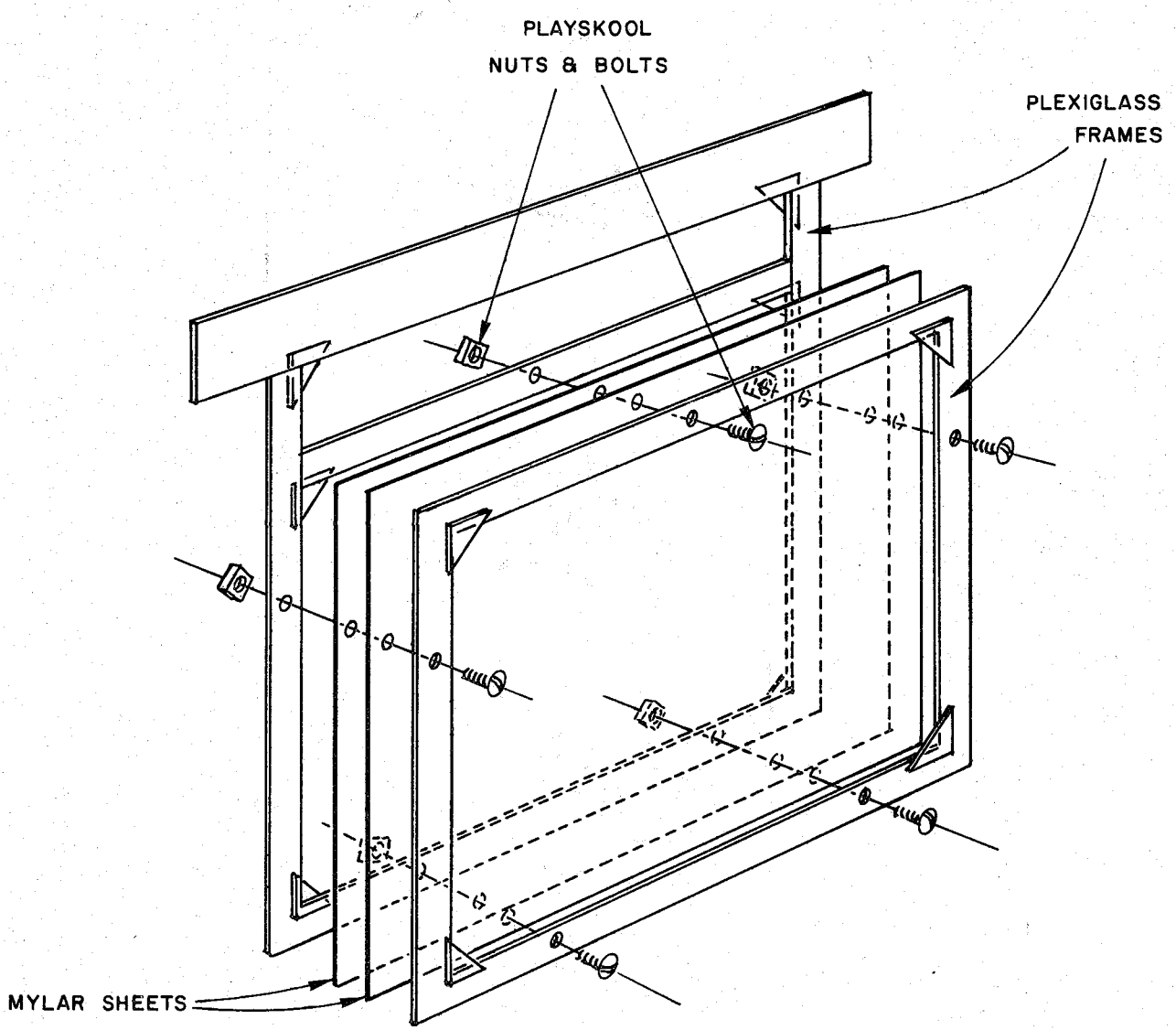
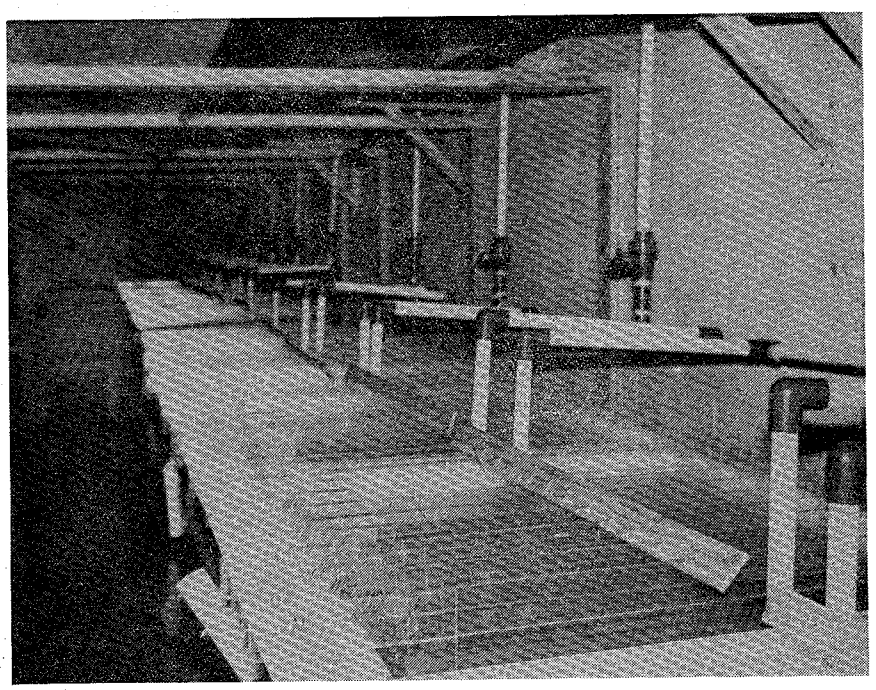
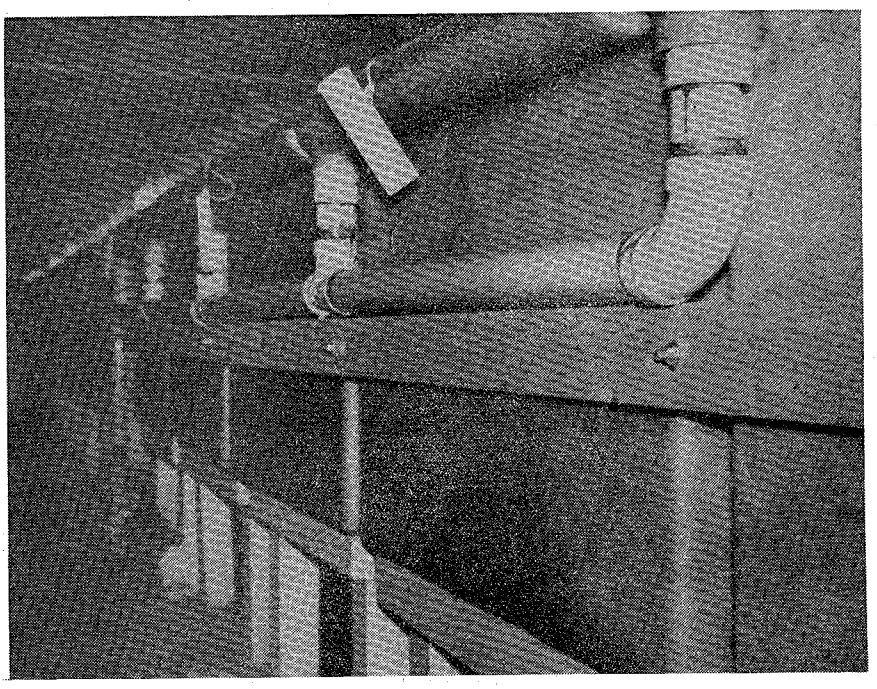


Figure 14. Plexiglass Frame for Holding Two Mylar Sheets.



Spat Tanks Containing Plexiglass Frames



Overflow Flume and Piping Arrangement of Spat Tanks

Figure 15. Overflow Flume and Piping Arrangement of Spat Tanks.

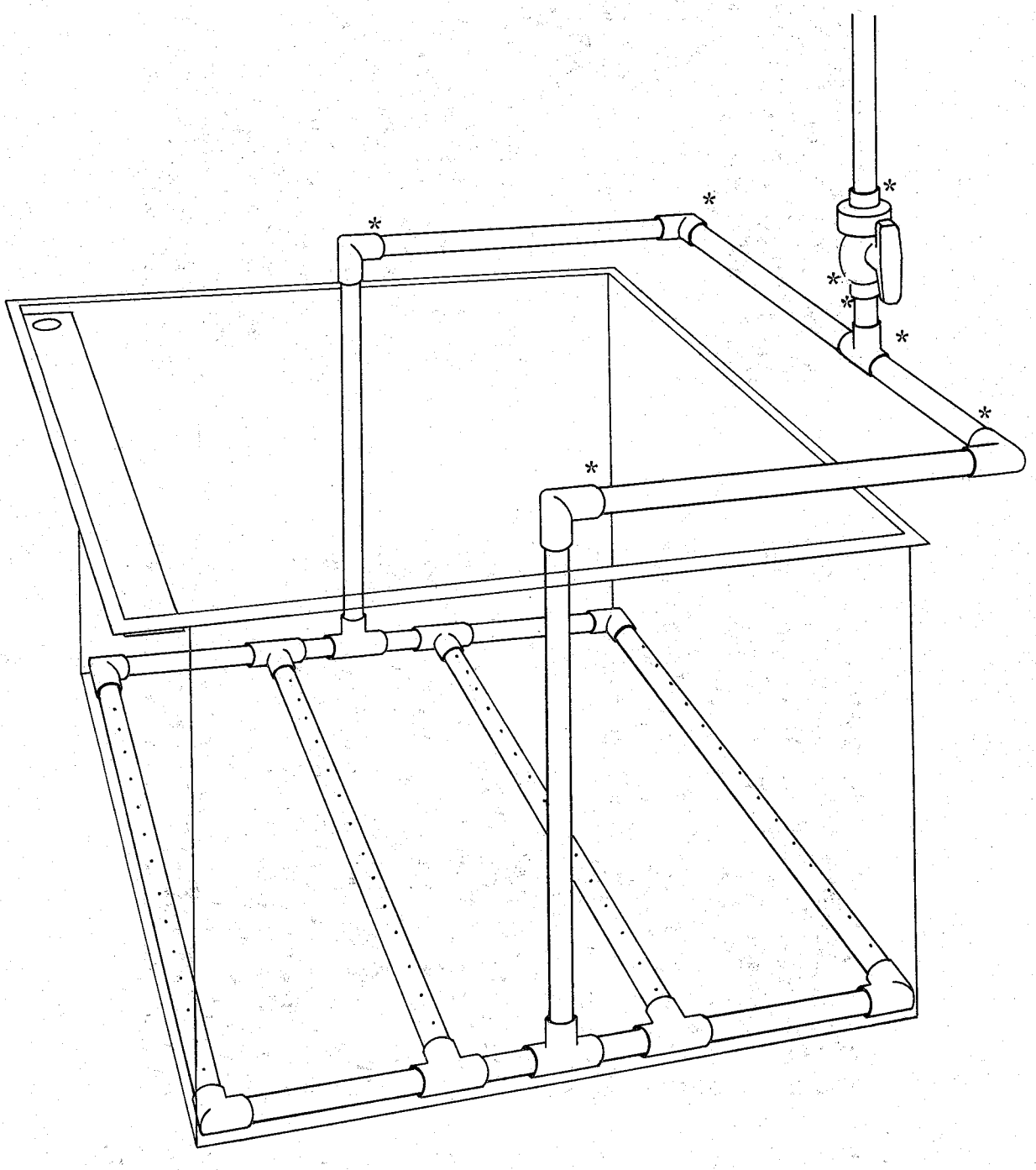


Figure 16. Diagram of Pipe System in Spat Tank.

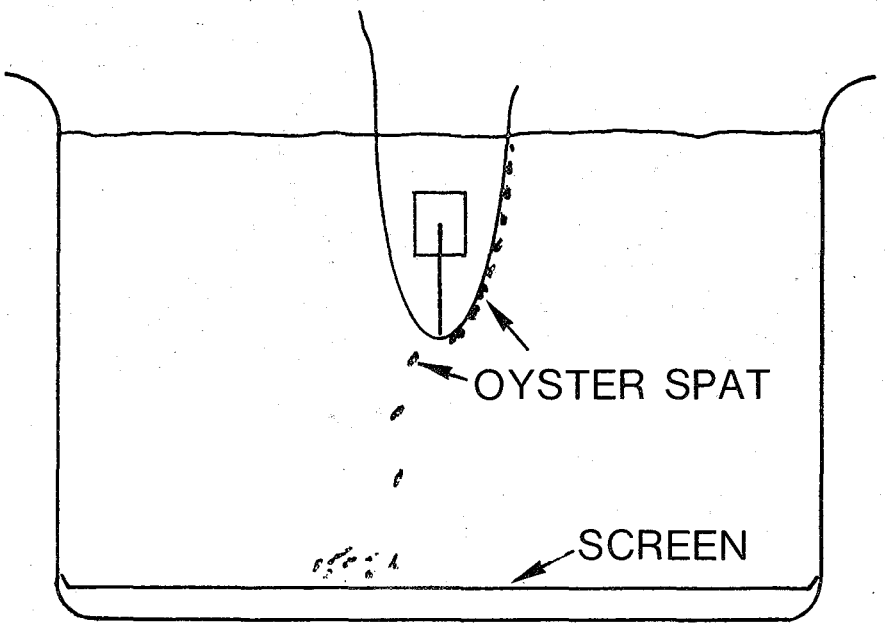


Figure 17. Method of Removing Oyster Spat from Mylar Sheets.

Methodology — Spat Holding Flumes

The DupMo Mark II flumes have been designed to insure optimal growth for 30 to 40 thousand free spat from 3/8 to 3/4 inch, with a minimum water flow of 200 gal per hour per flume. These flumes have the capability of holding 6 Nestier trays measuring 2 feet square [Figure 18]. There are several advantages to using sectional trays in the flumes. First, it allows the partitioning of the contents of the flumes into manageable tray units of 5,000 to 7,200 feet spat. The order that the trayed oysters receive the natural food in the ambient estuarine water can be changed by reversing the order of the trays in the flumes. Second, the trays allow a space underneath into which the silt, feces, and pseudofeces can be flushed out by a hose and drained without moving the oysters. Last, the trays containing the oysters can be easily moved to thoroughly clean the flumes.

The time required for the 3/8 inch free spat to reach marketable seed size ranges from 60 to 80 days after they have been taken off the sheets. This time period is dependent upon two major factors. First, the oysters must be kept clean. The accumulation of feces, pseudofeces, and silt will interfere with the ability of these small oysters to pump and feed and therefore yield optimal growth. The smaller oysters also can be smothered under these crowded conditions. Second, the presence of feces, psuedofeces, and silt between the small oysters contributes to mortality problems by worms (polychaetes) which set and live in this mud. Experience in two hatcheries has shown these worms will erode the thin shell of these small oyster spat while trying to make a home, resulting in an increase in mortality. One of the polychaetes, *Polydora,* burrows into the oyster's shell. Since the oyster's shell is extremely thin at this time, the worm is capable of burrowing through the shell into the shell cavity. Debility occurs and death may result. The small oyster at this time cannot deposit sufficient shell to wall off the burrow of the worm, resulting in large mortalities of the oyster population. This type of infestation is minimized if the oyster spat are kept clean. The schedule of cleaning the flumes should be once a week for each flume. This cleaning includes spraying the trays with a hose after the flumes have been drained. The incoming raw salt water should not be turned off since this aids in the flushing of the material underneath the trays. Nylon mosquito window

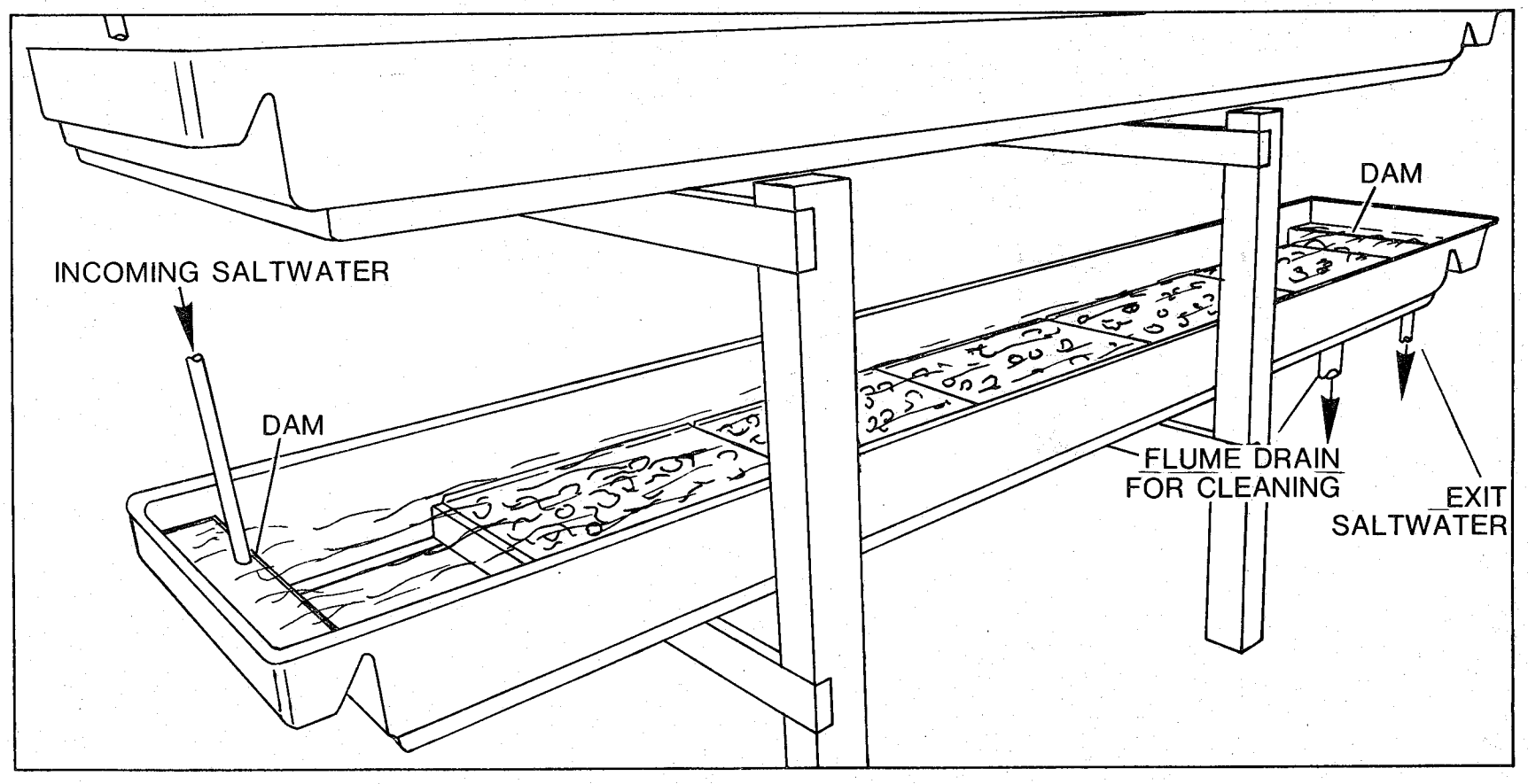


Figure 18. DupMo Mark II Flumes Containing Six Nestier Trays.

screening is recommended to be put over the oysters in the flumes when they are small. This procedure reduces the loss of spat. If the screen is not utilized, the small light spat are sprayed out of the trays and flushed into the exit raceways. The cleaning schedule may have to be adjusted, depending on the silt load in the incoming raw ambient water. It is also recommended that the flumes be thoroughly cleaned once a month. Removal of trays is required in this procedure in order to clean mud accumulated beneath trays. Failure to do so will result in the production of hydrogen sulfide from the anaerobic conditions. The hydrogen sulfide produced in these flumes will affect the pumping rate of these oysters and therefore their feeding rate and growth.

The total period needed before a market seed oyster may be sold has been found to be between 100 and 120 days. This period includes the time from spawning until the oyster reaches 3/4 inch in size. Figure 19 illustrates the growth rate and demonstrates measurements of batches brought through at a commercial hatchery in Maryland during the period of the latter part of April through the end of November. One must note as the figure illustrates, when too many seed oysters are placed in each flume (80,836 seed oysters) only 65% will reach 3/4" in the 115 days. Further measurements have shown oyster seed will grow at almost the same rate when the salt water is heated during the winter months. To date there appears to be sufficient natural food in the raw ambient salt water at the site of the aforementioned hatchery except for the last two weeks in December and the month of January.

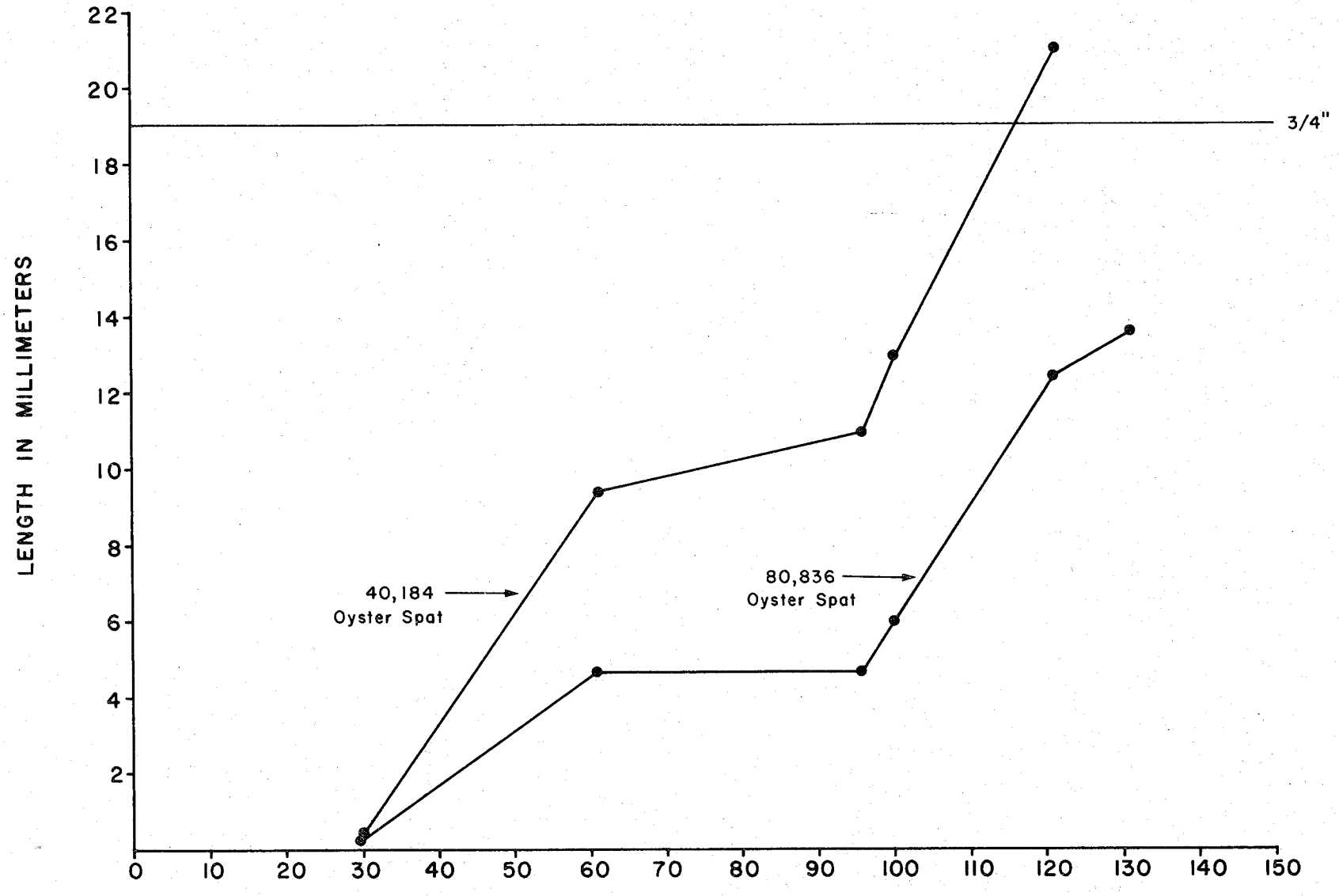


Figure 19. General Growth Rate of Oyster Spat in DupMo Mark II Flumes.

CHAPTER V. CULTURE OF ALGAL FOOD

Description of Three Species

The biology and the culture of algae in general are well known. Several sources detail mass culture of algae for commerical hatchery scale productions (Matthiessen and Toner, 1966; Ukeles, 1969, 1973). We do not recommend this methodology be utilized for those species of algae utilized for this hatchery system. The biology and culture of particular species of algae vary sufficiently from standard non-specific algal culture procedures. Each algal food species used in this hatchery system, the amount of light, temperature, and type of nutrients must be tailored to the individual species and to the requirements of this hatchery (c.f. pages 128-130). An oyster hatchery requires not only relatively large volumes of cultured algae but also high cell counts to operate efficiently. In addition the culturist must keep in mind changes in these procedures, which will be subsequently described, will change the nutritional value of the algal food and may change the growth and successful setting of oyster larvae.

The three algal species — *Pyramimonas virginica* nom. prov. (Va-17), *Psuedoisochryis paradoxa* nom. prov. (Va-12), and *Chlorella* sp. (Va-52) — have been isolated from the York River in Virginia. These algal species in their natural environment come from waters which carry a high silt and detrital load. Penetration and quantity of light are therefore low. It is for this reason the optimal growth of these species under artificial conditions occurs at low light levels. These important factors, the intensity and quantity of light, can make the difference between failure and success for the production of larval food for the hatchery.

Of the three species utilized in our hatchery system, only *Chlorella* sp. (Va-52) has been definitely classified into the class Chlorophyceae (Green Algae). Both *Pyramimonas virginica* (Va-17) and *Pseudoisochrysis paradoxa* (Va-12) were isolated in 1971, but have yet to be placed definitively in their proper classification. They were chosen primarily for their small size. Oyster larvae require small cells. After several years of bioassay it was established these cells had more than an adequate nutritional value (Windsor, 1977). All three species have an overall dimension of less than 6 μ.

The description of these three species can be best presented by the three electron micrographs [Figures 20, 21 and 22]. These photomicrographs are intended to give the general shape characteristics and not the fine structures of the cells. From these electron photomicrographs, one notes *Chlorella* sp. (Va-52) does not have flagella and therefore is non-motile (the cells do not move under their own power) and *Pyramimonas virginica* (Va-17) and *Pseudoisochrysis paradoxa* (Va-12) do have flagella and are motile. One may further note *Pyramimonas virginica* has 4 flagella and *Pseudoisochrysis paradoxa* 2 flagella.

These three species were isolated from an area with a 13 to 20 $^{o}/oo$ salinity range. Experimentation at the Virginia Institute of Marine Science and experience with actual mass culture at a hatchery in Maryland have shown these algae are capable of growing optimally in a range from 10.5 to 25 $^{o}/oo$ salinity.

The optimal rate of growth (increase in the number of cells per unit time) in any culture container is a function of the size of the inoculum, given optimal lighting conditions, salinity, and nutrients. Normally one can expect a minimum period of 2 weeks to reach the maximum number of cells per unit volume when growing these algae in test tubes and Fernbach flasks. The minimum period to obtain maximum cells per unit volume is 2 to 4 weeks in the larger 5 and 10 gal culture containers. The period required to obtain the maximum number of cells in a 10 gal culture container can be shortened by increasing the amount of inoculum (volume of cells and media added to the new culture container containing fresh medium).

On the average, the following cell densities (numbers per ml) can be expected after a 15-day period and constitutes a measure of the vigor of the mass algal cultures:

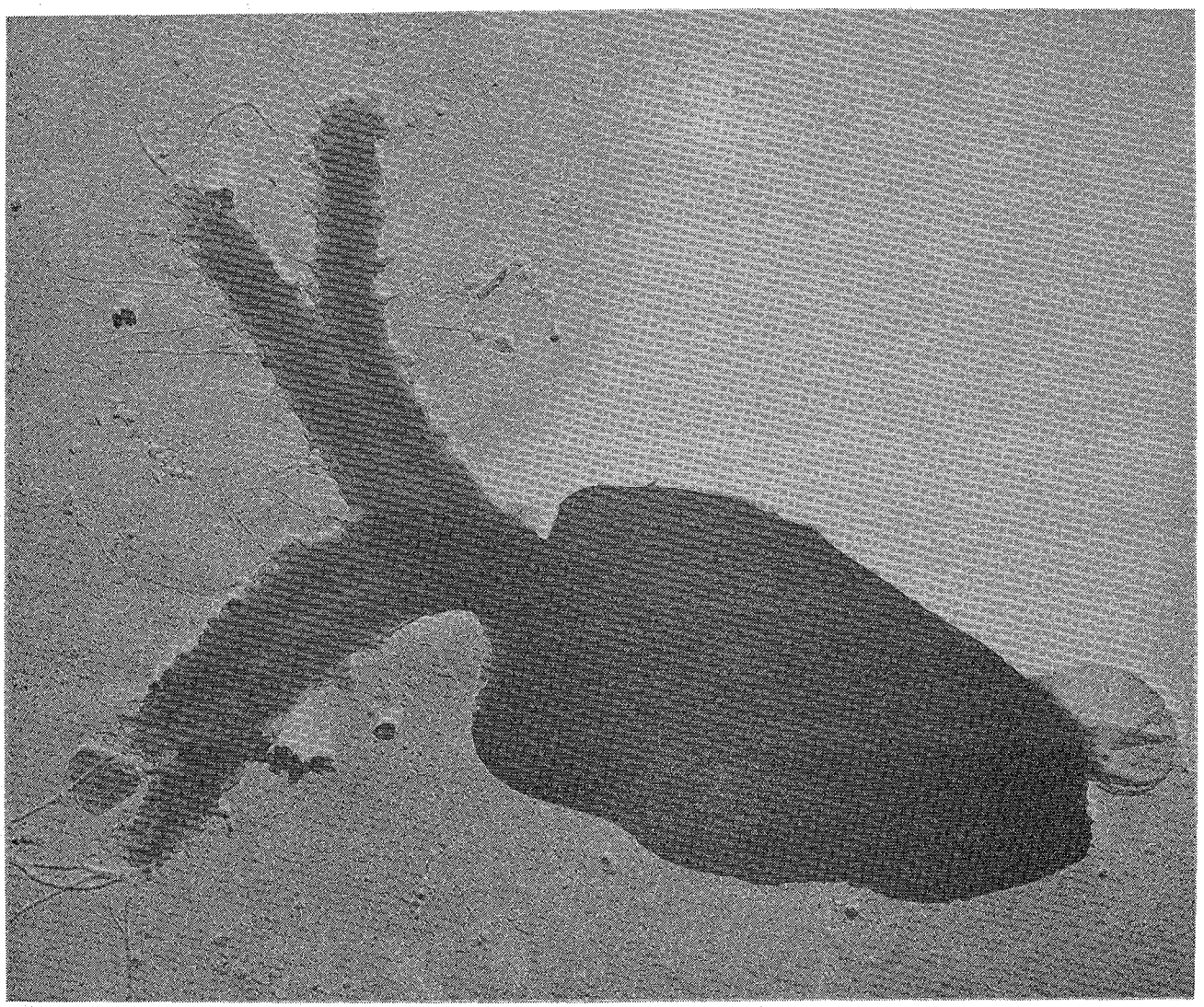


Figure 20. *Pyramimonas virginica* nom. prov.

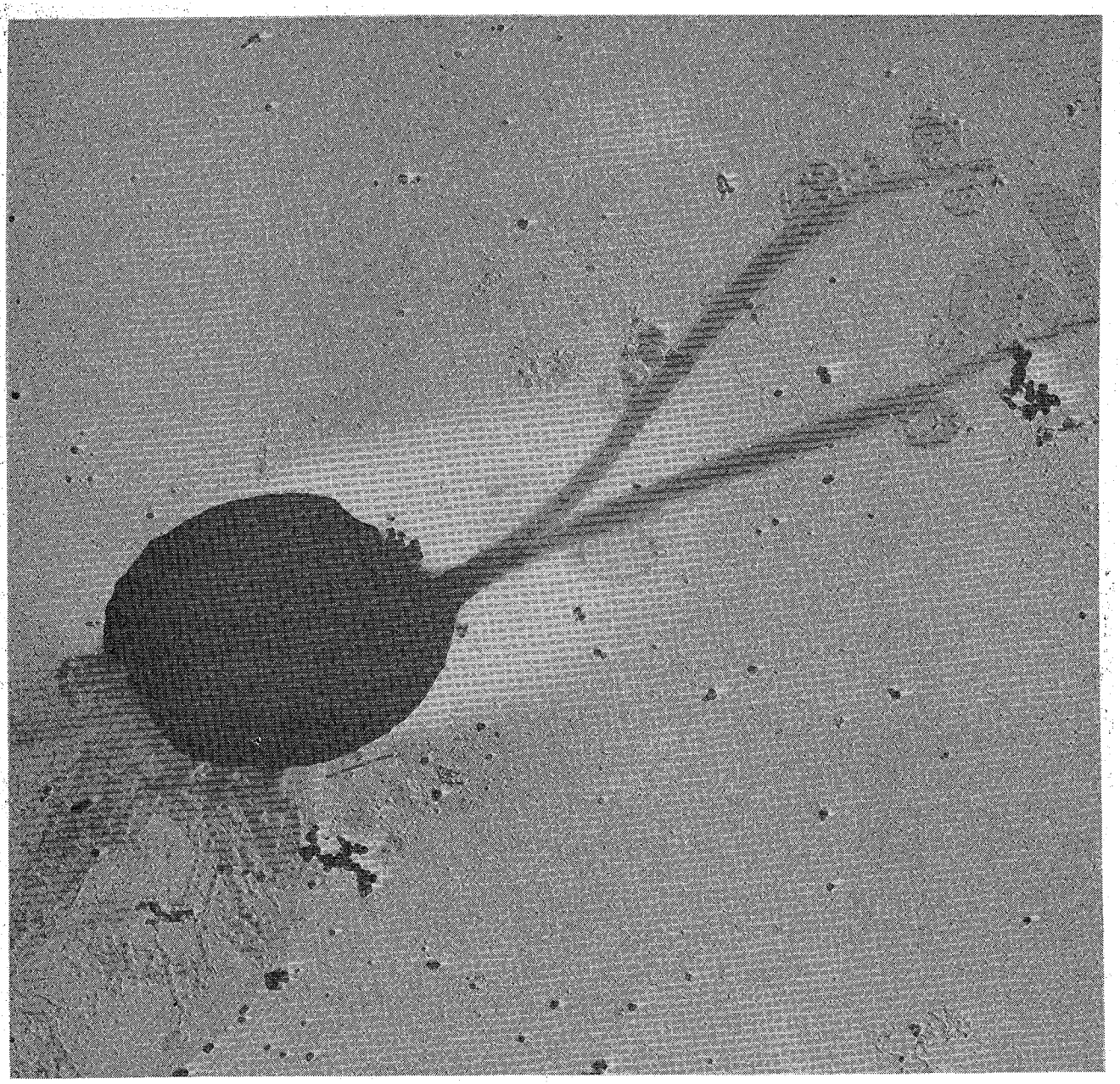
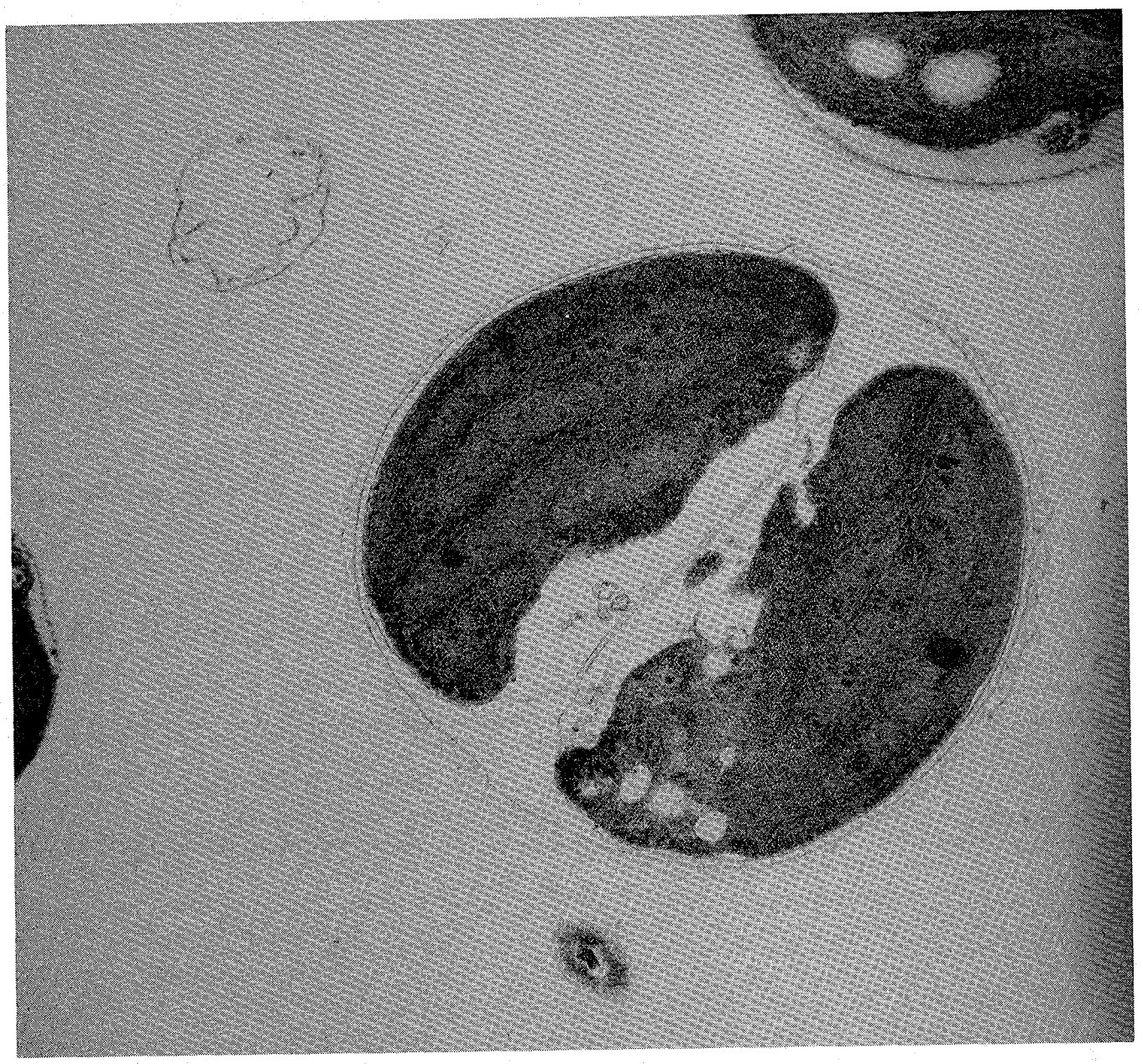


Figure 21. *Pseudoisochrysis paradoxa* nom. prov.



(1 to 2 u), 17, 432 X.

Figure 22. *Chlorella* sp.

Mass Culture	*Pyramimonas virginica* (Va-17)	*Pseudo-isochrysis paradoxa* (Va-12)	*Chlorella* sp. (Va-52)
5 gal (18 liters)	4-5 x 10^6/ml	8-9 x 10^6/ml	50-60 x 10^6/ml
10 gal (40 liters)	6-8 x 10^6/ml	8-11 x 10^6/ml	50-70 x 10^6/ml
250 gal (1000 liters)	4-6 x 10^6/ml	4-6 x 10^6/ml	50-60 x 10^6/ml

The temperature range of these three species is relatively narrow when optimal cell densities are required. *Pyramimonas virginica* (Va-17) and *Pseudoisochrysis paradoxa* (Va-12) require an optimal temperature of 20 C but can grow vigorously at 18 C to 21 C. *Chlorella* sp. (Va-52) requires a temperature range of 23 C to 25 C. *Chlorella* sp. will grow at lower temperatures; however, the cell densities will reach the maximum at 20 million cells per ml.

Preparation of Culture Medium

There are 3 steps in the preparation of the final algal medium to grow the three species of algae in the 5 types of culture containers [Figures 23 and 24].

The first step is the filtration of water. Only a general description of the procedure will be given here, since the detailed procedure will be described later under the "Filtration System Unit". The raw ambient salt water is first pumped into 3 fiberglas (250 gal) holding tanks from the general salt water system. This allows the ability to mix ambient water with heated or cooled water from the raw water heating and cooling systems (see Chapter VI) during all seasons to obtain the desired water temperature. The water is then pumped by Metless pumps through 10 μ filter units in series to a second set of tanks where prefiltered water moves through 2 — 1 μ filter units in series to a 250 gal tank to hold water for the algal medium. A third filtration utilizing one Metless pump then pushes this filtered water through another 2 — 1 μ filter unit in series to a storage tank above the pasteurizing unit. This salt water, which has been filtered 3 times, can now be used to fill the test tubes and Fernbach flasks, or it can be used for the pasteurization process to fill the 5, 10 and 250 gal algal culture containers.

The needed volume of filtered salt water is withdrawn into a 5 gal carboy for test tubes and Fernbach flasks where the proper amount of nutrient mixture can be added. It is then dispensed into the test tubes (10 ml) and Fernbach flasks (1.5 liters). These filled culture containers are then autoclaved prior to inoculation. The water is gravity fed through the pasteurizing heat exchangers and heated to 75-80 C to process the filtered water for filling the 5, 10 and 250 gal culture containers. The steam-sterilized 5 to 10 gal culture containers are directly filled at the pasteurizer through glass valves. A separate glass line conveys the pasteurized water to the 250 gal mass algal culture tanks in the culture rooms. The detailed description of the pasteurizing unit can be found in Chapter VII.

Nutrient Enrichment

The nutrient enrichment to be described below has been found to work extremely well for all types of algal culture, both at the Virginia Institute of Marine Science and at the commercial size hatchery in Maryland. The N_2M enrichment is made up from 5 basic stock solutions, as follows:

Ketchum and Redfields Solution "A" (Ketchum and Redfield, 1938)

KNO_3	— 202 g
Distilled water	— dilute up to 1 liter

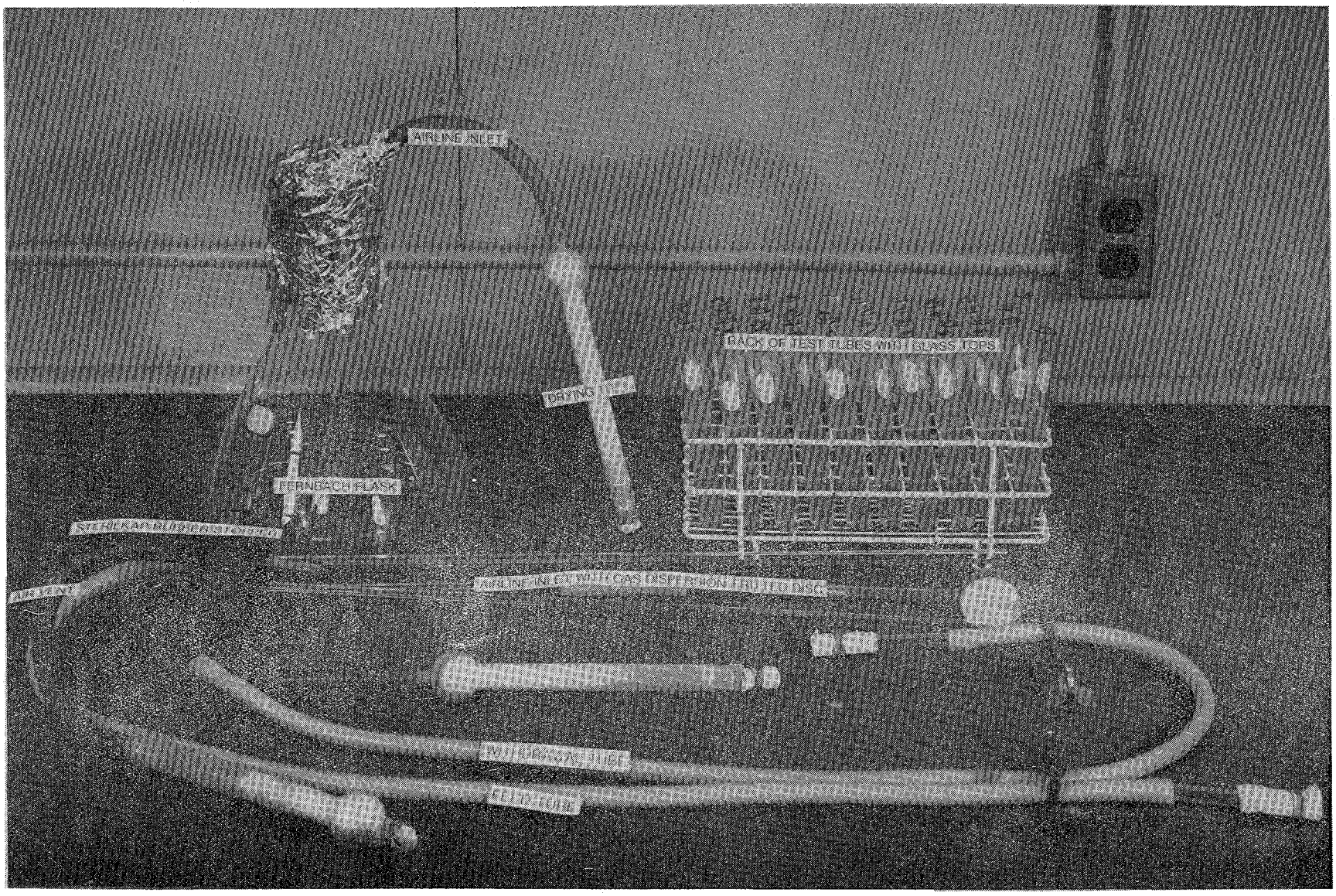


Figure 23. Arrangement of Tubing of Culture Containers and Type Glassware.

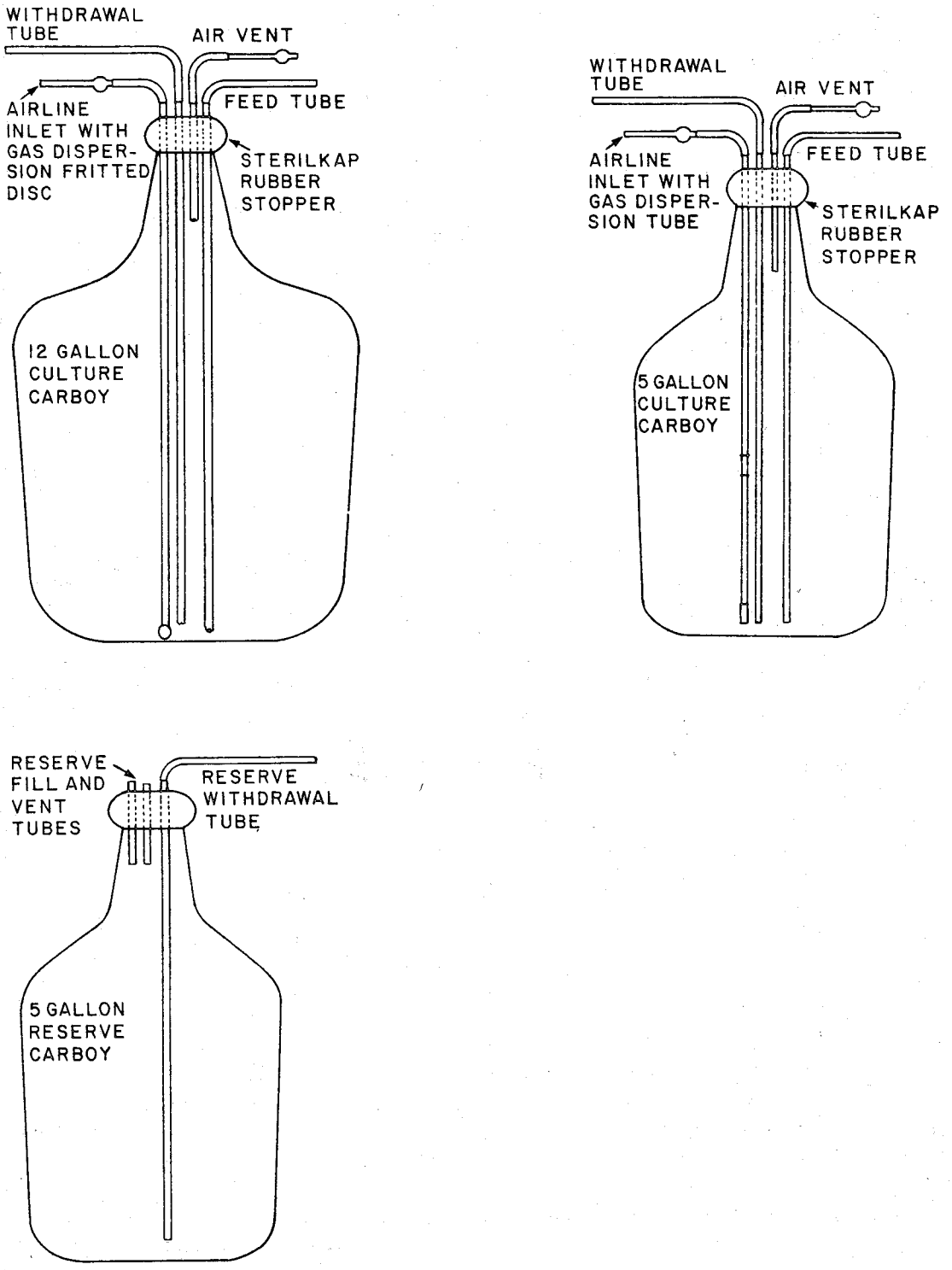


Figure 24. Type of and Arrangement of Tubing for 5, and 10 gallon Mass Culture Carboys.

Ketchum and Redfields Solution "B" (Ketchum and Redfield, 1938)

$FePO_4$	— first dissolve 5 g in 20 ml conc. HCl then add:
$Na_2HPO_4 \cdot 7H_2O$	— 30 g
$CaCl_2 \cdot 2H_2O$	— 27 g
$MgSO_4$ (anahydrous)	— 29 g
Distilled H_2O	— dilute to 1 liter

Sodium Molybdate Solution

1% $Na_2MoO_4 \cdot 2H_2O$ sol'n	— 11.9 ml
(10 g $Na_2MoO_4 \cdot 2H_2O$/1 liter — keep as stock sol'n)	
Distilled H_2O	— dilute to 1 liter

Arnon's Micronutrient Solution (modified) (Hoagland and Arnon, 1938)

1% H_3BO_3 stock sol'n	— 28.6 ml
1% $MnCl_2 \cdot 4H_2O$ stock sol'n	— 18.1 ml
1% $ZnSO_4 \cdot 7H_2O$ stock sol'n	— 2.22 ml
1% $CuSO_4 \cdot 5H_2O$ stock sol'n	— 0.79 ml
1% $COCl_2 \cdot 6H_2O$ stock sol'n	— 0.40 ml
Distilled H_2O	— dilute up to 100 ml

Soil Extract Solution

Soil extract normally acts as a chelator and is useful in areas where the water is not of optimal quality. Do not use agricultural soil or garden soil. These soils may have chemical additives such as fertilizer, pesticides and herbicides toxic to the algae. The top soil (woodland loam) should be obtained from an area that is known to be fairly clean.

Mix 1000 grams of finely sifted top soil (see above) to 2 liters of distilled water. Plug the container with cotton and autoclave for 30 min at 15 psi pressure. When cool, pour off the liquid carefully and filter this liquid through cotton and then through a Whatman No. 1 filter paper.

Final Stock Enrichment Solution (N_2M)

Ketchum and Redfields Solution "A"	— 200 ml
Ketchum and Redfields Solution "B"	— 100 ml
Sodium Molybdate Solution	— 50 ml
Arnon's Micronutrient Solution (modified)	— 50 ml
Soil Extract	— 200 ml
Distilled H_2O	— 100 ml
total	— 700 ml

This final solution should be well mixed and put into the proper size Pyrex containers with a cotton plug and a wrap of aluminum foil over the cotton. The solution should then be autoclaved for 15 min at 15 psi and refrigerated afterwards.

The enrichment solution is then used at the rate of 2 ml per liter of filtered water for test tubes, Fernbach flasks, and 5 gal (18 liters) Pyrex culture carboys. For the 10 gal (40 liter) Pyrex carboys and the 250 gal mass algal tanks, the enrichment solution is added at the rate of 4 ml per liter of filtered water.

Generally any amount of enrichment solution can be made up, but it must be in the same proportions as the above described mix. For example, in order to make up 7 liters of enrichment solution (N_2M), just multiply everything on the list by 10. The same goes for any stock solution. To make a greater quantity, just multiply everything in the solution by the same number. For example, for 3 liters of Ketchum and Redfields Solution "A", multiply KNO_3 (20.2 grams) by 30 to give 606 grams. Then dilute to 3 liters with distilled water. To make 3 liters of Ketchum and Redfields Solution

"B", multiply everything by 30 and dilute to 3 liters with distilled water. For the Sodium Molybdate Solution, just take what was used from the working solution. For the Arnon's Micronutrient Solution (modified), multiply everything by the desired factor. For example, if 3 liters is needed, multiply everything by 30, as shown:

H_3BO_3	0.286 g x 30 = 8.580 g
$MnCl_2 \cdot 4H_2O$	0.181 g x 30 = 5.430 g
$ZnSO_4 \cdot 7H_2O$	2.220 g x 30 = 66.600 g
$CuSO_4 \cdot 5H_2O$	0.790 g x 30 = 23.700 g
$CoCl_2 \cdot 6H_2O$	0.400 g x 30 = 12.000 g
dilute to 3 liters with distilled H_2O	

The soil extract can be made up in an 18 liter bottle; however, do not fill past the 12 liter mark when both soil and water are added. This prevents spillovers. For example, 4000 g of soil and 8 liters of distilled water can be added and sterilized as before.

Vitamin Enrichment Mix

Biotin Stock Solution

10 mg, dilute to 100 ml with distilled H_2O
(1 ml = 0.1 mg)

Thiamin-HCl Stock Solution

1 g, dilute to 100 ml with distilled H_2O
(1 ml = 10 mg)

B_{12} Stock Solution

0.1 g, dilture to 100 ml with distilled H_2O
(1 ml = 1 mg)

Using these stock solutions, make up the Vitamin Enrichment Mix as follows:

Biotin Stock Solution	— 10 ml
Thiamin-HCl Stock Solution	— 20 ml
B_{12} Stock Solution	— 1 ml
Mix and dilute to 1 liter with distilled H_2O	

Again any amount can be made up. For example, for 10 liters of Vitamin Mix, multiply quantities in mix by 10. Then take 100 ml Biotin, 200 ml of Thiamin-HCl, and 10 ml B_{12}; dilute to 10 liters with distilled water. Mix well, dispense appropriate amounts into Pyrex bottles, plug, sterilize for 10 min at 15 psi and refrigerate.

The Vitamin Mix is routinely used at the rate of 1 ml per liter of sea water for test tubes, Fernbach flasks, and 18 liter cultures. For 10 gal (40 liter) and 250 gal mass cultures, it is used at the rate of 2 ml per liter of estuarine water.

Sterilization of Medium and Glass Containers

Prior to sterilization either by steam or by autoclave, all glass containers should be cleaned in the following manner:

(1) Wash with Alconox, using brush until surface is clean
(2) Rinse 3 times with hot tap water
(3) Rinse 2 times with cold tap water
(4) Rinse 2 times with distilled or de-ironized water

The sterilization by autoclave of the glass containers and medium applies only to the test tubes and Fernbach flasks. In both cases the water containing the nutrients and vitamin is added to the test tubes containing 10 ml of medium (water plus enrichment) are autoclaved for 10 minutes at 15 psi. The Fernbach flasks containing 1.5 liters of media are autoclaved for 30 min at 15 psi. The autoclave that is used is an upright unit.[1]

Sterilization of empty 5 gal (18 liter) and 10 gal (40 liter) Pyrex carboys is accomplished in a steam sterilizer unit. The dimensions for the stainless steel steam chamber are 36" wide x 36" high x 39" deep, and it is run by a steam unit at 5 psi pressure (Steam Master PP-6 steam generator)[2]. The glass carboys with accessory units are held in this steam chamber automatically for 1 hour by the use of a 60 minute Nutone Timer.

The pasteurization of the triple-filtered estuarine water is accomplished by gravity feeding it through two Corning Flass 13.5 GBR heat exchangers. The flow of filtered water is adjusted to obtain a temperature of 80 C at the exit point.

To fill the steam-sterilized glass 5 and 10 gal Pyrex carboys, two glass valves are provided. This gives the ability to fill two 5 or 10 gal carboys at one time. It takes approximately 6 min to fill a 5 gal carboy and 13 min to fill 10 gal carboys. The carboys must be allowed to cool naturally overnight to insure complete pasteurization. These carboys can be put into a refrigerated room the following day to shorten the cooling period. The addition of Nutrient Enrichment Solution (N_2M) is added at the time of algal inoculation.

The pasteurization of the triple filtered water for use in the 250 gal (1000 liter) algal culture containers is accomplished in a similar manner. A separate glass valve and glass pipe line feeds the heated filtered water to the two algal tank culture rooms.

The flow of filtered water through the heat exchangers is adjusted so that a temperature of 80 C is obtained where this water enters the 250 gal algal tank. The average time taken to fill these tanks is about 90 min, and the tank must be kept covered during and after filling. These tanks should be allowed to cool naturally over a 2 day period to insure complete pasteurization. The insulating jacket may be removed and aeration initiated at the end of this period. The Nutrient Enrichment Solution (N_2M) is added at the time of inoculation with algae. A complete description of each type of container is given in the next section.

Methodology for Inoculation

Test tube culture of three species of algae — *Pyramimonas virginica, Pseudoisochrysis paradoxa,* and *Chlorella* sp. — is the base from which the algal food is started and is the continuous supply of non-contaminated algal seed culture for further inoculation during the operation of the hatchery. The original cultures may be obtained from the Marine Culture Department, Virginia Institute of Marine Science, Gloucester Point, Virginia 23602. Ten tubes of each species should be available at the hatchery to begin the culture program. After the system is started the hatchery should have 40 test tubes of each species ready for use continuously. As previously stated, the autoclaved test tubes should contain 10 ml of enriched water medium. Add initially 2 ml of algal inoculum from the algal seed culture source obtained from the Virginia Institute of Marine Science to each of these test tubes. This will require a total of 80 ml or 8 tubes of each species. The remaining 2 tubes of the original 10 starter cultures should be kept until these newly inoculated tubes are successfully growing. Thereafter, every 2 weeks a new set of 40 test tubes for each species should be inoculated from the previous set. Five tubes should be held until the new test tube cultures are successfully growing. The total number of test tubes needed will then be at least 480 to operate this part of the algal culture section. The medium enrichment and filtered water should be prepared no earlier than 3 days prior to inoculation.

Fernbach Flasks

The next step in the procedure is the inoculation of Fernbach flasks which should contain 1.5

[1]Autoclave Vertical, Electric Precision Scientific. 14 x 26 #67012, Cat. No. 58619-034. VWR Scientific, P.O. Box 8603, Baltimore, Maryland 21240. (301) 796-8500.
[2]Steam Master Co., Inc. 110 West 30th St., New York, N.Y.

liters of filtered water with enrichment medium. A total of 3 Fernbach flasks should be prepared to inoculate the 5 gal (18 liter) Pyrex carboys at any one time from each of the three algal species. This will then require at least 9 Fernbach flasks per species or a total of 27 flasks for the three species. The autoclaved Fernbach flasks should be inoculated at the rate of one flask every 7 days for each of the three species of algae. This will insure a constant supply to inoculate the 5 gal (18 liter) Pyrex carboys. Each of the Fernbach flasks should be inoculated with the contents from 10 culture tubes. The total inoculum will then be 100 ml. First remove the aluminum foil cover from the autoclaved flasks and then the cotton plug. Afterwards the contents of 1 test tube should be poured quickly into the flask. The cotton plug must then be quickly replaced. This procedure should be repeated until the remaining 9 tubes have been poured in. After this procedure is finished the aluminum foil cover should be put back over the flask neck and cotton plug.

The time required to obtain optimal cell densities from the Fernbach flasks for the three species will vary between 3 and 4 weeks. A gas dispersion tube may be used to aerate the cultures to reduce the time for obtaining optimal cell densities by 1 week.

Five Gallon Culture Carboys (18 liters)

These culture containers should be first made up according to Figure 24. The Pyrex carboys should be calibrated to show 1 liter increments. This is done by taking a 1 liter volumetric flask and pouring the contents into the Pyrex carboy. After each liter is poured into the bottle the level should be marked with a Blaisdell Hi-Temp Marker (green). This should be repeated until 18 liters have been poured into the Pyrex carboy. The bending of the glass tubing can be accomplished by utilizing a gas-operated Bunsen burner with a wing nozzle. The glass tubing should be slowly heated over a 3 to 4 inch length allowing the tube to bend slowly. Some practice is required before success is achieved.

Once these Pyrex carboys have been assembled they should be steam sterilized and then filled with 2 liters of pasteurized, triple filtered water. After they have cooled they are ready to be inoculated. One Fernbach flask containing a dense culture of one of the species required should be poured into the 5 gallon carboy. At the same time the algae are being poured in 8 ml of enrichment solution (N_2M) and 4 ml of Vitamin Mix should be added. These inoculated cultures should then be placed in the bottle culture room which is kept at 20 C. Three days later and every other day thereafter 2 liters of triple filtered water containing N_2M and Vitamin Mix should be added until the full 18 liters of algal culture is obtained. Usually these cultures can be used at the end of 18 days. Each species will require a total of 8 five gal Pyrex carboys, of which 6 should contain algal culture with optimal densities of algae. Eight extra 5 gal Pyrex carboys should be ready to inoculate at any time. This will require a total of 40 five gal Pyrex carboys.

Five Gallon Reserve Carboys

For each 5 gal algal culture carboy in use there should be 1 five gal reserve carboy which can be utilized to feed culture medium to these cultures. These reserve Pyrex carboys should be prepared in the same manner as algal culture carboys, except for the number of tubes that are emptied into the Pyrex carboy. These reserve carboys should be steam sterilized and then filled with 18 liters of pasteurized, triple-filtered salt water. The exit tube of the reserve carboy should then be joined to the culture medium intake tube of the algal culture Pyrex carboy. For each liter of filtered reserve salt water this is siphoned from the reserve carboy into the algal culture carboy, 4 ml of N_2M and 2 ml of Vitamin Mix should be injected into the rubber hose connecting the two carboys prior to siphoning the reserve filtered water into the algal culture carboys. Sterile disposable syringes should be used for injection of the N_2M and Vitamin Mix. A total of 30 reserve carboys will be required to feed the 5 gal algal culture carboys.

Ten Gallon Batch Culture Carboys (40 liters)

The 10 gal (40 liter) Pyrex algal culture carboys should be calibrated at 5 liter intervals using the same procedure as used to calibrate the 5 gal algal culture carboys. The preparation of the glass

tubes, rubber tubing, drying tubes, and stoppers should be identical to that described previously, except that the glass tubing in these carboys will be longer.

These 10 gal mass algal culture Pyrex carboys should be steam sterilized prior to filling with pasteurized, triple filtered salt water. These bottles should contain 30 liters of this pasteurized water.

After these 10 gal algal culture bottles have been cooled to 20 C over a 2 day period, they should be inoculated with 10 liters of inoculum from one of the required species obtained from one of the 5 gal algal cultures. This inoculation is accomplished by siphoning the algal inoculum out of the withdrawal tube from the 5 gal algal culture bottle through the connection to the culture medium intake tube of the 10 gal mass culture carboy. During this siphoning period 120 ml of N_2M and 60 ml of Vitamin Mix should be injected into the siphon rubber tubing. These algal cultures should be ready in 15 days for use as inoculum for the 250 gal batch culture containers. A total of 10 ten gal mass culture carboys will be required for each species.

The following number of mass culture bottles should have optimal cell densities at any one time:

Pyramimonas virginica (Va-17)	8 carboys
Pseudoisochrysis paradoxa (Va-12)	6 carboys
Chlorella sp. (Va-52)	4 carboys

Besides the 40 ten gallon carboys that will be in operation at one time, there should be 8 extra 10 gal carboys which can be inoculated as needed. The total number of 10 gal Pyrex culture carboys required for this part of the operation is 48.

250 Gallon Mass Culture Tanks

A total of twelve 250 gal mass culture tanks are required to properly schedule the culture of and grow *Pyramimonas virginica* (Va-17), and *Pseudoisochrysis paradoxa* (Va-12). Two rooms 24' x 20' with refrigeration have been included in the design on the second floor to schedule the pasteurization and mass culture of these two algal species. Each of the rooms contain six 250 gal tanks (c.f. pg 143). The *Chlorella* sp. tank room contains three 250 gal tanks. As previously described, these 250 gal mass culture tanks are filled with 210 gallons of pasteurized, triple-filtered water [Figure 25]. These fiberglas tanks, which have a gel coat on the inner surface, are made with a special heat-resistant resin able to withstand temperatures up to 195 F and have a fiberglas cover made of the same material with a 40" by 40" plate glass window. The culture tank lid has a silicone gasket (Corning—Industrial Grade) to obtain a tight seal on the tank with 3 openings to accommodate 2 air tubes and an air vent or inoculation point. One air line comes to each tank and is connected to a drying tower. From this drying tower the air line is divided via a tee to 2 air lines connected to 2 glass tubes passing through the fiberglas lid. The glass tubes are cut so the ends are about 3 inches off the bottom of the tank. The glass tubes which are below the tank lid are steam sterilized prior to use and are in place when the pasteurized water is being added.

At maximum feeding rates the projected needs for the 18-250 gal larval culture tanks for any given larval brood are:

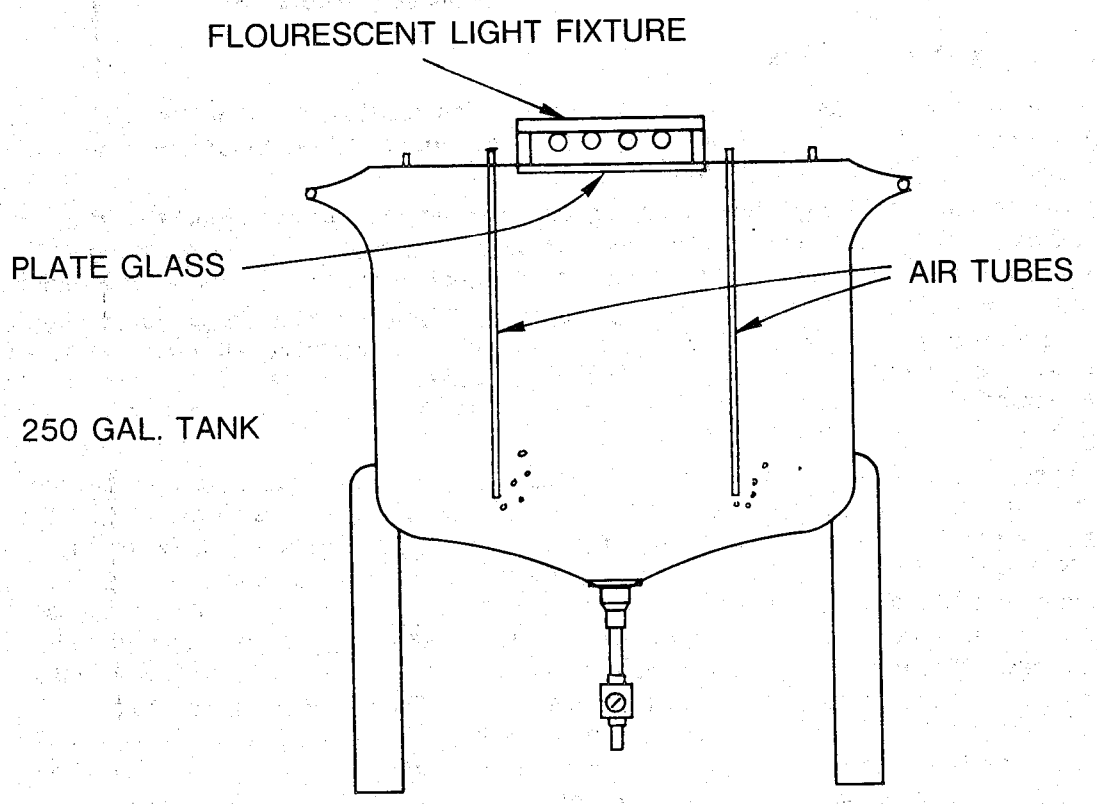
Pyramimonas virginica (Va-17)	1,600 liters per 14 days
Pseudoisochrysis paradoxa (Va-12)	1,900 liters per 14 days
Chlorella sp. (Va-52)	900 liters per 14 days

Two to 4 ten gal batch cultures will be required as inocula depending on the species to be inoculated. The number of 10 gal batch cultures usually required per species is as follows:

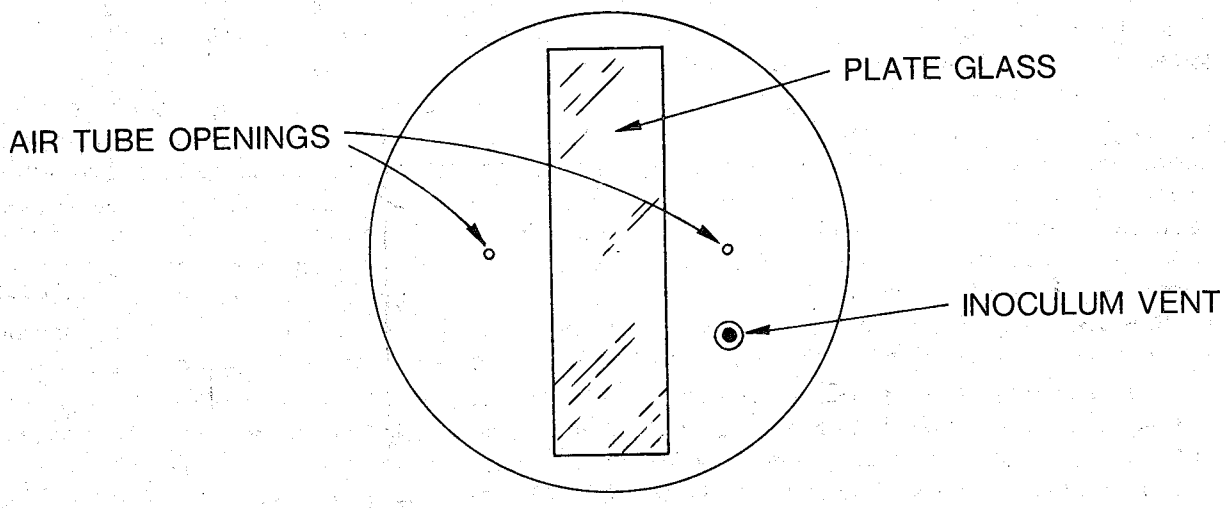
Pyramimonas virginica (Va-17)	= 4
Pseudoisochrysis paradoxa (Va-12)	= 3
Chlorella sp. (Va-52)	= 2

These numbers are based on obtaining the optimal count as described on page 118. If the counts should be lower at the time of inoculation, then 1 additional 10 gal batch culture will have to be added for each species.

The N_2M enrichment (4 liters) and Vitamin Mix (2 liters) should be added at the time of inoculation of these tanks. The scheduling of the inoculation is as follows:



a. Side View Showing Fluorescent Lamp Placement



b. Top View Showing Plate Glass Window and Air Vents

Figure 25. Algal Mass Culture Tank (250 gal).

tubes, rubber tubing, drying tubes, and stoppers should be identical to that described previously, except that the glass tubing in these carboys will be longer.

These 10 gal mass algal culture Pyrex carboys should be steam sterilized prior to filling with pasteurized, triple filtered salt water. These bottles should contain 30 liters of this pasteurized water.

After these 10 gal algal culture bottles have been cooled to 20 C over a 2 day period, they should be inoculated with 10 liters of inoculum from one of the required species obtained from one of the 5 gal algal cultures. This inoculation is accomplished by siphoning the algal inoculum out of the withdrawal tube from the 5 gal algal culture bottle through the connection to the culture medium intake tube of the 10 gal mass culture carboy. During this siphoning period 120 ml of N_2M and 60 ml of Vitamin Mix should be injected into the siphon rubber tubing. These algal cultures should be ready in 15 days for use as inoculum for the 250 gal batch culture containers. A total of 10 ten gal mass culture carboys will be required for each species.

The following number of mass culture bottles should have optimal cell densities at any one time:

Pyramimonas virginica (Va-17)	8 carboys
Pseudoisochrysis paradoxa (Va-12)	6 carboys
Chlorella sp. (Va-52)	4 carboys

Besides the 40 ten gallon carboys that will be in operation at one time, there should be 8 extra 10 gal carboys which can be inoculated as needed. The total number of 10 gal Pyrex culture carboys required for this part of the operation is 48.

250 Gallon Mass Culture Tanks

A total of twelve 250 gal mass culture tanks are required to properly schedule the culture of and grow *Pyramimonas virginica* (Va-17), and *Pseudoisochrysis paradoxa* (Va-12). Two rooms 24' x 20' with refrigeration have been included in the design on the second floor to schedule the pasteurization and mass culture of these two algal species. Each of the rooms contain six 250 gal tanks (c.f. pg 143). The *Chlorella* sp. tank room contains three 250 gal tanks. As previously described, these 250 gal mass culture tanks are filled with 210 gallons of pasteurized, triple-filtered water [Figure 25]. These fiberglas tanks, which have a gel coat on the inner surface, are made with a special heat-resistant resin able to withstand temperatures up to 195 F and have a fiberglas cover made of the same material with a 40" by 40" plate glass window. The culture tank lid has a silicone gasket (Corning—Industrial Grade) to obtain a tight seal on the tank with 3 openings to accommodate 2 air tubes and an air vent or inoculation point. One air line comes to each tank and is connected to a drying tower. From this drying tower the air line is divided via a tee to 2 air lines connected to 2 glass tubes passing through the fiberglas lid. The glass tubes are cut so the ends are about 3 inches off the bottom of the tank. The glass tubes which are below the tank lid are steam sterilized prior to use and are in place when the pasteurized water is being added.

At maximum feeding rates the projected needs for the 18-250 gal larval culture tanks for any given larval brood are:

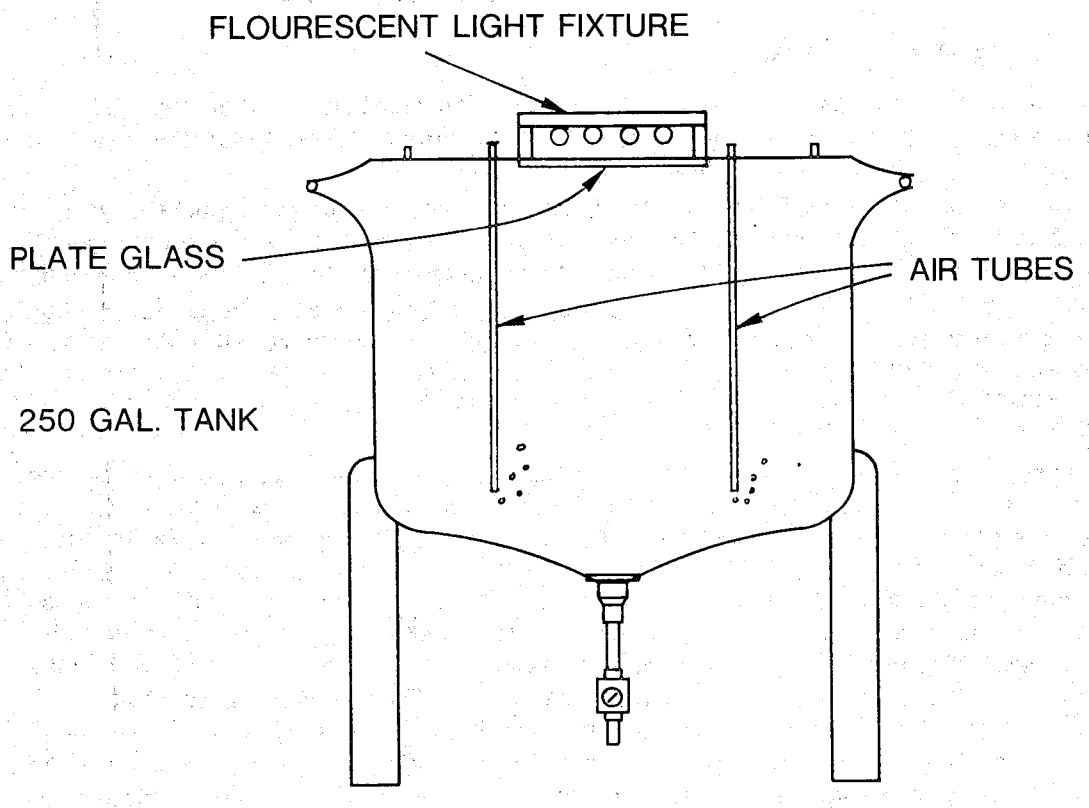
Pyramimonas virginica (Va-17)	1,600 liters per 14 days
Pseudoisochrysis paradoxa (Va-12)	1,900 liters per 14 days
Chlorella sp. (Va-52)	900 liters per 14 days

Two to 4 ten gal batch cultures will be required as inocula depending on the species to be inoculated. The number of 10 gal batch cultures usually required per species is as follows:

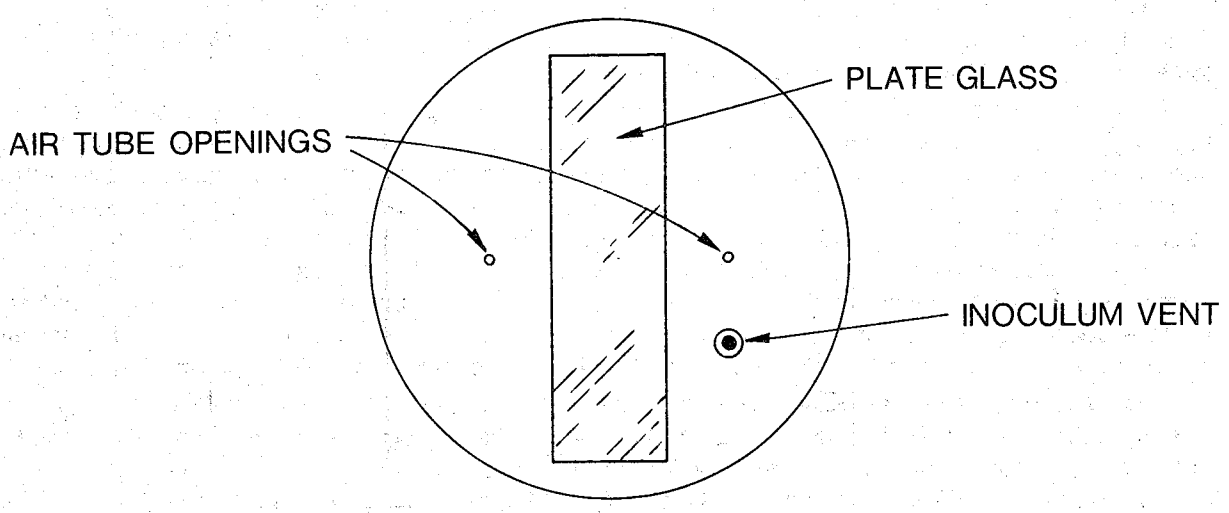
Pyramimonas virginica (Va-17)	= 4
Pseudoisochrysis paradoxa (Va-12)	= 3
Chlorella sp. (Va-52)	= 2

These numbers are based on obtaining the optimal count as described on page 118. If the counts should be lower at the time of inoculation, then 1 additional 10 gal batch culture will have to be added for each species.

The N_2M enrichment (4 liters) and Vitamin Mix (2 liters) should be added at the time of inoculation of these tanks. The scheduling of the inoculation is as follows:



a. Side View Showing Fluorescent Lamp Placement



b. Top View Showing Plate Glass Window and Air Vents

Figure 25. Algal Mass Culture Tank (250 gal).

Pyramimonas virginica (Va-17)	1 tank per week
Pseudoisochrysis paradoxa (Va-12)	1 tank per week
Chlorella sp. (Va-52)	1 tank every two weeks

Determination of Algal Cell Densities

Regular counts of the three species of algae must be made in order to schedule inoculation of the 5, 10 and 250 gal algal cultures, to monitor growth of the algal cultures, and to determine the quantity of algae to be fed to the oyster larvae.

To take a sample of the 5 gal (18 liter) and 10 gal (40 liter) cultures, first flush the algae from the withdrawal tube on the culture bottle by withdrawing a 125 ml Erlenmeyer flask full pouring this into a waste container. Fill the flask again up to the 50 ml mark to obtain the sample used for counting.

In sampling the mass or tank cultures, cut a length of rubber tubing comfortable to work with and place it on the mouthpiece end of a sterile serological pipette. Place the other end of the rubber tubing in your mouth and the pipette in the culture. To get a representative sample move the pipette around the tank while withdrawing algae up to the mark on the pipette and then place it in a flask. This is the sample to be counted.

These samples must now be prepared for counting. For the three algal species there are 2 methods of preparation according to cell size. *Pseudoisochrysis paradoxa* (Va-12) and *Pyramimonas virginica* (Va-17) are both prepared by the following procedure. Making sure the sample previously obtained is mixed well, withdraw a 5 ml aliquot with a volumetric pipette and transfer it to a 10 ml volumetric flask. Dilute to volume with the fixative (10% formalin) to the mark [Figure 26]. slip immediately removing the pipette from the groove. This allows the chamber to be filled quickly and evenly without any spillovers of the sample outside of the silvered counting area. Care must be taken not to overfill the chamber so that the coverslip bulges upward. If a spillover occurs, the chamber must be cleaned and refilled. Before refilling the chamber, the volumetric flask must be reshaken and a clean disposable Pasteur pipette used. The quicker the chamber is inoculated following withdrawal of the sample aliquot, the less chance of error. Other sources of error occur in the sampling technique and in the counting of the cells. These errors can be eliminated with practice. Several counts should be made on each sample initially to determine the degree of variation in the counts.

When the chamber is correctly inoculated the contents are allowed to settle out before counting. *Pseudoisochrysis paradoxa* (Va-12) and *Pyramimonas virginica* (Va-17) should be allowed to settle for 5 min, while *Chlorella* sp. (Va-52) requires 7 min. to settle.

The A-O "Brightline" Hemacytometer has three dividing lines surrounding each square, allowing an easier determination of those cells which are to be counted in that square with the center line being the boundary line. To avoid counting an algal cell which lies in the region of the lined area twice, count only those cells within the boundary lines on the top and left sides of the square (or similarly those cells within the boundary lines on the bottom and right sides of the square). A good routine for methodically counting the area without retracking is to begin in the upper left square of the twenty-five square area and work down that column, move to the right and up the following column, move to the right and down the following column, and so on until all squares are counted. Figures 27 and 28 indicate the counting area and the direction of the count.

When calculating the results consider *Pyramimonas virginica* (Va-17) and *Pseudoisochrysis paradoxa* (Va-12) were diluted by 1:2 (5 ml/10 ml) and *Chlorella* sp. (Va-52) was diluted by 1:100 (1 ml/100 ml). Therefore, to obtain the actual number of algal cells present per ml, multiply the count number on the hand tally by the dilution factor of 2 for *Pyramimonas* and *Pseudoisochrysis* and then 10^4. For *Chlorella* the count number is multiplied by a dilution factor of 100 and then by 10^4 to obtain the number of algal cells per ml. To put it in equation form,

count x dilution factor x 10^4 = cells/ml

The following examples demonstrate the calculations:

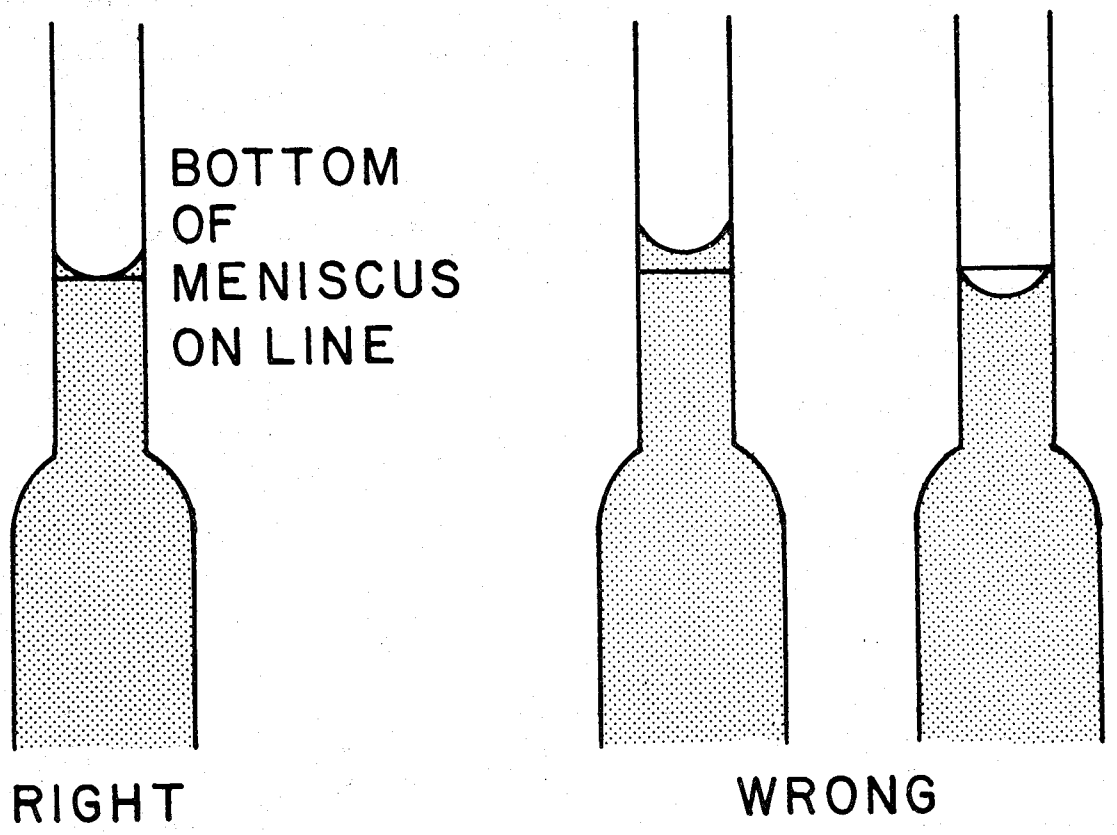


Figure 26. Reading Meniscus for Volumetric Pipettes and Flasks.

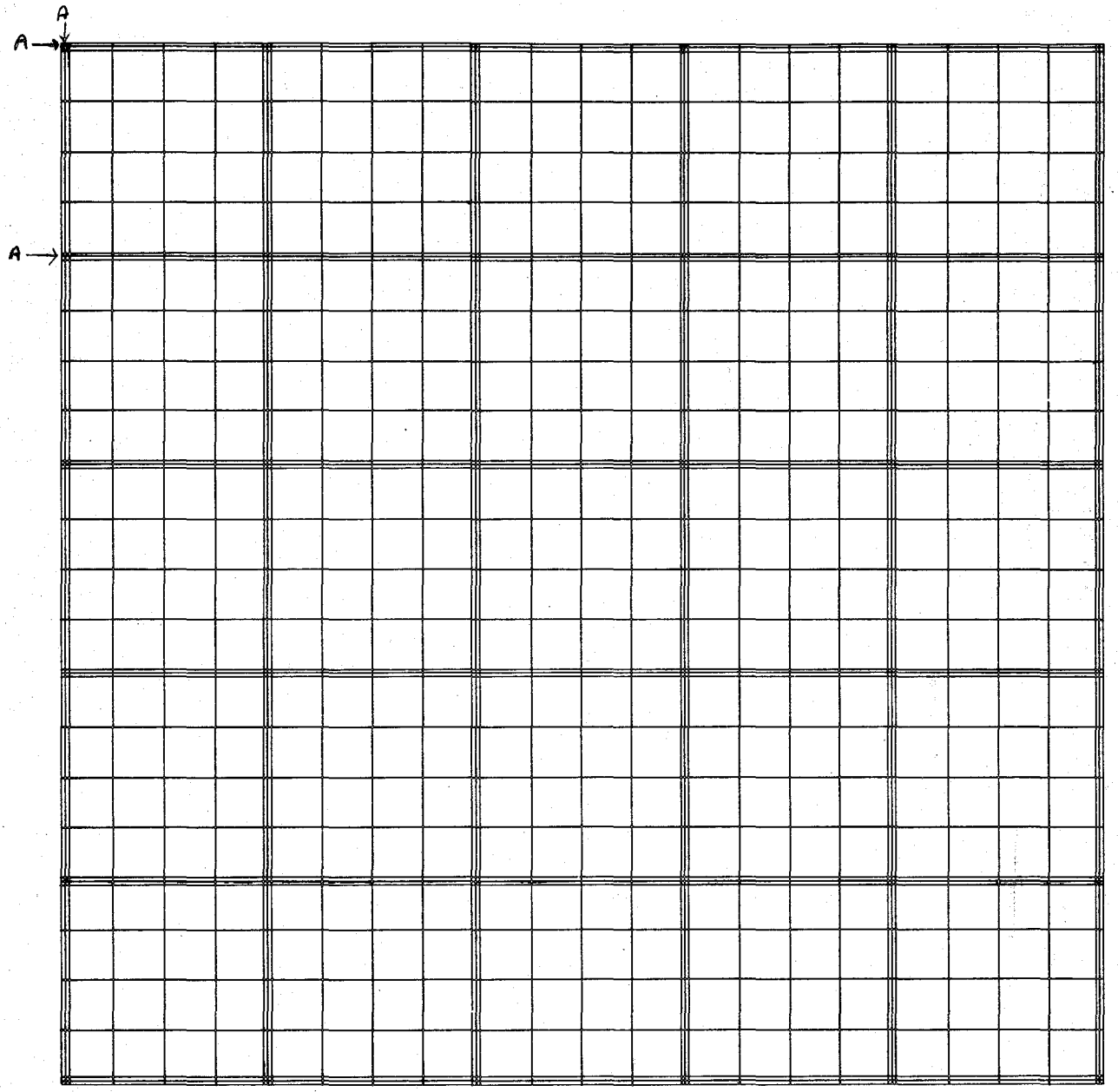


Figure 27. Counting Area of Brightline Hemacytometer (25 large squares).

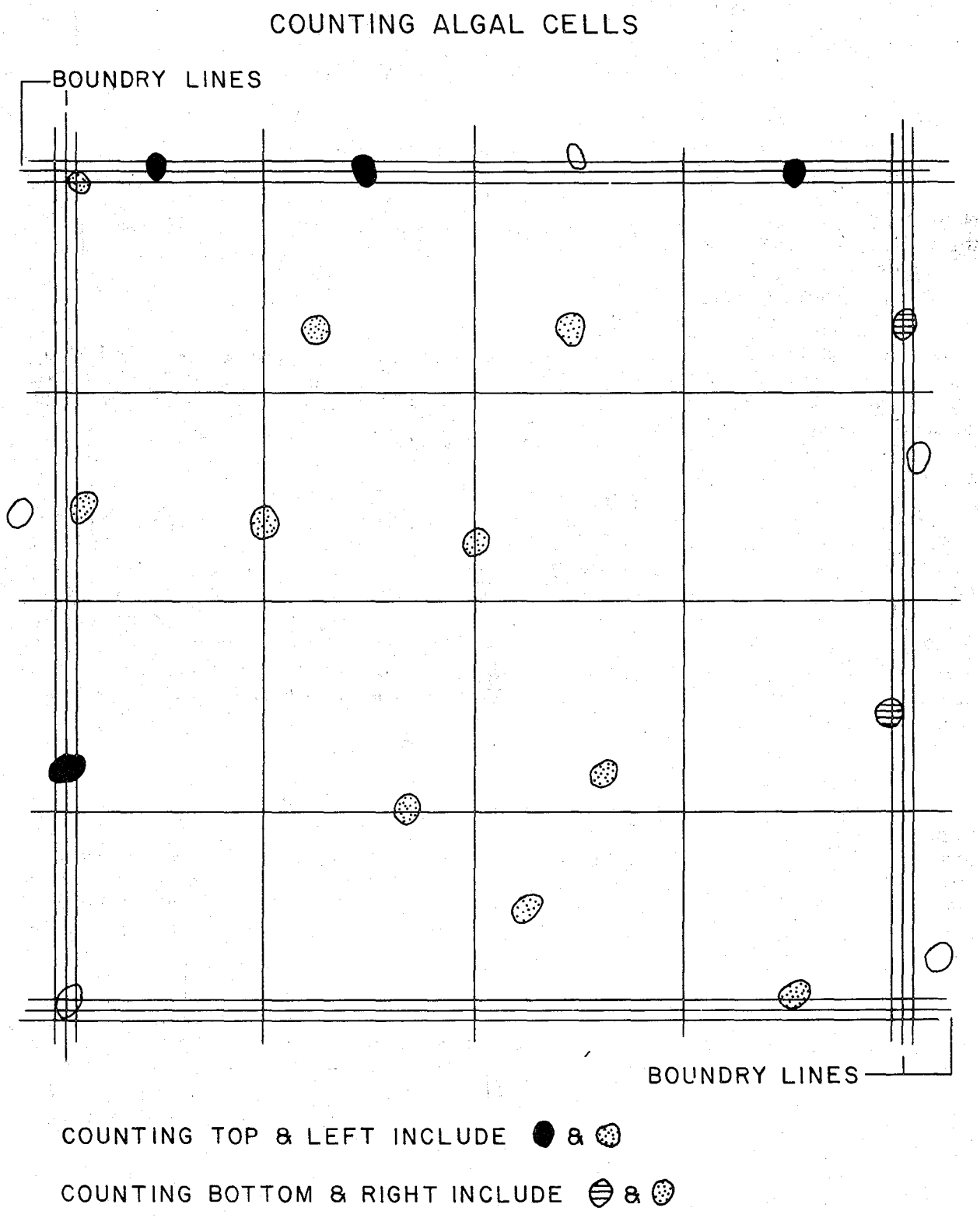


Figure 28. One Large Square of Brightline Hemacytometer.

Pseudoisochrysis paradoxa (Va-12)
Count shown on hand tally = 200
$200 \times 2 \times 10^4 = 400 \times 10^4 = 4.00 \times 10^6$ or 4,000,000 cells/ml
Count shown on hand tally = 240
$240 \times 2 \times 10^4 = 480 \times 10^4 = 4.80 \times 10^6$ or 4,800,000 cells/ml
Pyramimonas virginica (Va-17)
Count shown on hand tally = 250
$250 \times 2 \times 10^4 = 500 \times 10^4 = 5.00 \times 10^6$ or 5,000,000 cells/ml
Count shown on hand tally = 260
$260 \times 2 \times 10^4 = 520 \times 10^4 = 5.20 \times 10^6$ or 5,200,000 cells/ml
Note: Initial counts upon inoculation can produce densities in the 10^4 range. Example, any count on the above species that is 49 or below = $49 \times 2 \times 10^4 = 98 \times 10^4$ or 980,000 cells/ml
Chlorella sp. (Va-52)
Count shown on hand tally = 60
$60 \times 100 \times 10^4 = 60 \times 10^2 = 60 \times 10^6$ or 60,000,000 cells / ml
Finally, the algal counts obtained for the 250 gal mass algal cultures will establish the feeding rate to the larval culture tanks from Tables 2, 3, 4, 5, 6 and 7. The algal counts obtained from the 5 and 10 gal algal cultures are used to indicate the status or health of the cultures (rate of growth and contamination) and inoculation size for the 10 gal and 250 gal mass algal cultures.

Illumination of Algal Cultures

One of the most important factors determining the successful culture of these 3 species of algae is the type and quantity of illumination. Most attempts and failures in the past have been due to too much light. As previously mentioned, these 3 species of algae have their origins in an estuarine habitat which normally contains high silt and detrital loads in the water column. Due to the physical interruption of light by this silt and detritus, the intensity and quantity of sunlight is reduced to very low levels. Paralleling the low light levels found in natural waters, successful culture of these algae in the hatchery also requires relatively low levels of light to achieve successful culture and optimal densities of cells. The mixture of light sources used and described below is necessary to duplicate as nearly as possible the spectrum of normal sunlight.

Test Tubes

The requirement of light for *Pyramimonas virginica* (Va-17) is 180 foot-candles. This is achieved by putting these test tube cultures behind the *Chlorella* sp. (Va-52) test tube cultures directly in front of two 40 watt fluorescent bulbs, one being a Sylvania Gro-Lux and the other a Ken-Rad warm white. *Chlorella* sp. requires 600 foot-candles and is successfully grown in front of these 40 watt fluorescent bulbs. *Pseudoisochrysis paradoxa* (Va-12), like *Pyramimonas virginica,* will grow optimally at 180 foot-candles. The placement of *Pseudoisochrysis* test tube cultures behind the *Chlorella* sp. test tube cultures will yield optimal results.

Fernbach Flasks

The light requirements and placement of these 3 species of algae in relation to the fluorescent lights are identical to those of the test tube cultures. *Chlorella* sp. (Va-52) should be placed directly in front of the fluorescent light, whereas *Pyramimonas virginica* (Va-17) and *Pseudoisochrysis paradoxa* (Va-12) should be placed behind Va-52. The lights are the same for the tube cultures. Typical growth curves for each species are included in Figures 29, 30 and 31.

Five and Ten Gallon Culture Carboys

Both of these algal mass cultures require two 40 watt Gro-Lux (Sylvania F 40-GRO) and one each of 40 watt warm white (Ken-Rad F 40 WW) and cool white (Sylvania F 40 D) fluorescent bulbs. The light intensity at any point in front of the fluorescent bulbs is an average of 800 foot-candles.

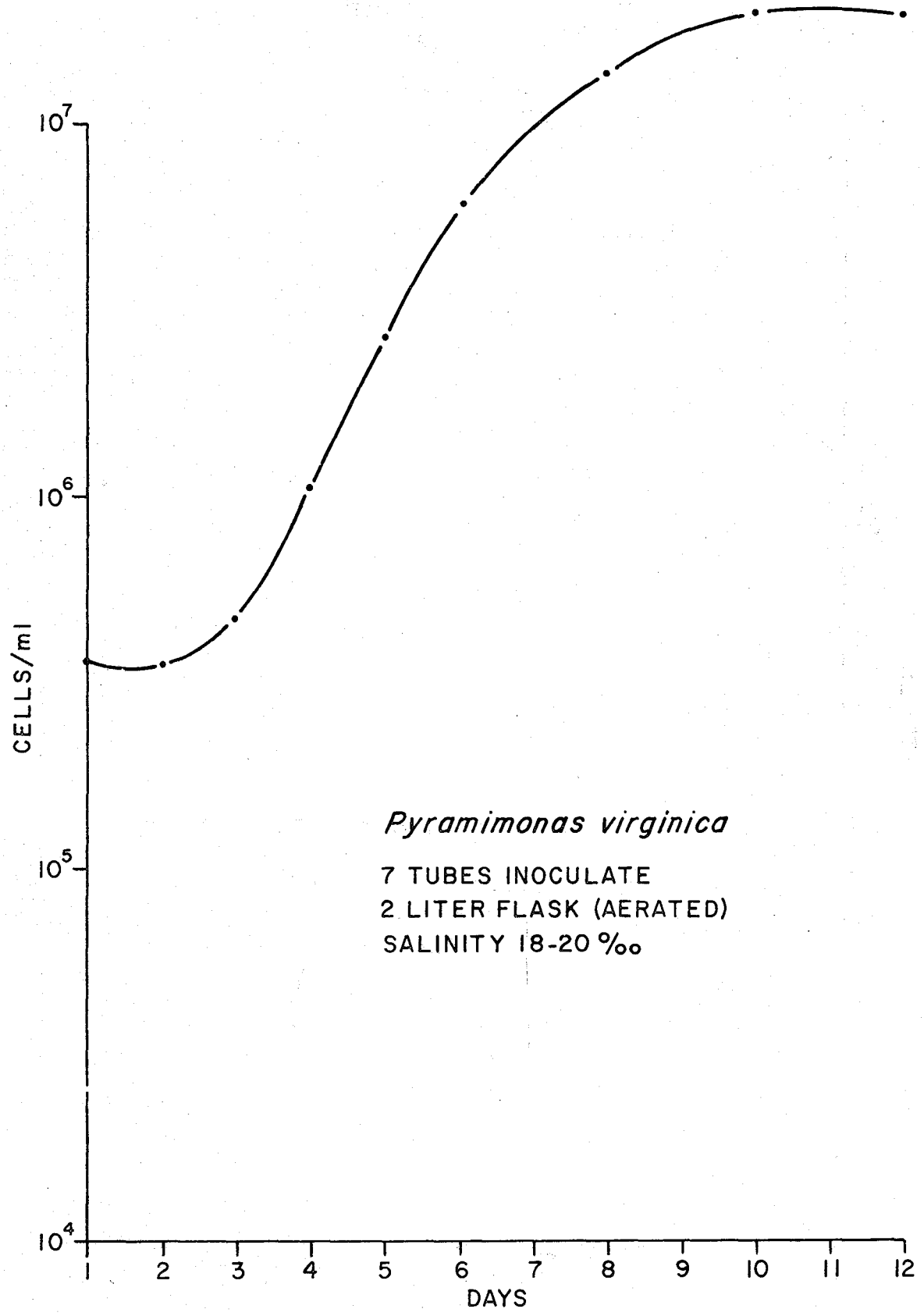


Figure 29. Growth Curve of *Pyramimonas virginica.*

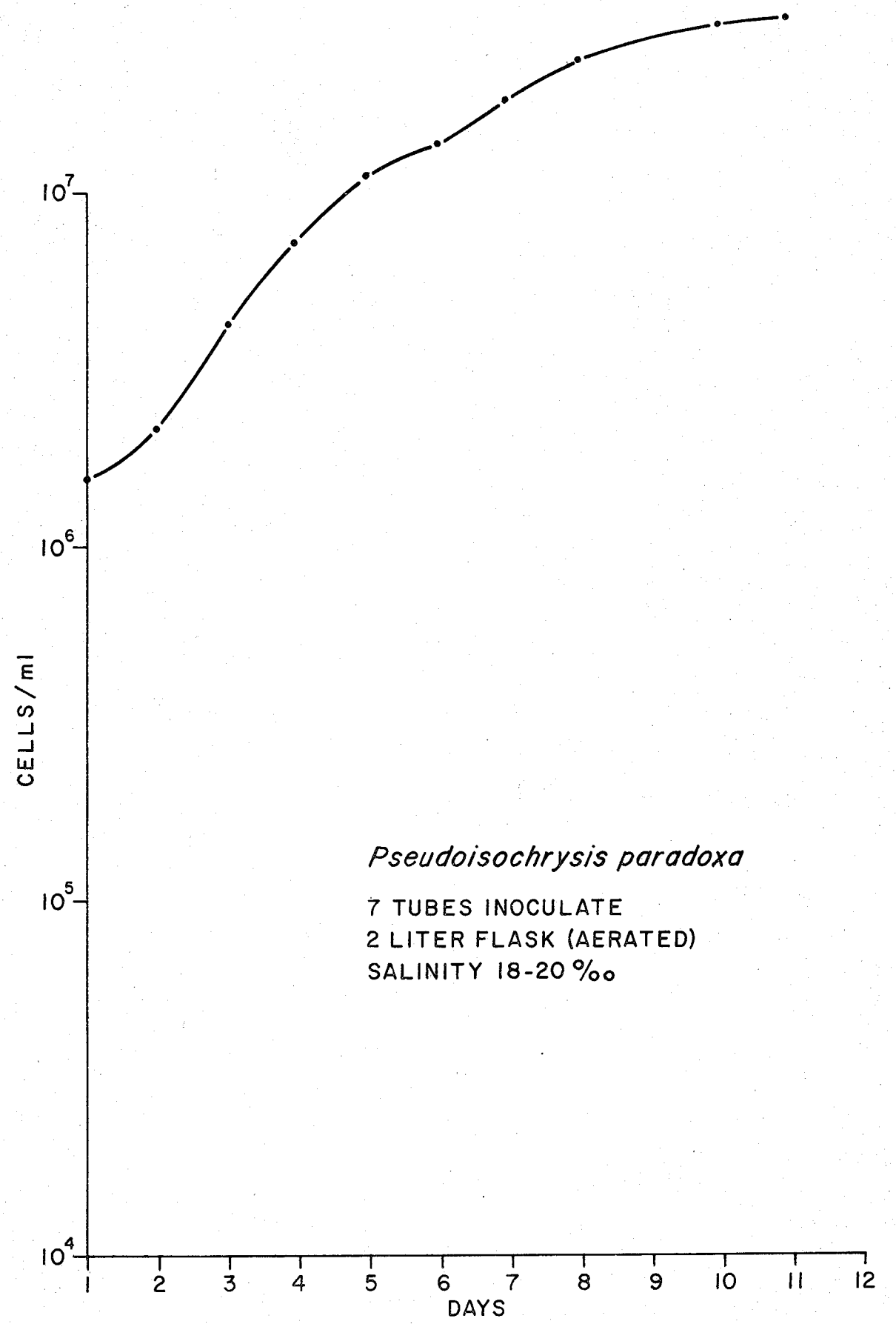


Figure 30. Growth Curve of *Pseudoisochrysis paradoxa.*

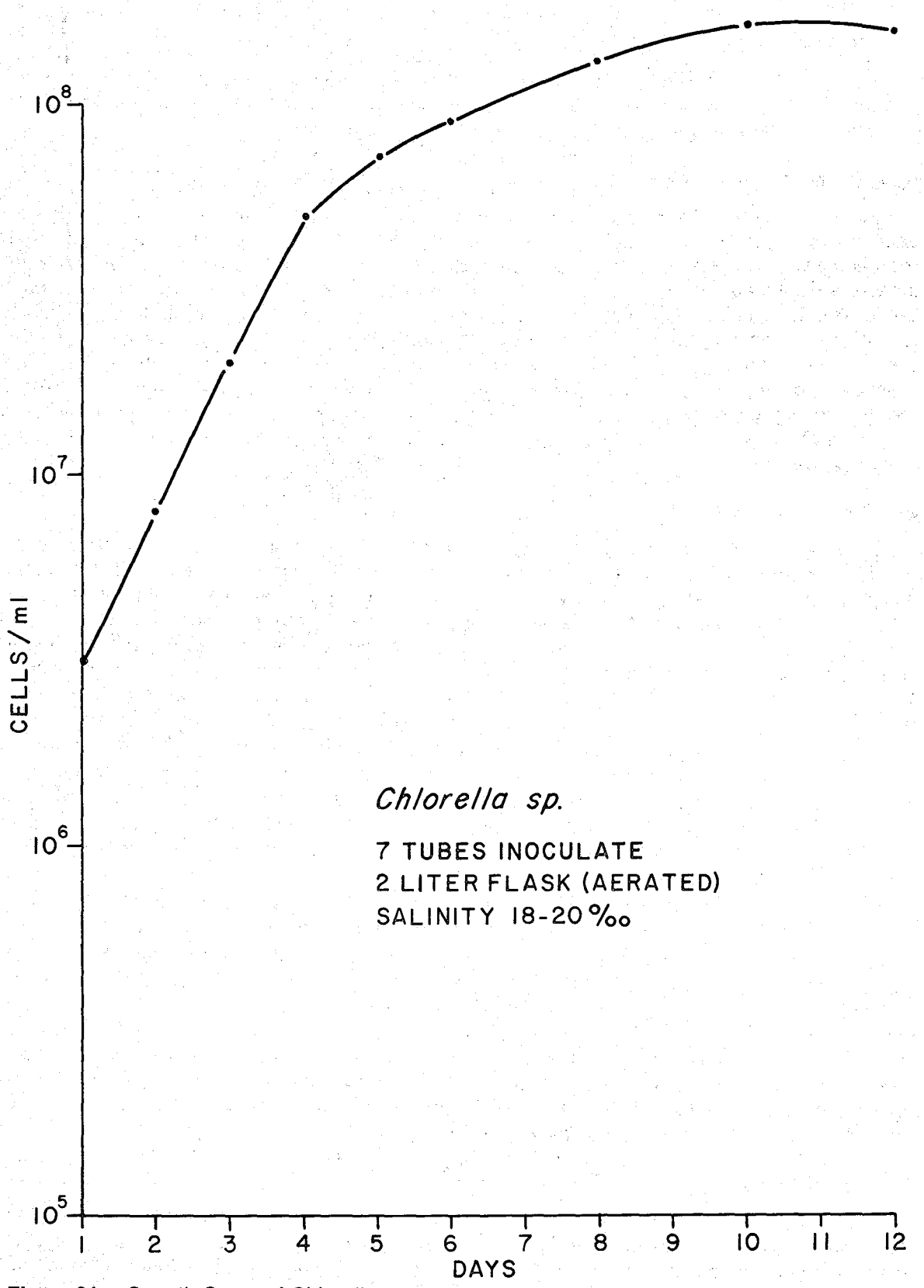


Figure 31. Growth Curve of *Chlorella* sp.

The width of the starter culture room is designed to insure there is a minimum of incident light reaching the bottle on the opposite side of the room (c.f. pg. 143).

250 Gallon Mass Culture Tanks

Eight 48 inch fluorescent lights are used on the outside and over the plate glass window of the tank lid. These include four 40 watt Gro-Lux (Sylvania F 40-GRO), two 40 watt warm white (Ken-Rad F 40 WW), and two 40 watt cool white (Sylvania F 40 D) fluorescent bulbs.

Aeration Distribution System

An air line should be installed in such a way to run the length of the bottle culture room over each row of algal culture bottles. The line consists of a 1/2 inch PVC Schedule 80 pipe. An air regulator maintains a constant 10 psi in the line. Over each 5 and 10 gal algal culture bottle is located a plastic aquarium valve. This permits the regulation of airflow to each 5 and 10 gal culture bottle. The flow of air to each bottle should be adjusted so that a moderate production of air bubbles is observed. The aeration of these cultures serves 3 functions. The first function is to obtain thorough mixing of algal cells in the container so sufficient light is received by each cell. The second function is to supply air or carbon dioxide required for photosynthesis by these algal cells. The third function is to prevent the settling out of the cells since one species is non-motile and all species would tend to settle out inhibiting cellular division and causing eventual death due to a lack of supply of carbon dioxide.

Anti-Contamination Procedures

In working with these 3 species of algae the most important aspect is the cleanliness of all surfaces, containers and especially of the personnel's hands when handling equipment, glassware, and cultures. Personnel should not work in areas where there is raw ambient salt water prior to working in the algal section. All work with algae should be done with 1 species at a time. Neither samples from, nor inoculations of culture containers containing different species of algae should be done at the same time. In addition, personnel should wash their hands after working with one species and before starting to work with another species of algae. Transferral of tube cultures should take place where there is a minimal movement of air to reduce chances of contamination. All regular pipettes, Pasteur pipettes, rubber tubing, and glass tubing should be sterilized prior to use.

The glass 1-inch pipe which brings pasteurized filtered water to the 250 gal algal cultures from the heat exchangers should be taken apart and cleaned with Clorox once a month. The 3/4-inch CPVC ball valves under the 250 gal fiberglas algal culture tanks should be taken apart and soaked in a 20% Clorox solution after the culture tank has been emptied and before refilling with pasteurized filtered water.

The 2 cotton filled air drying tubes, which are on each 5 and 10 gal Pyrex algal culture carboy, should be changed every 2 weeks and replaced with sterile drying tubes.

Last, all 5, 10 and 250 gal algal cultures should be checked once a week under the microscope for contamination. The checking of these cultures can be combined with the counting procedure on a regular basis. The manager of the algal culture section will be better able to recognize contaminants if he or she has become familiar with the characteristics of the cells of each algal species in uncontaminated populations. Samples of the original tube cultures from the Institute should be examined microscopically under a 40 x objective for the typical shape and movement patterns of the flagellated algae. *Chlorella* sp., which is non-motile, allows for a relatively easy distinction from contaminants, be they motile flagellates or diatoms.

Culture Problems

Many of the problems occurring in algal culture operations can be attributed to a lack of attention to details of protocol or methodology. A careful record of all the cultures and things done to and with them must be kept to simplify the procedure of solving culture problems. All tube racks, bottles, and other culture containers must be labelled with date of sterilization by pasteurization and

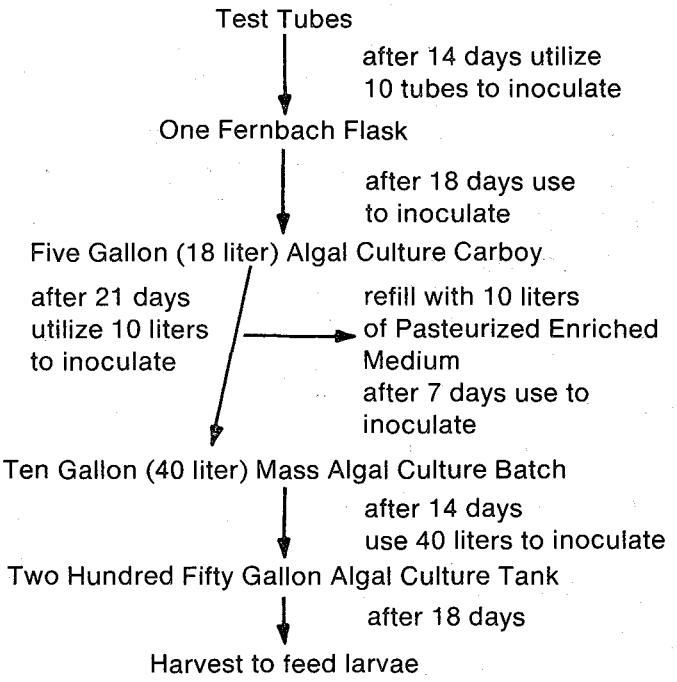
autoclaving. Meticulous records should be kept. In addition, the culture containers must be labelled as to the species inoculation date and from what culture the inoculum was obtained. To make this procedure simpler, each type of culture container should be labelled with a reference number corresponding to the log book where other pertinent information is kept on a daily basis. For example: The 5 gal culture carboys can be labelled C-1, C-2, C-3, etc; the 10 gal mass culture bottles, M-1, M-2, M-3, etc; and the 250 gal tank cultures, T-I, T-2, T-3, etc. Since each of the 3 species of microalgae has been assigned a number, i.e. Va-12 for *Pseudoisochrysis paradoxa,* Va-17 for *Pyramimonas virginica,* and Va-52 for *Chlorella* sp., the prefix consisting of the identity number for each algal species added to C-1 (e.g. 17-C-1), to M-1 (e.g. 17-M-1), or to T-1 (e.g. 17-T-1) will aid in the identification of any culture. This identification gives the ability to trace the history of each culture should problems of contamination and/or the reduction of growth occur.

It has been our experience problems with the growth of cultures occur mostly with *Pseudoisochrysis paradoxa* and*Pyramimonas virginica. Pseudoisochrysis paradoxa* normally gives trouble if the 5 gal cultures are allowed to remain in the same culture container for longer than 6 weeks, even if new enriched medium is added periodically. For this reason a 5 gal culture of each species should be terminated at the end of the sixth week and a new one started from a Fernbach flask culture. *Pyramimonas virginica* should be treated the same as *Pseudoisochrysis paradoxa.* When the color of *Pyramimonas virginica* changes from a normal light green to a yellowish color with a trace of light green, this culture should be terminated. This change indicates that the culture is too old for that particular strain. The timing of this color change will vary for no known reason. In some cases the major reason may be the presence of too much light for a particular culture, even though other cultures appear to be growing optimally. The presence of growth of algae on the sides of the container will also indicate the necessity to terminate a particular culture.

Chlorella sp. (Va-52) in the 5 gal culture carboys should be terminated after 8 weeks. As in the other species, one may note an algal growth appearing on the walls of the container. This condition indicates this particular culture is growing old. If this occurs before 8 weeks, the culture should be terminated and a new one started from an inoculum from the Fernbach flasks.

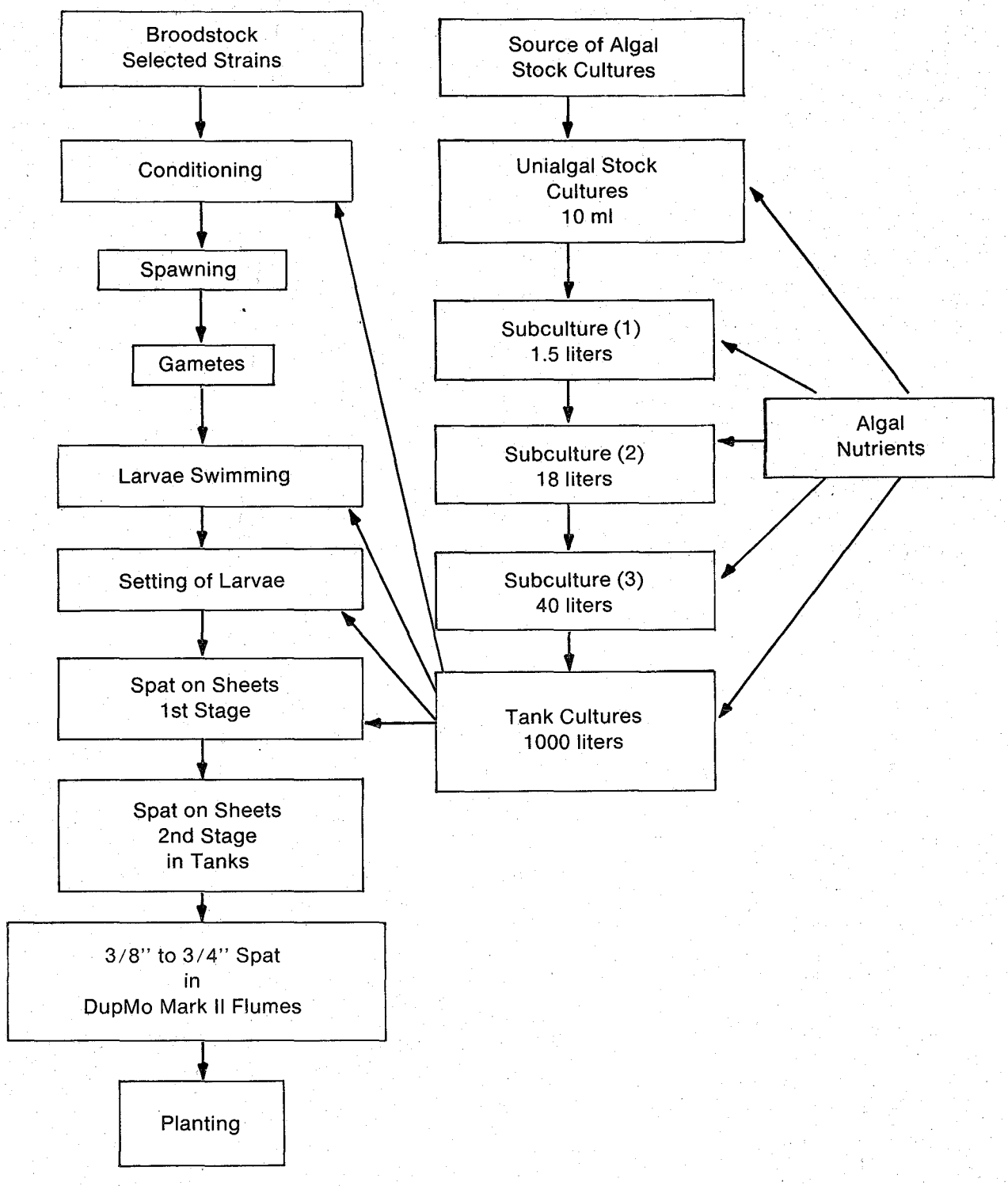
Two flow diagrams, one for the algal operation and a summary flow diagram of the total hatchery system operation are included.

Flow Diagram of Production



One must note in this flow sheet approximately six 10 gal (40 liter) mass algal culture batches can be produced before the 5 gal (18 liter) algal culture carboy must be terminated and a new one started in its place.

Summary of Hatchery System



CHAPTER VI. CULTURE FACILITIES

General Physical Facilities

The hatchery consists of two buildings, a general culture building and a holding building comprising a total of 19,750 square feet. These two buildings are designed to give the capability of producing 44 million oyster spat per year. Smaller units can be built and will be discussed at the end of this chapter. For the purpose of decreasing construction costs, one of the side walls is utilized as a common wall for both buildings. They can, however, be built separately.

The general culture building (150 ft. long by 50 ft. wide by 20 ft. high) is divided functionally into parts, one-third for holding of broodstock oysters and set oyster spat and two-thirds for culture facilities for algae and oyster larvae [Figure 32]. The second floor which houses the algal culture and estuarine water treatment facilities does not cover the holding area for broodstock oysters and set oyster spat [Figure 33].

The holding building (150 ft. long by 50 ft. wide by 20 ft. high) houses 300 flumes in modular units of 10 flumes consisting of paired flumes, 5 tiers high [Figure 34].

General Recommendations and Comments

The following types of PVC piping and valves are recommended for both buildings.

1. All major 3 inch incoming lines and fittings should be Schedule 80 PVC.
2. All 2 inch branch pipe lines can be Schedule 40 PVC.
3. All PVC ball valves should be Hayward ball valves, recommended because of the high quality and longevity of the product. Double union valves are preferable.
4. All drain lines and their fittings can be ASTM Schedule 40 PVC. This PVC product is utilized for drain systems commercially.
5. Where unions are required in low pressure pipes (drain system), "No Hub" drain unions can be used. These come in 1-, 1 1/2-, 2-, 3- and 4-inch sizes. They are relatively cheap and allow for quick "take down" of pipes for cleaning purposes.

Basic Salt Water System

The major salt water pumping system for the culture building is a flow-through system, driven by a 4 x 6, 50 HP electric Dorr-Oliver pump. Another 4 x 6, 50 HP electric Dorr-Oliver pump supplies the salt water system in the holding building. Each pump supplies 70,000 gal per hour, if the distance from the pump to the buildings is less than 200 feet. An additional 3 x 2, 15 HP Dorr-Oliver electric pump serves for each building as an emergency unit.

The intake for each 4 x 6 Dorr-Oliver pump is illustrated in Figure 35. The lengths for the 6 inch Schedule 80 polyvinychloride (PVC) intake pipe will vary according to locality. An inline ball check valve or flap valve should be installed 2 feet above the opening of the intake pipe.

The output portion of the 4 x 6 OB Dorr-Oliver pump is described in Figure 36. All pipes and fittings are Schedule 80 PVC, with glue or slip joint type fittings for valves, unions and reducers. The size of the pipe and fittings are 4-inch Schedule 80 PVC until just after the wye. At this point it is reduced to 3-inch lines running to each building (see diagram). It is important a PVC union be put every 20 feet to assure these pipes can easily be taken apart and cleaned. If at all possible, the main lines going to the buildings should have 45° elbows only instead of 90° elbows. This reduces frictional losses and the dynamic head. All pipes on the outside of the building should be adequately secured to the ground and the building. They should also be insulated to minimize the deleterious effects of ultraviolet light on PVC pipes and fittings and to eliminate freezing.

The intake piping for the 3 x 2 OB Dorr-Oliver reserve pumps is identical for the main pump, except all pipes and fittings are 3-inch Schedule 80. The output piping should be directly connected to the main-line pumping system, as indicated in Figure 36. The 2-inch output line should have a tee and a 2-inch ball valve for priming, as indicated in Figure 35 for the 4" x 6" Dorr-Oliver mainline pump.

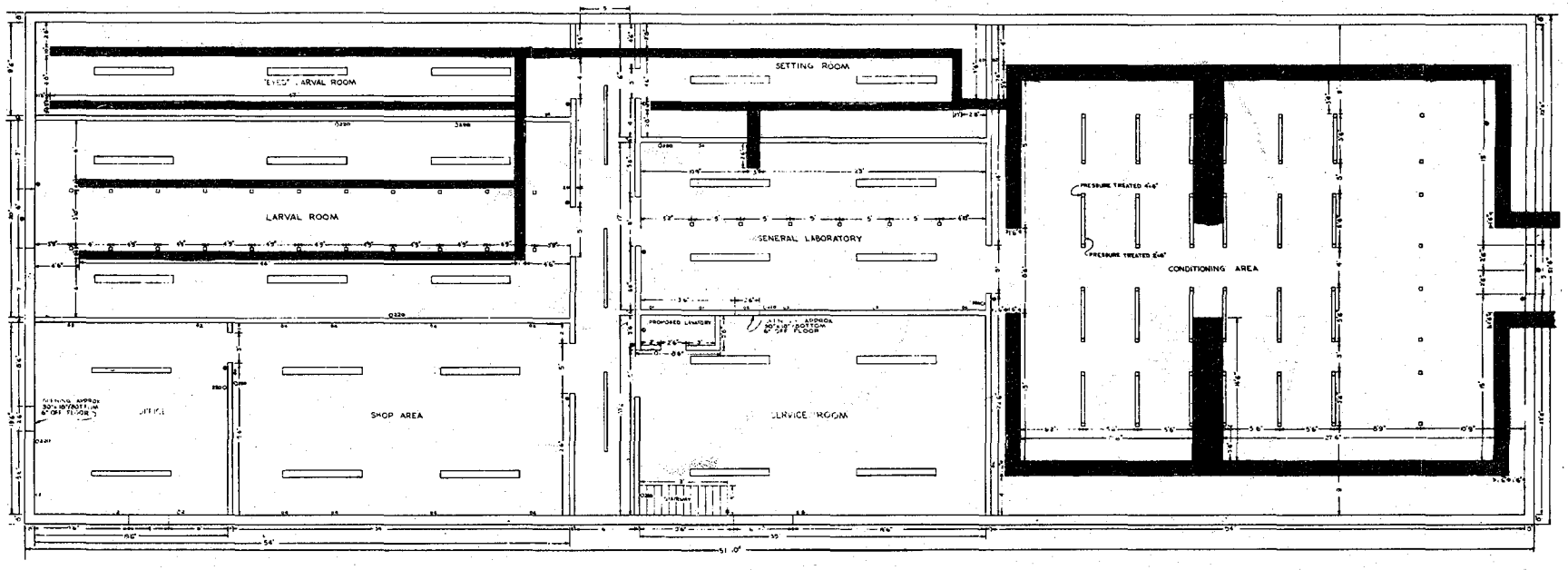


Figure 32. Plans for First Floor of Culture Building of Oyster Hatchery.

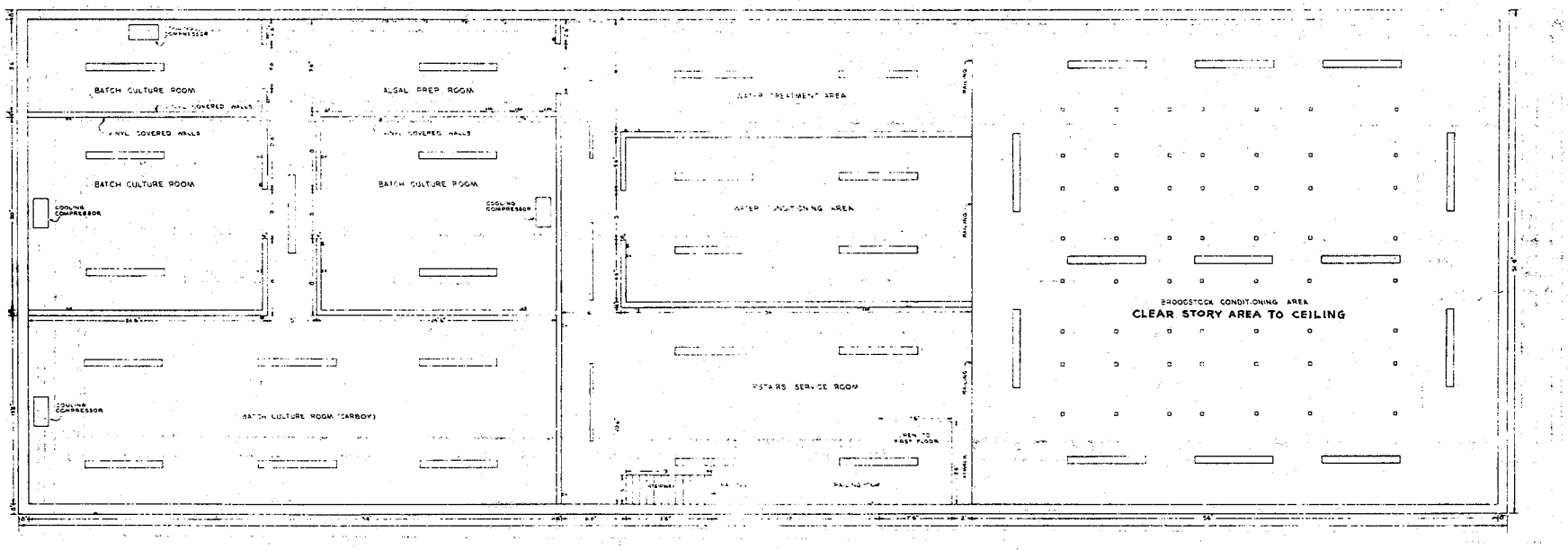


Figure 33. Plans for Second Floor of Culture Building of the Oyster Hatchery.

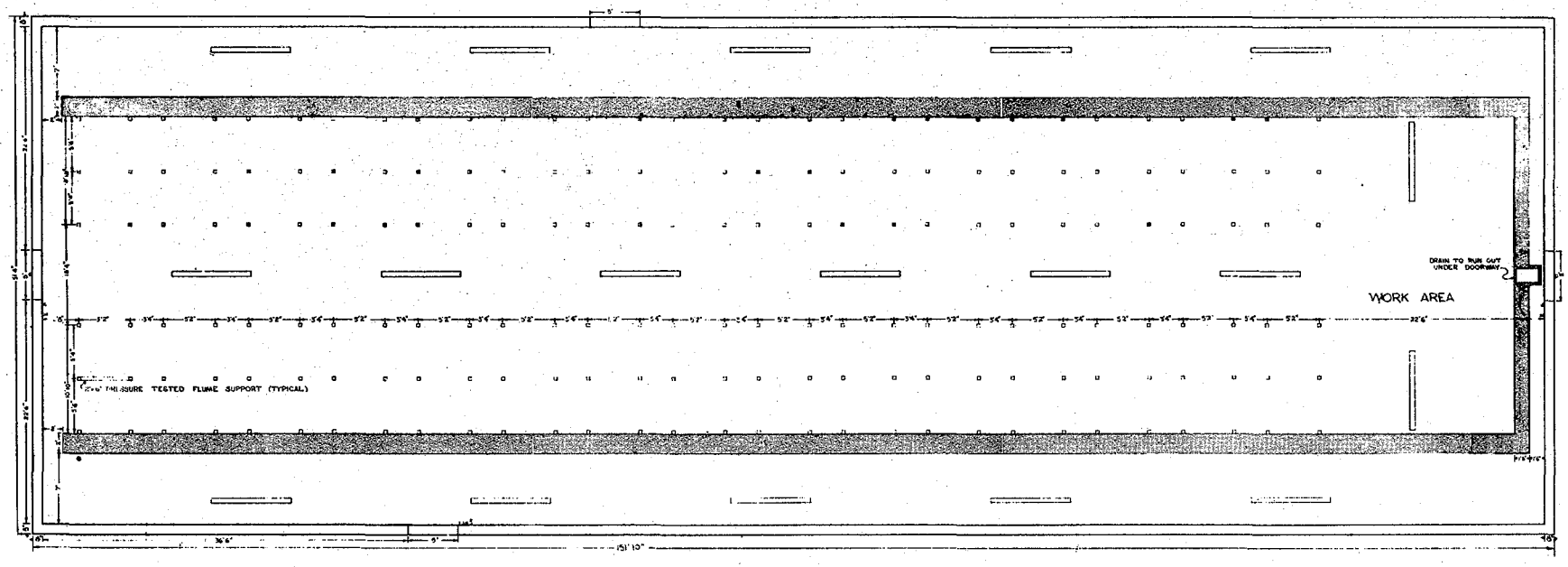


Figure 34. Plans for Holding Building of Oyster Hatchery.

The two 3-inch lines reaching the building should be run up the outside of the culture and holding buildings to avoid interference with the arrangement of the PVC pipes and valves in the building. There is little danger of freezing during the winter with the rapid flow of estuarine water in these pipes on the outside and appropriate insulation. Each 3-inch PVC line will feed half of each of the culture and holding buildings.

General Pipe System in Culture Facility

The placement of the main 3-inch lines in the building is described in Figure 37. All fittings (valves, tee, reducers, and unions) are 3-inch PVC Schedule 80. The branch lines to the flumes, spat-tanks, filtration unit, and heat exchangers are reduced to 2-inch Schedule 40 PVC. All joints are slip or glued.

The flow-through system exists into the discharge runways, as indicated in Figure 38. The pressure of the salt-water in the pipe system is regulated by a Powers Series 200 Pressure Controller, which drives a 3-inch pipe size Grinnel Air Motor (spring to close). Both the Pressure Controller and air motor are driven by compressed air. The recommended pressure in the salt-water pipe system is 12 psi.

Spat Tanks

There are 10 branches off the main salt water lines, feeding the 50 spat tanks in the culture building. Figures 39 and 40 describe the hook-up for 3 units of 5 spat tanks each.

In the hook-up for each spat tank (c.f. as described in Figure 15a), it should be noted none of the joints below the water should be glued. Not gluing the joints allows quick dismantling and cleaning of the spat tank piping system. The only joints to be glued below the 3/4 inch ball valves are indicated by an asterisk in the diagram in Figure 16.

The 2-inch Schedule 40 PVC overflow and draining pipe system for the spat tanks is designed so 5 spat tanks can be drained at one time. This reduces to 10 the number of 2-inch ball valves required for all the spat tanks in the culture building. Similarly, the overflow system is also piped in units of 5 spat tanks (c.f. Figure 15b). A hook-up of more than 5 spat tanks to a 2-inch pipe overflow system would create problems in moving 300 gal per hour per spat tank into the concrete exit trough.

The frames used to hold the Mylar sheets are described in Figure 14. A total of 650 frames are required. They are made of 1/4 inch Plexiglass and can be manufactured at the hatchery, since the cementing of Plexiglass is relatively simple. The 5/8 inch plastic nuts and bolts which secure the Mylar sheets were purchased from Playskool, Inc.

The 50 spat tanks have an holding capacity of 6.25 million spat up to 3/8 inch size. Each spat tank contains 13 frames with 2 Mylar sheets per frame, giving a total of 26 Mylar sheets. A setting of 10,000 spat per Mylar sheet therefore yields 125,000 spat per tank. The spat tank and its piping system is designed to distribute the sea water containing food to all the small oysters on the Mylar sheets. A flow rate of 300 gal per spat tank is required to assure a turbulent flow between the frames in the tank.

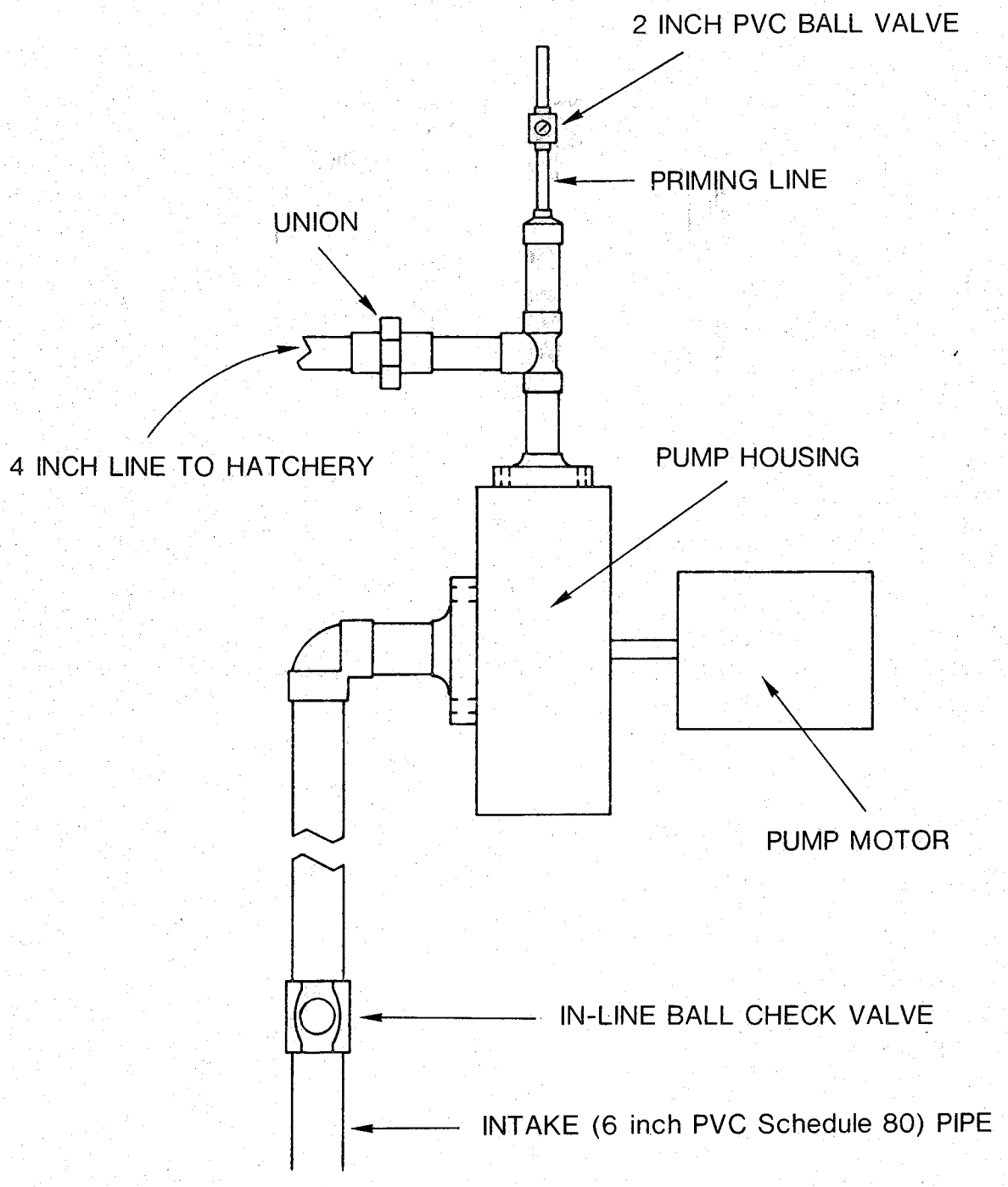


Figure 35. Intake Portion of Salt Water Pumps Side View.

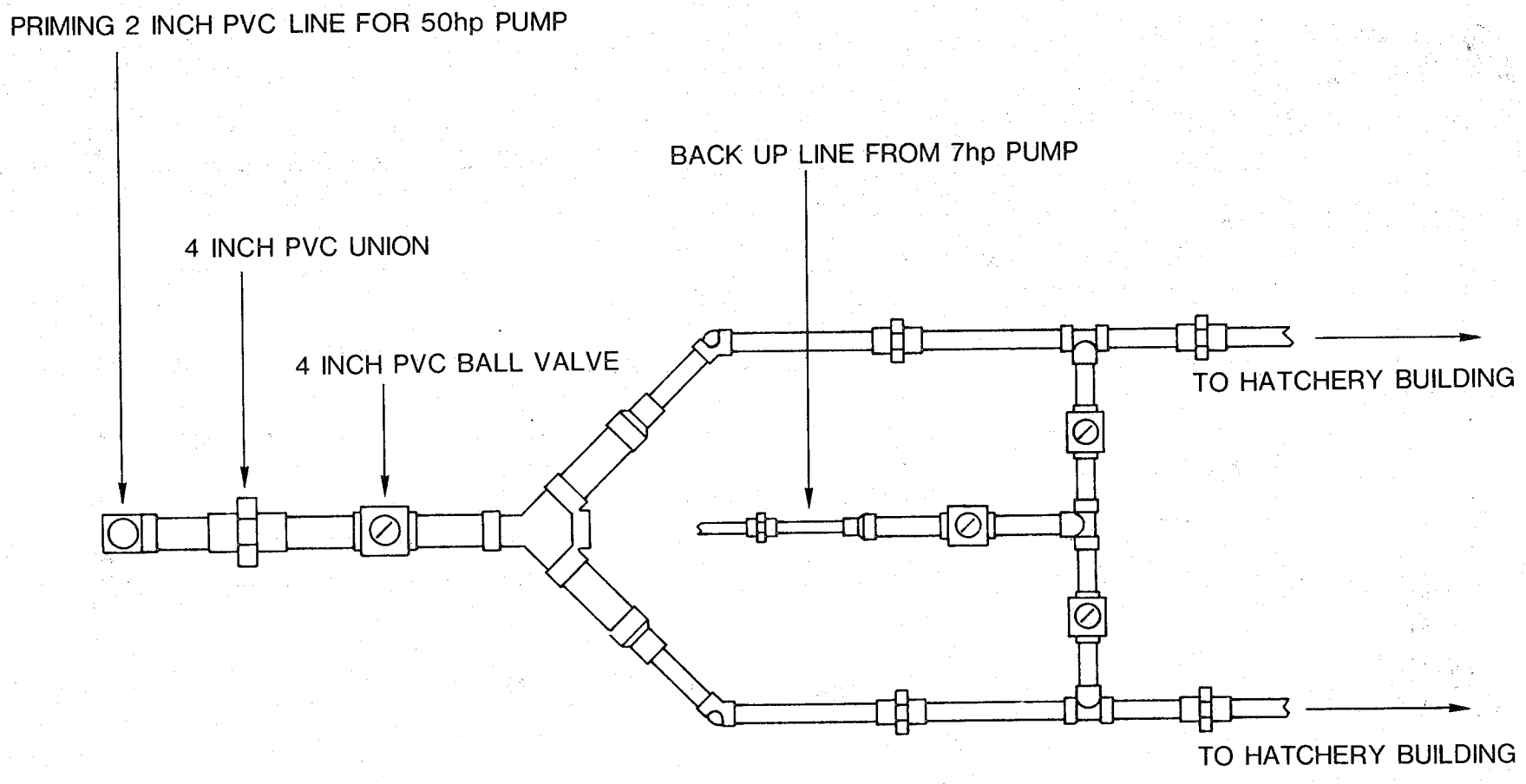


Figure 36. Top View of Output Section Leaving Pump to Culture Building.

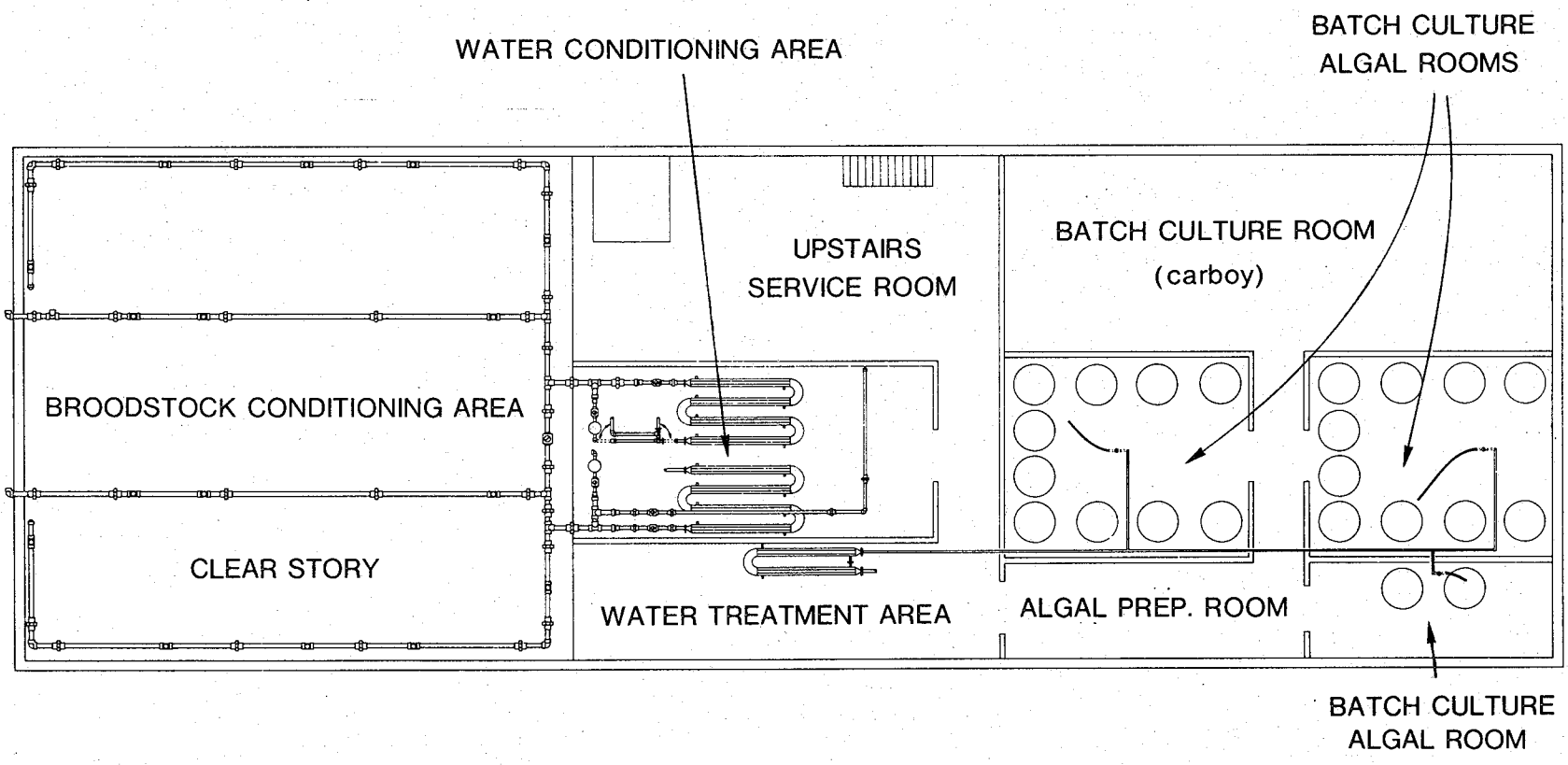


Figure 37. Schematic of General Raw Salt Water Supply to the Culture Building and of Pasteurized Filtered Salt Water to Algal Culture Tank Rooms.

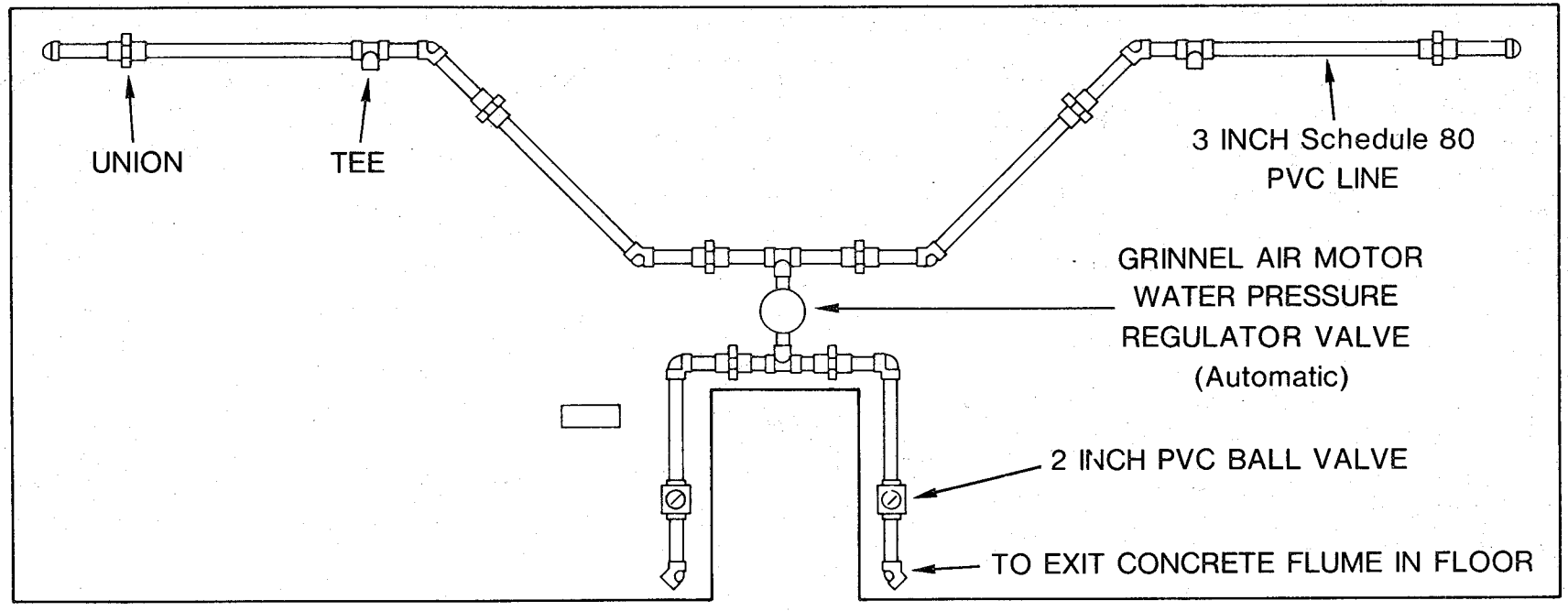


Figure 38. Schematic of Discharge End of General Salt Water System in the Culture Building of the Wall Facing the Bay in the Broodstock Conditioning Area.

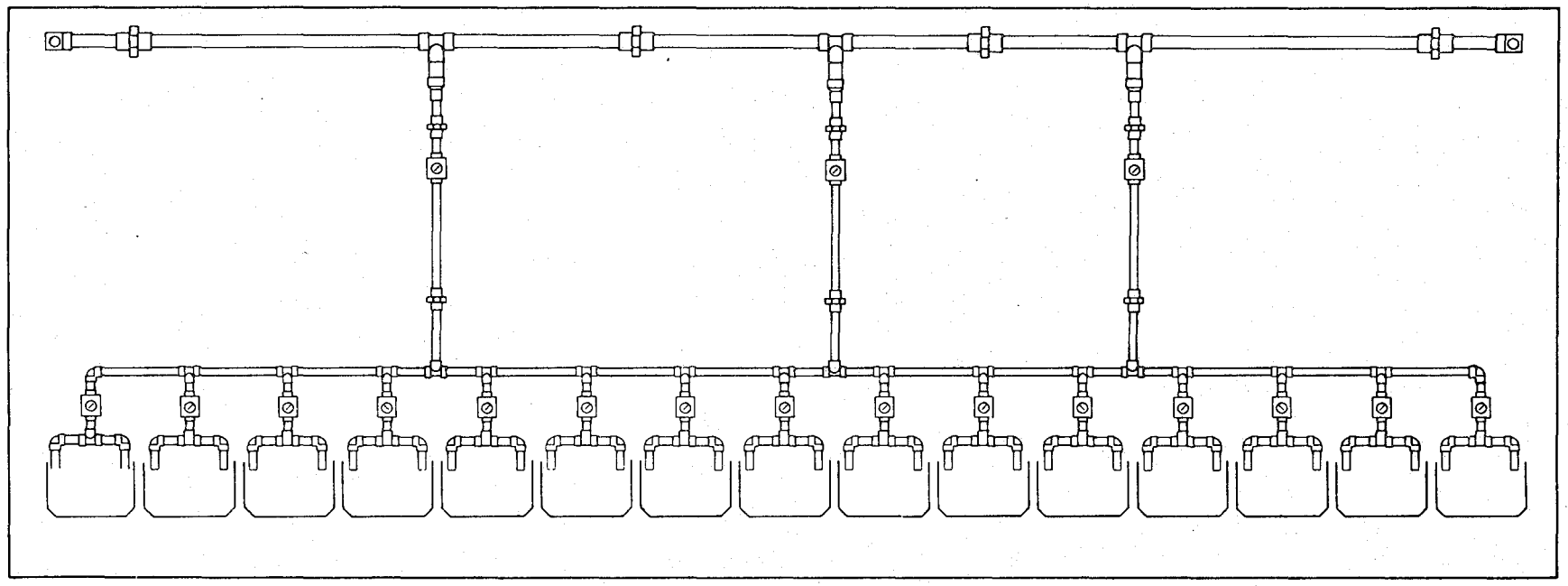


Figure 39. Schematic of Hook-Up of Spat Tanks from the Major Salt Water Lines (View of Side of Wall of Culture Building Holding Area).

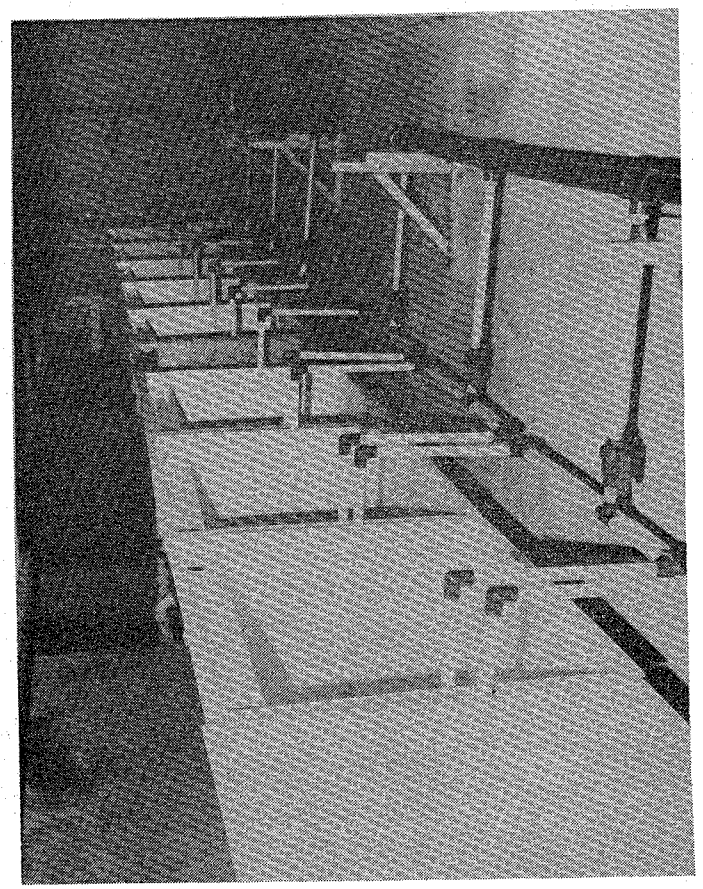


Figure 40. Arrangement of Spat Tanks in Culture Building Holding Area.

Holding Flumes

The flumes used both as conditioning flumes and seed growing flumes are arranged in units of 10 each. The flumes are arranged in pairs and are stacked 5 tiers high (Figure 41). There are 8 units, or a total of 80 flumes, in the culture building. Each 2 units of 20 flumes in the culture building has a 2-inch PVC line with a 2-inch ball valve, to control the flow of raw ambient bay water to a distribution flume. The water from this distribution flume is piped through 1-inch lines to the individual flumes. Flow control to these individual flumes is regulated by in line, 1-inch ball valves [see Figures 42 and 43].

One section of the hatchery culture building, i.e. 20 flumes, is reserved for holding broodstock oysters. Of these, 15 flumes receive sea water from the heating and cooling heat exchangers, which are temperature regulated by an automatic temperature controller.

Both the overflow pipe system and the draining and cleaning system are shown in Figure 44. The layout of these pipes is horizontal so 4 flumes on one tier can be interconnected, both for overflow and the clean out drains. This reduces the number of 2-inch ball valves and the amount of pipe required for the drain system to 1 valve per 4 flumes or 5 ball valves per 2 units.

Each flume contains 6 Nestier trays. Since each Nestier tray can hold 5,000 seed oysters 1/4 to 3/4 inch size, a total of 30 thousand seed oysters can be held per flume. There are 60 flumes containing 360 Nestier trays to give a total growing capacity in the culture building of 1,800,000 seed oysters. The broodstock holding section (20 flumes) can hold 150 broodstock oysters per flume, or a total of 1,500 oysters in 120 Nestier trays.

The flow rates to the individual flumes should be 300 gal per hour, or 24,000 gal per hour for the 80 flumes in the culture building. This flow rate is considered to be minimal to preserve a laminar flow along the upper part of the water column over the oysters, thereby assuring an adequate supply of food for the seed oysters regardless of their position in the flume.

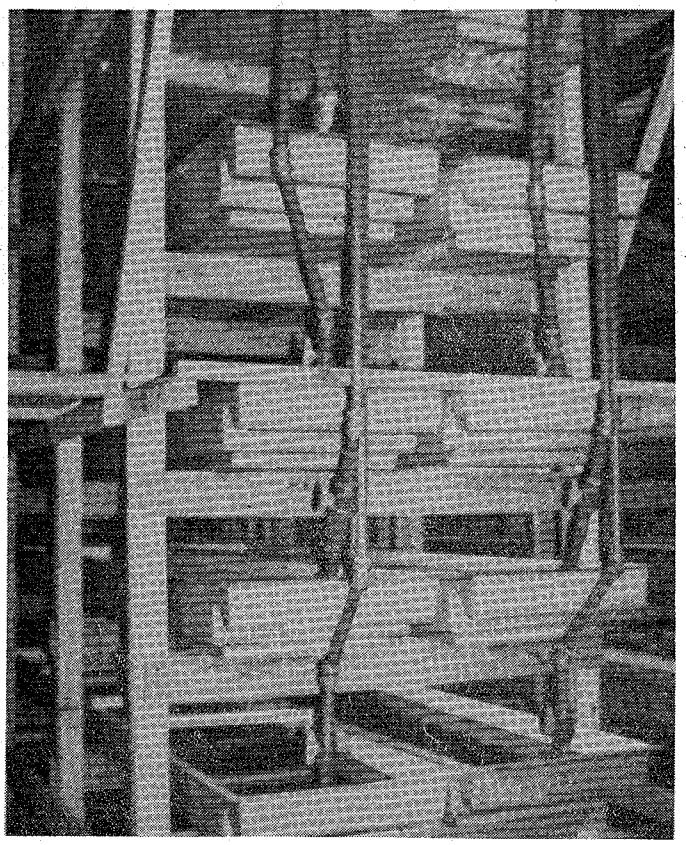


Figure 41. Arrangements of Intake End of DupMo Mark II Flumes.

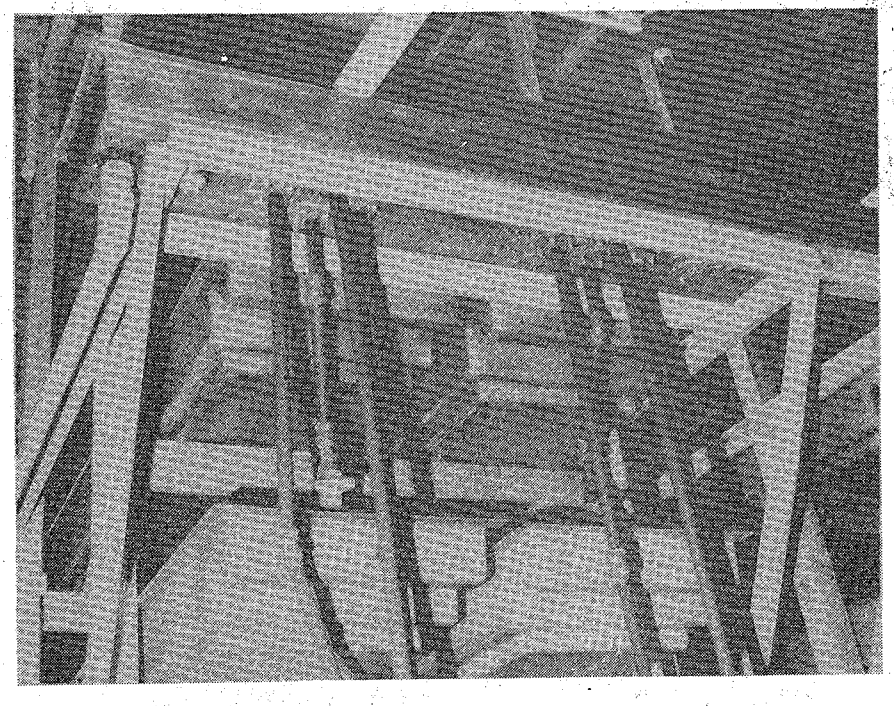


Figure 42. Distribution Flume Arrangement in Relation to DupMo Mark II Flumes.

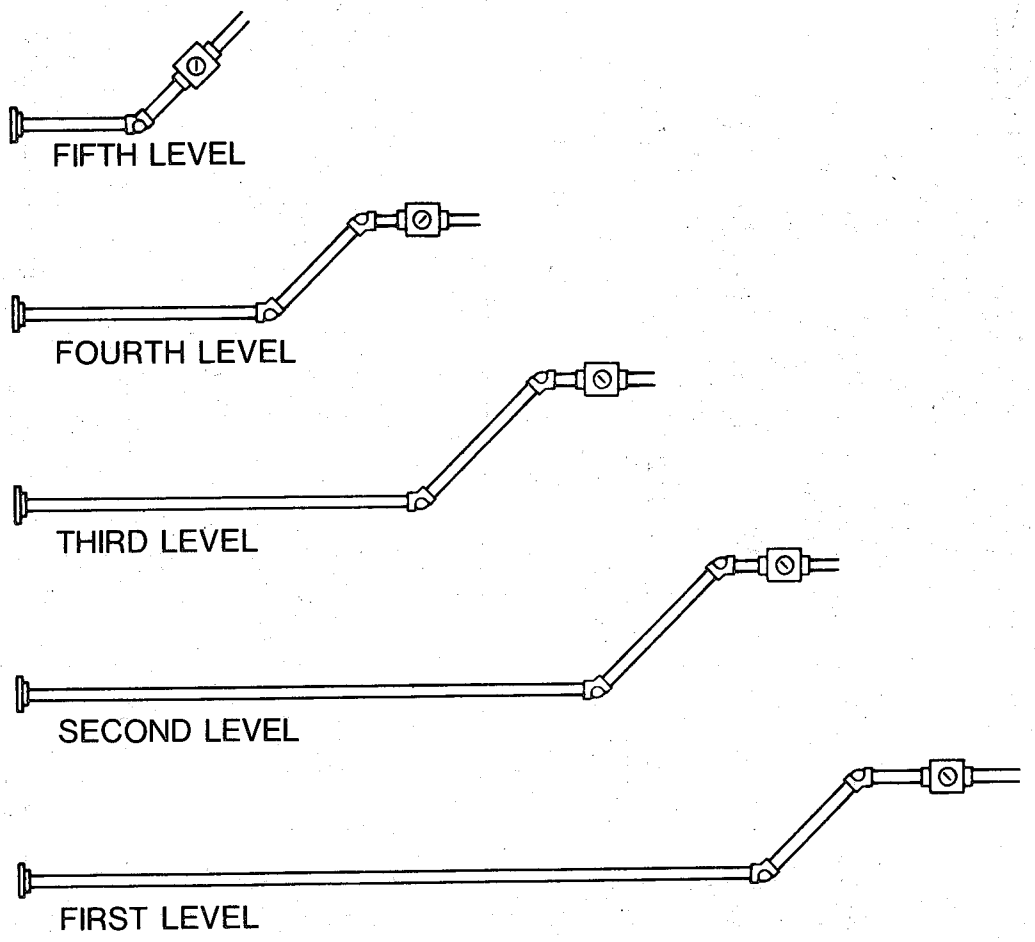


Figure 43. Arrangement of Pipes (1-inch pipes and valves) to DupMo Mark II Flumes.

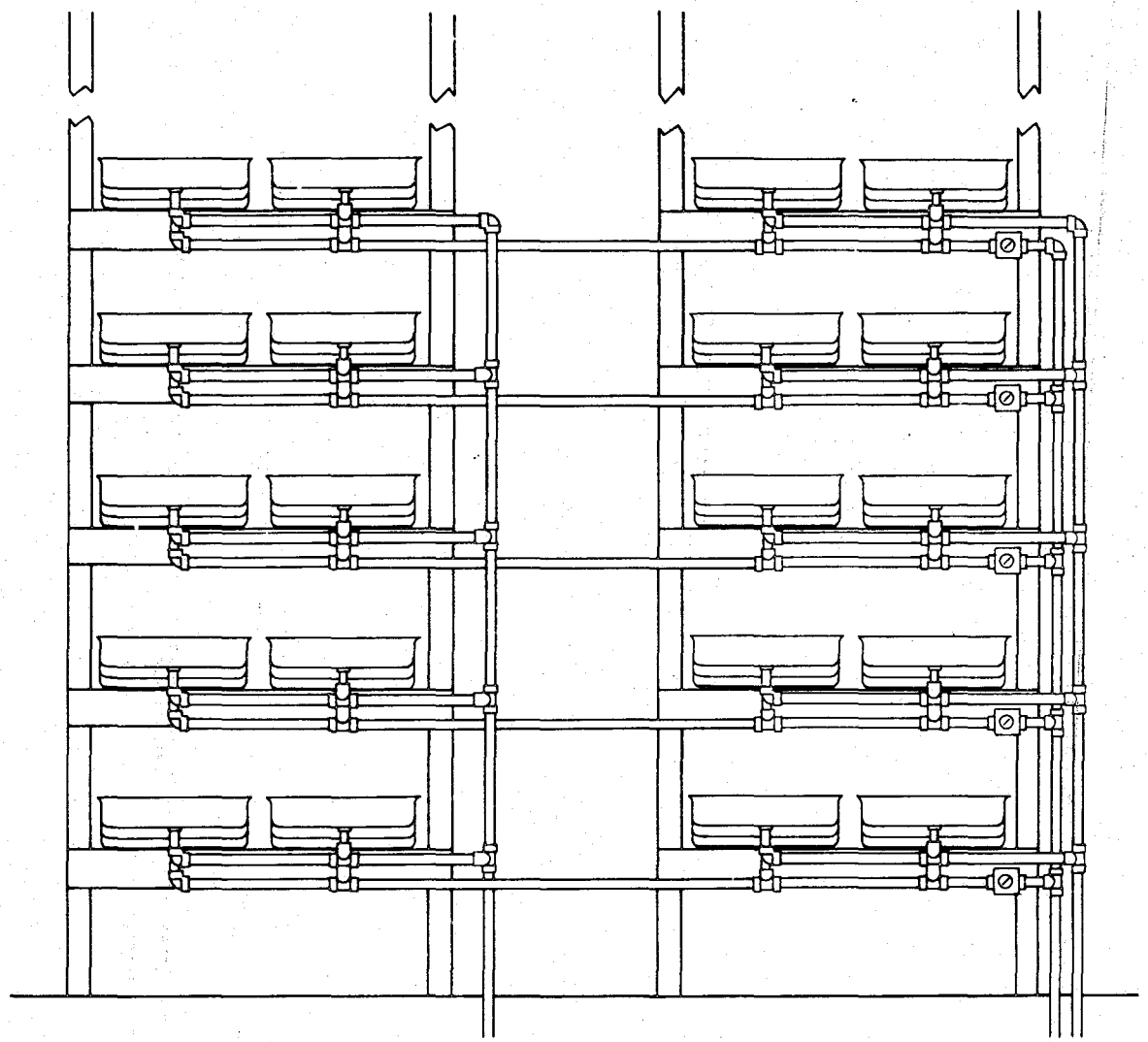


Figure 44. Overflow and Drain Pipe Systems for Two Modules of DupMo Mark II Flumes.

General Piping System for the Holding Building

As previously described, two 3-inch Schedule 80 PVC lines feed the salt water to the holding building. Figure 37 illustrates the arrangement of the major pipes (3-inch Schedule 80 PVC) to the 300 flumes in the holding building. The branch lines, as in the culture building, are 2-inch Schedule 40 PVC. The distribution system to the flumes is identical to the culture building flumes [see Figure 32]. The total number of branches in the holding building is 16 with one 2-inch line or branch for every 20 flumes. The exit flow design in the holding building, which includes an automatic pressure valve, is also identical to the culture building.

The cleanout and overflow drain system of the flumes in the holding building is identical to Figure 44 for the culture building. Again note the system is designed in units of 20 flumes.

The holding building has been designed to allow for the possible future addition of spat tanks along the side walls of this building. This addition, however, would require the installation of additional salt water pumps to feed the spat tanks.

The flumes in this building have the capacity to hold 9 million 3/8 to 3/4 inch size seed oysters. A total of 1,800 Nestier trays are required for the flumes. The flow rates to each flume should be 250 gal per hour per flume.

Water Treatment Section for the Culture Building

This salt water treatment system is the basis for the successful temperature manipulation of the water during the conditioning and spawining of the broodstock; the pasteurization of the salt water for the algal culture; and the temperature control and filtration of the salt water during the larval culture and larval setting. It is divided into a heating system for raw salt water, a cooling system for raw salt water, a water filter system for larval and algal culture, and a pasteurization system for production of the algal growing medium.

Heating System for Raw Salt Water

The heating system for raw salt water serves three basic functions. The first is to heat raw salt water so the broodstock can be conditioned during those times when the ambient salt water is below 19 C. The second function is the production of heated salt water to be fed into the filtration system for use in the larval culture and setting systems. Finally, the third function is the production of preheated raw salt water to be used in the final pasteurization of the filtered algal salt water culture medium.

The heating system is located in the service room and consists of a 1,500,000 BTU furnace which distributes hot fresh water to both the raw heat exchangers and pasteurization heat exchangers. A main 3-inch copper line feeds the fresh hot water (200 F) to a series of Bell and Gossett # 60 pumps, size 1 S KM with 1/4 HP, 1725 RPM, single phase — 4.2 amp motors. Each heat exchanger has one of these pumps feeding it hot water. All the electric pumps are controlled by individual switches. The main reason for this arrangement is the ability (1) to get maximum heat to each heat exchanger and (2) to regulate the amount of heat according to the ambient salt water temperature. This type system will also reduce the cost of operating the furnace. The furnace has a 2,000 gal oil tank buried outside the building, a supply sufficient for operations from 10 to 14 days.

The 6 heat exchangers for heating the raw salt water are Corning 135 GRB, 13 1/2 ft^2 glass shell delux heat exchangers, hung from the ceiling on the right side of the water conditioning room. They are hooked in series for the raw salt water to circulate through [see Figure 37]. It is absolutely necessary this type of heat exchanger be used for the following reasons: (1) no metal should come in contact with salt water; (2) there is a minimum of scaling on the fresh water side; (3) the utilization of 5/8 inch tubes in the Corning heat exchanger preclude clogging in areas where there are heavy silt and detritus loads; (4) any fouling can easily be observed; and, (5) the heat exchangers are easily taken apart and cleaned.

The direction of the flow of fresh water in the heat exchangers should be in the opposite direction of the salt water flow. In addition, the fresh water input into the heat exchanger should

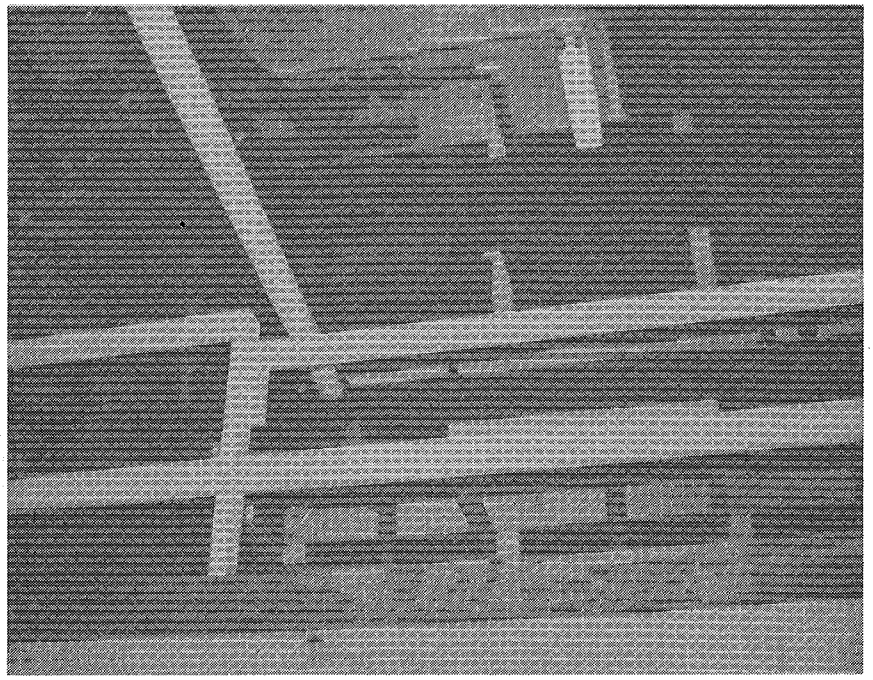


Figure 45. Degassing Flumes on Second Floor.

come in underneath the exit out the top. This allows for movement of air to the bleeding valves which are located at the highest point of the return copper lines. Furthermore, the incoming hot water should be piped to the heat exchanger on the end where the raw salt water is leaving. This provides for the maximum heat exchange in the system.

The piping of the salt water through the heating exchangers is described in Figure 37. A 2-inch Schedule 80 line feeds the heat exchangers. The flow of salt water to the heat exchangers is regulated by a 2-inch ball valve placed in the salt water line at a point just prior to its entry into the heat exchange unit. As the salt water exits, it is divided into two 2-inch lines at a tee. From the tee one line goes into a distribution flume and the other line goes to the raw water filtration holding tanks. Each of these lines have a 2-inch ball valve right after the tee. This allows a regulation of the 29 C raw salt water to either the filtration unit or to the broodstock flumes [Figure37].

The regulation of the temperature of the raw salt water which goes to the broodstock flumes is achieved by an automatic mixing valve. As previously mentioned, the raw salt water temperature exits from the heat exchangers into a distribution flume at a constant 29 C. To maintain constant temperatures to the broodstock condition flumes, a 2-inch line comes off a tee on the branch line which feeds the heat exchangers. This line goes to a Grinnel Air Motor valve (spring to open), which in turn feeds raw ambient salt water to the distribution flume where the heated raw salt water enters [see Figure 37]. This air motor valve is regulated by a Powers Series 200 Temperature Controller. A sensing bulb is installed at the exit of the cold and hot salt water lines. This controller can be set to the temperature required by the schedule of temperature for conditioning the broodstock oysters. This system will automatically maintain a constant temperature regardless of the ambient salt water temperature fluctuations.

Once the raw ambient and heated salt water has been mixed in the distribution flume, the salt water is then distributed to the 8 flumes where the heated water is degassed [Figures 37 and 45].

Since the heating of a liquid decreases the solubility of the air in solution, small micro-bubbles start to come out of solution. If these micro-bubbles are not removed before the salt water reaches the broodstock oysters, high mortalities will occur from air embolisms in the intestinal tract and in the tissues of these broodstock oysters. The aeration of this heated salt water by airstones provide large bubbles on which the micro-bubbles can coalesce in the degassing flumes. The rate of flow through these flumes is adjusted just below that rate at which the broodstock will produce feces and pseudofeces that float up in the water column in the conditioning flumes.

Cooling System for Raw Salt Water

The cooling system is used only for keeping the salt water temperature below that point where the broodstock will spawn during the summer season. In addition, it is also utilized to recycle the broodstock through winter water temperatures and to recondition these oysters to be utilized during the fall. The number of heat exchangers required is identical to the heating system.

The major difference in the two systems is a 20-ton capacity cooling compressor must be used to cool the incoming raw salt water. This will be sufficient to achieve the required temperatures described in the preceding chapters. Unlike the heating system, the cooled raw salt water can be mixed by using the same type regulator and can be delivered directly to the distribution flume which feeds the 1-inch PVC lines going to the 10 broodstock cooling flumes. The degassing system is not utilized because gas will not come out of the water when the temperature is lowered and the salt water has a greater capacity to hold gases. Figure 37 illustrates the incoming branch line to the 4 cooling heat exchangers and the exit system to the distribution flumes. Five flumes are recommended, i.e., one vertical row, be able to receive 11 C water directly from the heat exchanger and that the mixing valve be piped to the other vertical row of 5 flumes.

Salt Water Filtration System

The salt water filtration system supplies one micron filtered water for the larval culture tanks, the setting tanks, and the algal tanks. The water delivered to these systems throughout the filtration system must remain uncontaminated from any toxic products during the procedure. It is for this

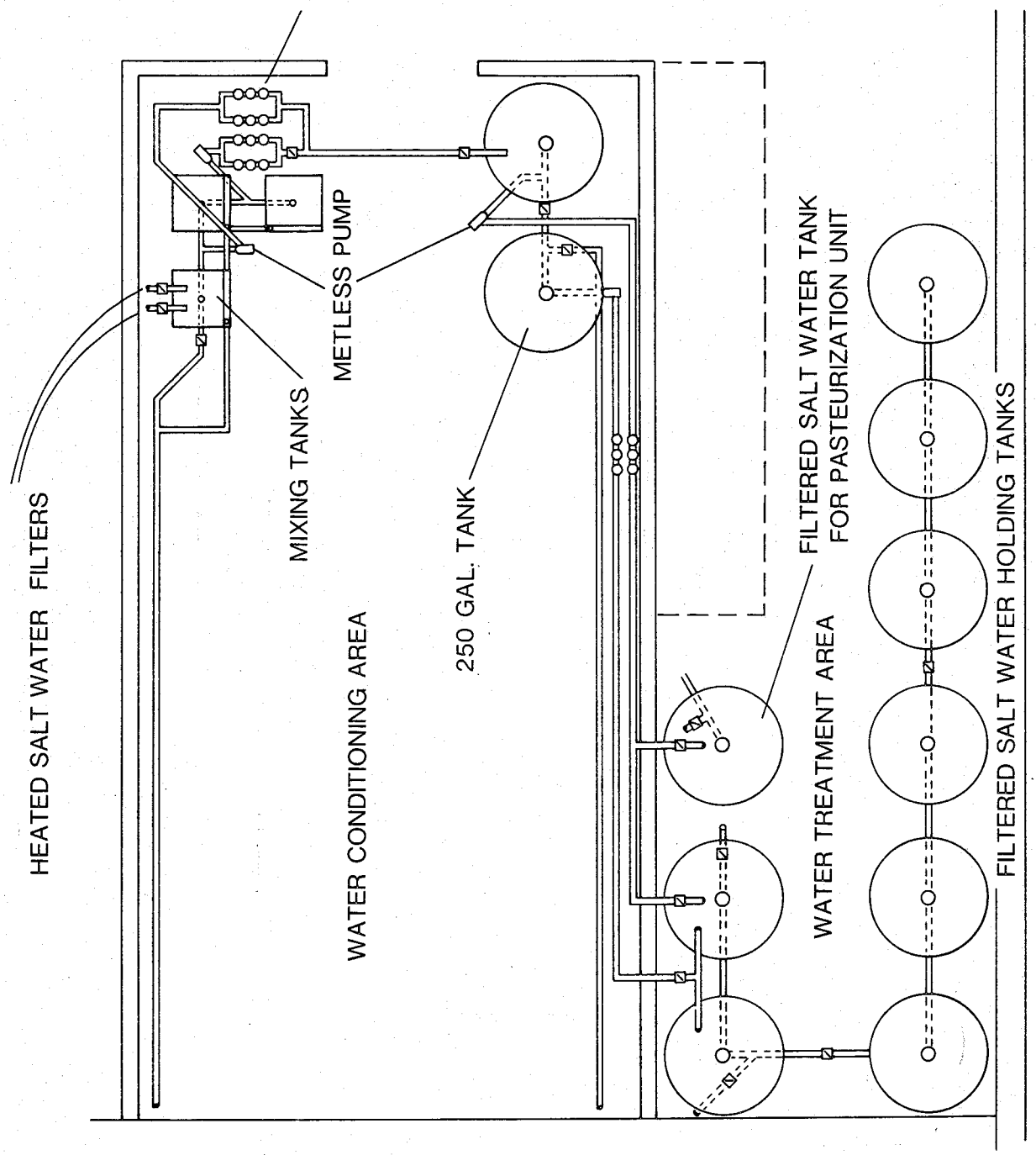


Figure 46. Water Filtration System in Treatment and Conditioning Rooms.

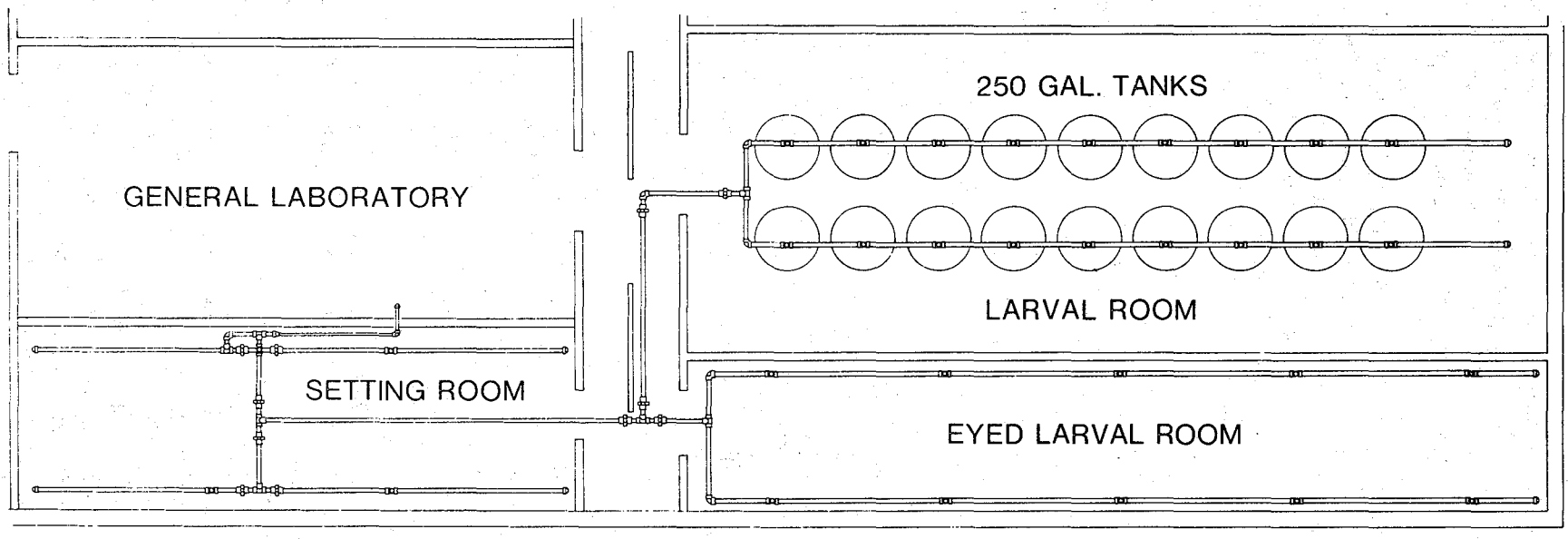


Figure 47. General Piping of Filtered Salt Water to Larval Culture Room; Setting Rooms and Spawning Table.

reason the quality composition and make of all the materials used must be known and must be used as prescribed. Substitutes are *not* acceptable unless otherwise fully tested. The various tanks used both for storing filtered water and for culture work must have a gel coat over the fiberglas resin. Once the water is filtered, the system is static and dilution of potential or actual toxicants cannot occur.

As previously described, two 2-inch lines, one from the main 3-inch raw salt water line and one from the heating heat exchanger are piped to three 250 gal (1000 liter) holding tanks in the water conditioning room. Each 2-inch line has a 2-inch ball valve, so ambient and heated water can be mixed to the desired temperature. From these 3 tanks, 3 Serfilco Metless Pumps (Model 44-581C, 3-phase, 3/4 HP) pump water to a second series of three 250 gal (1000 liter) tanks. Each pump is piped to 2 sets of filter housings (Filtrite Corporation Model — IPS) containing 10μ and 1 μ filter cartridges (Honey Comb Filter Tubes). Each set has the 2 housings in series, so the salt water passes consecutively through the 10 μ and then the 1 μ filter cartridge [Figures 37 and 46.].

The second series of three 250 gal (1000 liter) tanks are then used to supply 3 more Serfilco Metless Pumps with the same arrangement of filter housings (2 sets per pump in series). These filter housings contain only 1 μ filter cartridges. After the water passes through these filters, it is piped into a common 2-inch line which can go to the 2 pasteurization unit holding tanks or to the six 250 gal filtered water holding tanks in the water treatment room. It is recommended the filter cartridges be used until they are clogged, then thrown away. These cartridges should *never* be cleaned and reused since they cannot be completely cleaned of organic material, especially that lodged towards the center of the core. This organic material will be decomposed by bacteria which in turn produce substances toxic to oyster larvae.

Once the filtered water reaches the 6 interconnected 250 gal fiberglas holding tanks in the water treatment room, it can then be piped directly to the larval culture room, setting room, or spawning table by gravity [Figures 33 and 47].

It is highly recommended only Schedule 80 PVC pipe tee, elbows, unions, ball valves, and couplings be used for the filtration system and the distribution system carrying the filtered water. The major reason is the quality of this particular type of PVC presently used is known to be non-toxic.

Pasteurization System

The pasteurization system is composed of three 13.5 GBR Corning Glass Heat Exchangers and provides the capacity to pasteurize 250 gal in 30 min at 185 F. The 1 μ filtered water, which has been pumped to the two 250 gal pasteurization holding tanks in the water treatment area, is again pumped by a single Serfilco Metless Pump through another set of filter housings containing 1 μ filter cartridges into a 150 gal tank located over the 2 heat exchangers. This filtered water is then gravity fed to the heat exchangers [Figures 48 and 49]. Once the filtered water has been heated it can be distributed in three ways: (1) returned to the 2 holding tanks and be returned for further heating; (2) fed through glass lines to the mass algal 250 gal tanks in the tank rooms, or (3) fed to 2 glass valves to fill the sterilized 5 and 10 gal Pyrex algal carboys [Figures 37, 48 and 49]. It is important all the PVC pipes, adaptors, ball valves, unions, couplings, tees, and elbows be Schedule 80 CPVC to be able to withstand temperatures of above 104 F (40 C).

The gel-coated fiberglass tanks utilized for the pasteurization unit and for the mass algal rooms should be constructed of a special, heat resistant resin which can withstand temperatures of 185 F. These specifications must be followed, or the tanks will become soft and collapse. Each of the 2 major 250 gal mass algal culture rooms contain nine 250 gal culture tanks, each fitted with a lid that can be sealed to keep out contaminants. The fiberglass lid contains a plate glass window to transmit the light from a 4 bulb fluorescent fixture. The 1-inch drain and ball valve pipes with adaptors should also be made of Schedule 80 CPVC [Figures 25, 32 and 37]. The third mass algal room will contain two 250 gal mass algal tanks, where higher temperatures are required for the optimal growth of one of the species of algae.

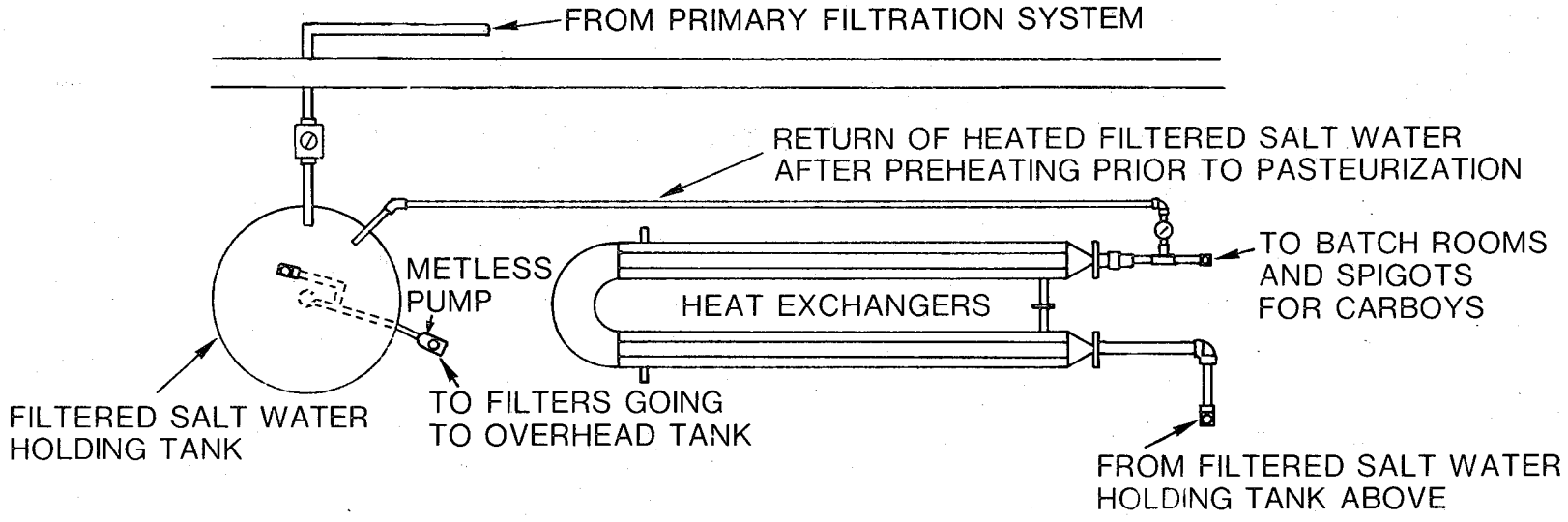


Figure 48. Overview Schematic of Algal Filtered Water Tank and Pasteurization Heat Exchangers in Water Treatment Area.

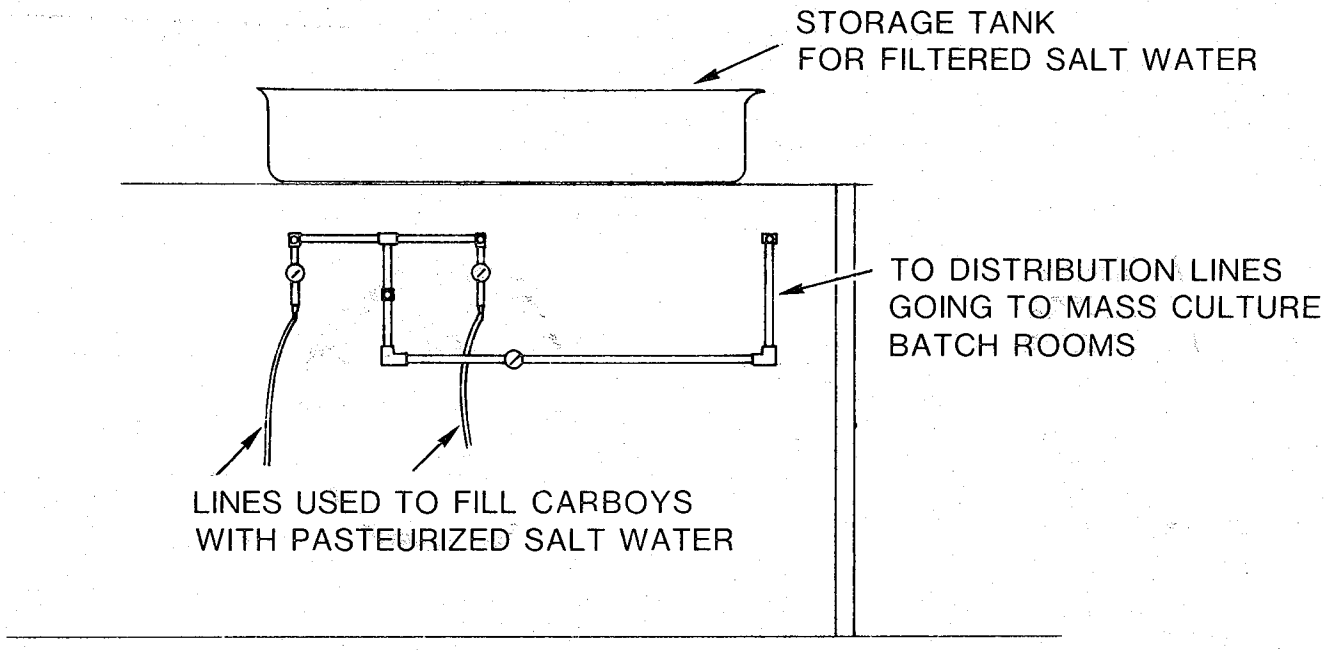


Figure 49. Frontal View of Heat Exchanger Distribution Pipes Located under Filtered Salt Water Gravity Holding Tank.

Air System

The supply of compressed air to the hatchery is produced by a 10 HP Quincy Air Compressor. Although these compressors are reliable, an emergency air compressor should also be available. The spare can be a duplicate of the Quincy Air Compressor or another type that can at least supply air to the algal bottles and tanks and the larval growing tanks. The main outgoing air line of the air compressor is fitted with an Air Milk Filter, and then a variable reducing pressure valve, which is usually set to 10 psi. Once the air line enters the building, 3/4 inch Schedule 80 PVC pipe and fittings can be used. The air system feeds the following units: the aeration of the larval tanks, setting modules, algal bottles and algal tank units, degassing flume system, and air motors for the mixing valves and pressure valves. Therefore, lines must be directed to the following areas: the larval culture room, the 2 setting rooms, the laboratory room, the 3 mass algal culture tank rooms, the mass culture bottle room, the water conditioning room, the exit side of the broodstock room, and the inland side of the holding building. These lines may be placed at the most convenient locations to reach the point localities.

General Remarks

The design and utilization of this hatchery system is intended for use at a commercial level of activity. The particular size and the number of unit modules incorporated into the operation is based on the assumption it is necessary to produce and sell at least 24 million 3/4 inch oyster seed to meet the operating costs for a one year period. Smaller units can be constructed and operated, however, there is a minimum to the size which the water treatment plant section (filtration, heating, cooling) and algal culture operation can be scaled down to and still operate with efficiency and profit for the total operation when the price of each seed oyster on the market at this period in time is so "low". It is only when the commercial venture incorporates a growout system to produce adult oysters for market that operations on a small scale and on a part time basis can be justified within reasonable cost-profit considerations. To attempt to describe within the confines of this manual all the possibilities of design and operation for various size hatchery units without the knowledge of the intention of how many oyster seed must be produced, for what purpose, and during what season of the year, is an impossible task. The system described above is not intended for use by "Mom and Pop" ventures but is a system designed to produce relatively large quantities of seed oysters so that "Mom and Pop" ventures and oysters growers will have a source of seed supply regardless of the natural cycles of oyster seed production.

CHAPTER VII. PRODUCTION AND COST AND INCOME FLOW SCHEDULE

The total production for this hatchery design with one holding building is approximately 44 million 3/4 inch seed oysters per year. The scheduling of the flow of 3/4 inch seed production is based on the following assumptions of system capability.

1. The hatchery can spawn, grow oyster larvae, and set oyster larvae on a year-round basis.
2. The hatchery can grow oyster spat on sheets in spat tanks on year-round basis.
3. The hatchery can grow free spat in the flumes during 9 months of the year (March through November).
4. The hatchery, with 50 spat tanks and 360 holding flumes, has a capacity to hold a total of 17 million oyster spat at any given time for growth to 3/4 inch seed.
5. Sales of 3/4 inch seed can be made for planting on the bottom in the estuary from September through April.
6. Sales of 3/4 inch seed for regulated grow-out systems of oysters can be made on a year-round basis.

If the hatchery sales to regulated grow-out systems are 3/8 to 1/2 inch seed oysters, then the total production becomes limited by the number of oysters sold per month. There is, however, the potential to hold an additional 12,500,000 seed oysters up to 3/8 inch with the incorporation of 100 spat tanks in the holding building. Furthermore, the growth period of these smaller seed to 3/8 inch requires only 5 weeks, so that 10 batches, or 125 million, 3/8 inch seed oysters can be produced per year in addition to the 44 million 3/4 inch oysters within the design of the hatchery described.

The total number of oysters set in a month depends on whether there are one or two spawnings per month. One spawning per month will produce 4 million seed oysters and will easily yield 44 million seed oysters per year. Previous experience from actual operations indicates at least 10 million "eyed" larvae can and will set from one batch. If a conservative estimate (50%) of the set oysters successfully metamorphose, then 5 million seed oysters will be available each month. Allowances for mortalities from set to seed size oysters for sale will then produce at least 4 million seed oysters per month, although mortalities during the growth period in the spat tanks and grow-out flumes rarely exceed 10 percent.

In summary, the hatchery, if it commences full production of 4 million in January, will have transferred 4 million seed oysters into the flumes by the end of February. This succession of 4 million set every month will then subsequently add 4 million every month to the flumes. By the end of April, 4 million oysters will be ready for sale. Each month thereafter another 4 million 3/4 inch oyster seed will be ready for sale.

During the second year a stockpiling of 8 million seed oysters in flumes from January and February will be ready the first week in March. Thereafter 4 million cultch-free oysters will be ready each month. If the hatchery goes to the sale of 3/8 inch oysters, then with 100 additional spat tanks the setting numbers can be doubled by 2 spawnings per month. The addition of a second holding building can double the 3/4 inch seed oyster production with the same basic hatchery system.

Personnel Requirements

The personnel requirements for the operation of this hatchery are a manager (chief biologist), one other biologist, and four technicians. Additional hourly labor may be required during the preparation of seed stocks for delivery. The algal section of the hatchery requires full-time services of one biologist and one half-time technician. The larval biology requires full-time services of a chief biologist (manager) and three and a half technicians. It is highly preferable the manager have sufficient experience with oyster larval culture and the other biologist have some microbiology background from college. The four technicians can easily be trained by the manager for the specific duties required by a hatchery operation.

Cost Projections and Income Flow

Before income and cost flows can be considered and described, the cost of the land, buildings, and equipment should be outlined. The cost of land as projected for two acres of water front land is currently (1976) estimated at $70,000 in the Chesapeake Bay region. The cost of the buildings, if subcontracted by the hatchery manager, is estimated presently at $80,000. This includes the cost of the major electrical wiring of the building. The cost of the plumbing has never been assessed, since it is expected hatchery personnel can install all the piping pertaining directly to the hatchery systems. The actual cost of the equipment is estimated to be about $160,000. This is based on the cost of equipment for a similar hatchery now in production with the inflation escalation factor included. The equipment costs can be separated for the housing building and culture building. They are respectively $40,000 and $120,000.

In considering the cost and income cash flows, the persons or corporate body must include the cost of borrowed money to the schedule that will be given below. This cost income flow table does not include the payment of the principle to the banks or investors.

EXAMPLE OF COST AND INCOME FLOW FOR HATCHERY

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Interest and Principle Loan 1 and Truck Loan Payment	$ 4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100	4,100
Interest Loan 2	800	800	800	800	800	800	800	800	800	800	800	800	800	800	800	800	800	800	800	800
Interest Loan 3		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	
Rent	1,800		1,800		1,800		1,800		1,800		1,800		1,800		1,800		1,800		1,800	
Insurance				1,000							3,000									
Misc. Plant Supplies, Repairs—Phone and Truck Operation	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700
Operation	4,000	4,000	4,000	3,200	3,200	3,200	3,200	3,200	3,200	3,500	4,000	4,500	4,500	4,500	4,500	3,500	3,500	3,500	3,500	3,500
Electricity																				
Fuel Oil																				
Part Time Workers																				
Salaries:																				
4 Basic	3,500	3,500	3,500	3,500	3,500	3,500	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,400	4,400	4,400
2 Add Technicians					1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Fringe 13%	500	500	500	500	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600
Subtotal	15,400	14,600	16,400	14,800	16,700	14,900	16,700	14,900	16,700	15,200	21,000	16,700	18,500	16,700	18,500	15,700	17,500	16,100	17,900	
Sales in millions	–	–	–	–	4	4	4	4	8	4	8	–	–	–	5	4	11	4	4	
Income Sales/ Less 5% down	–	–	–	–	28,500	28,500	28,500	28,500	57,000	28,500	57,000	–	–	–	36,675	28,500	78,375	28,500	28,500	
Monthly Income Less cost		-14,600	-16,400	-14,800	11,800	13,600	11,800	13,600	40,300	13,300	36,000	-16,700	-18,500	-16,700	18,125	12,800	60,875	12,400	10,600	
Cum. Net Cash before taxes and loan payment		-14,600	-31,000	-45,800	-34,000	-20,400	-8,600	+5,000	45,300	58,600	94,600	77,900	59,400	42,700	60,825	73,625	134,500	146,900	157,500	

Note:

1. Loan 1 includes payment of Principle and interest on $175,000 at 12%.
2. The Rent is for the leasing of the property and buildings that were built to hatchery specifications.

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APPENDIXES

CHEMICALS

(1) Buy only Certified or Reagent Grade

(2) Addresses of companies are as follows:

Fisher Scientific Co.
7722 Fenton St.
Silver Springs, Maryland

J. T. Baker
222 Red School Lane
Phillipsburg, New Jersey 08865

E. H. Sargent & Co.
35 Stern Ave.
Springfield, New Jersey 07081

ICN Pharmaceuticals, Inc.
Life Sciences Group
26201 Miles Rd.
Cleveland, Ohio 44128

Sigma Chemical Co.
P.O. Box 14508
St. Louis, Missouri 63178

(3) Quantities indicated are for initial start up

FIBERGLASS TANKS

DupMo Mark II Flume	12" x 24" x 12; 2" drain
Larval and Algal Holding Tanks	48" x 30", round with conical bottom, 250 gallon capacity, 2" drain, with legs
Spat Tank	29" x 36" x 27" flanged with interior trough, 2" drain

Available at: Moorman Manufacturing Incorporated
Rt. 1 Box 21-M
Hayes, Virginia 23072

(804) 642-2622

ALGAL CULTURE

Equipment Needed for Each Bottle

Article	12 gal Culture (40 liter)	5 gal Culture (18 liter)	5 gal Reserve (18 liter)
12 gal Pyrex Bottle	1	—	—
5 gal Pyrex Bottle	—	1	1
Sterilkap Stopper	1	1	1
Glass Tubing 8 mm	23" inlet, 4" outlet	20" inlet, 4" outlet	—
10 mm	23" feed, 28" withdrawal	20" feed, 24" withdrawal	24" withdrawal
14 mm	—	—	2 - 4" vents
Rubber Tubing			
5/16" x 3/32"	2 - 36" pieces for feeding & withdrawal tubes	2 - 36" pieces for feeding & withdrawal tubes	1 - 36" piece
1/4" x 1/8"	2 - 12" pieces for air inlet & outlet	2 - 12" pieces for air inlet & outlet	—
Drying Tubes	4	4	—
Pinchclamps	2	2	—
Bags to Wrap Top	1	1	1
Air Dispersion	1 fritted disc	1 dispersion tube	wrap with aluminum foil
			—

(1) Any leftover 10 mm tubing can be cut into 3" pieces for tips on the feeding and withdrawal tubes

(2) Wrap all exposed glass tubing tips with Kimwipes; Wrap all exposed rubber tubing tips with aluminum foil

(3) Adapt gas dispersion tubes (18 liter Culture Bottles) & fritted discs (40 liter Culture Bottles and Tanks) to the 8 mm air inlet tubing

EQUIPMENT REQUIRED FOR OPERATION OF ALGAL CULTURE

Equipment	Description - Size	Amount
12 gal Bottle		48
5 gal Bottle		40 Culture Bottles 30 Reserve Bottles
Steril-kap	size 12, Neoprene green (order from BBL through A. H. Thomas) BBL #60447	118
Glass Tubing	Borosilicate, Standard Wall Size 8 mm 10 mm 14 mm	10 pounds 1 case 1 pound
Rubber Tubing	5/16" x 3/32" 1/4" x 1/8"	618 feet 176 feet
Mohr Pinchcock Clamp		206
Drying Tubes	Nalgene #6201	352
Kimwipes		1 case
Drying Tower	300 mm	25
Rubber Stopper	for drying tower Size 3 8	1 pound 3 pounds
Test Tubes	Corning #9820 Size 18 x 150 mm	480
Test Tube Tops	shell vial, 4 dram Size 21 x 70 mm	480
Test Tube Racks		12
Fernbach Flasks	Corning #4420	36
Erlenmeyer Flasks	125 ml	12
Cotton	non-absorbent	1 pound rolls
Cheesecloth	grade 80 (order from any sewing or fabric center)	60 yards
Sylvania Gro-Lux Lamps	40 watt F 40 Gro	
Ken-Rad Warm White	40 watt F 40 WW	

EQUIPMENT REQUIRED FOR OPERATION OF ALGAL CULTURE

Equipment	Description - Size	Amount
Sylvania Cool White	40 watt F 40 CW	order through any electrical supply store
Sylvania Daylight	40 watt F 40 D	
Gas Dispersion Tubes	coarse fritted cylinder (Corning #39533-C)	80 (18 1 & 2 1)
	coarse fritted disc - vertical 40 mm diameter (Corning #39530-C)	70 (40 1 & tanks)
Pipette washer		1
Pipette dryer		1
A-O Brightline Hemacytometer		3
Hemacytometer Cover Slips		12
Disposable Pipettes	5" and 9"	1 case each size
Volumetric Flasks	10 ml (Corning #5640)	6
	100 ml (Corning #5640)	6
Volumetric Pipettes	(Corning #7100)	
	1 ml	6
	10 ml	6
Hand Tally		1
Microscope	Standard with mechanical stage 10X or 15X eyepieces 10X and 43-45X objectives	1
Pipettes	Mohr type for transfer	
	Size: 10 ml	1 case
	25 ml	1 case
Disposable Syringes	(30 cc) sterile	1 case
Needles	sterile	1 case
Cork Borer Set		1 complete set
Markers	Hi-Temp, green, Blaisdell	12
Bottles, Serum	resistance glass	
	Size: 100 ml	1 case
	1000 ml	30
Stopper	Sleeve type for serum bottle, size 20	1 case

EQUIPMENT REQUIRED FOR OPERATION OF ALGAL CULTURE

Equipment	Description - Size	Amount
Bunsen Burner	with regular and wing tip	1

Order the above supplies, except where noted, from any of the following:

Fisher Scientific Co.
7722 Fenton St.
Silver Springs, Maryland

Scientific Products
8855 McGaw Rd.
Columbia, Maryland 21045

General Scientific Corporation
8741 Landmark Rd.
Richmond, Virginia 23228

Arthur H. Thomas Company
Vine St. at 3rd
P. O. Box 779
Philadelphia, Pa. 19105

CHEMICALS REQUIRED FOR ALGAL CULTURE

Compound	Description	Chemical Notation	Amount	Catalog number Fisher	Catalog number J. T. Baker
Potassium Nitrate	crystal	KNO_3	10 lb	P-263	3190
Sodium Phosphate	dibasic heptahydrate	$Na_2HPO_4 \cdot 7H_2O$	1 lb	S-373	3824
Calcium Chloride	dihydrate, crystal	$CaCl_2 \cdot 2H_2O$	1 lb	C-79	1332
Magnesium Sulfate	anhydrous, powder	$MgSO_4$	1 lb	M-65	2506
Hydrochloric Acid	concentrated	HCl	1 pt	A-144	9535
Sodium Molybdate	crystal	$Na_2MoO_4 \cdot 2H_2O$	1 lb	S-336	3746
Boric Acid	powder	H_3BO_3	1 lb	A-74	0084
Manganese Chloride	crystal	$MnCl_2 \cdot 4H_2O$	1 lb	M-87	2540
Zinc Sulfate	crystal	$ZnSO_4 \cdot 7H_2O$	1 lb	Z-68	4382
Cupric Sulfate	ferric crystal	$CuSO_4 \cdot 5H_2O$	1 lb	C-493	1843
Cobalt(ous) Chloride	crystal	$CoCl_2 \cdot 6H_2O$	1 lb	C-371	1670
Acetone-technical grade (for cleaning lettering on bottles)			1 gal	A-17	9008
Hydrochloric Acid-technical grade (for cleaning inside of bottles)			1 case	A-142	9548
Ferric Phosphate	powder	$FePO_4$	1 lb	E. H. Sargeant & Co.	

VITAMINS	Description	Amount	ICN	Sigma
d-Biotin	crystalline	1 gram	101023	B-4501
Thiamine-HCl	vitamin B_1	25 grams	103028	T-4625
Vitamin B_{12} (Cyanocobolamine)	crystalline	1 gram	103271	V-2876