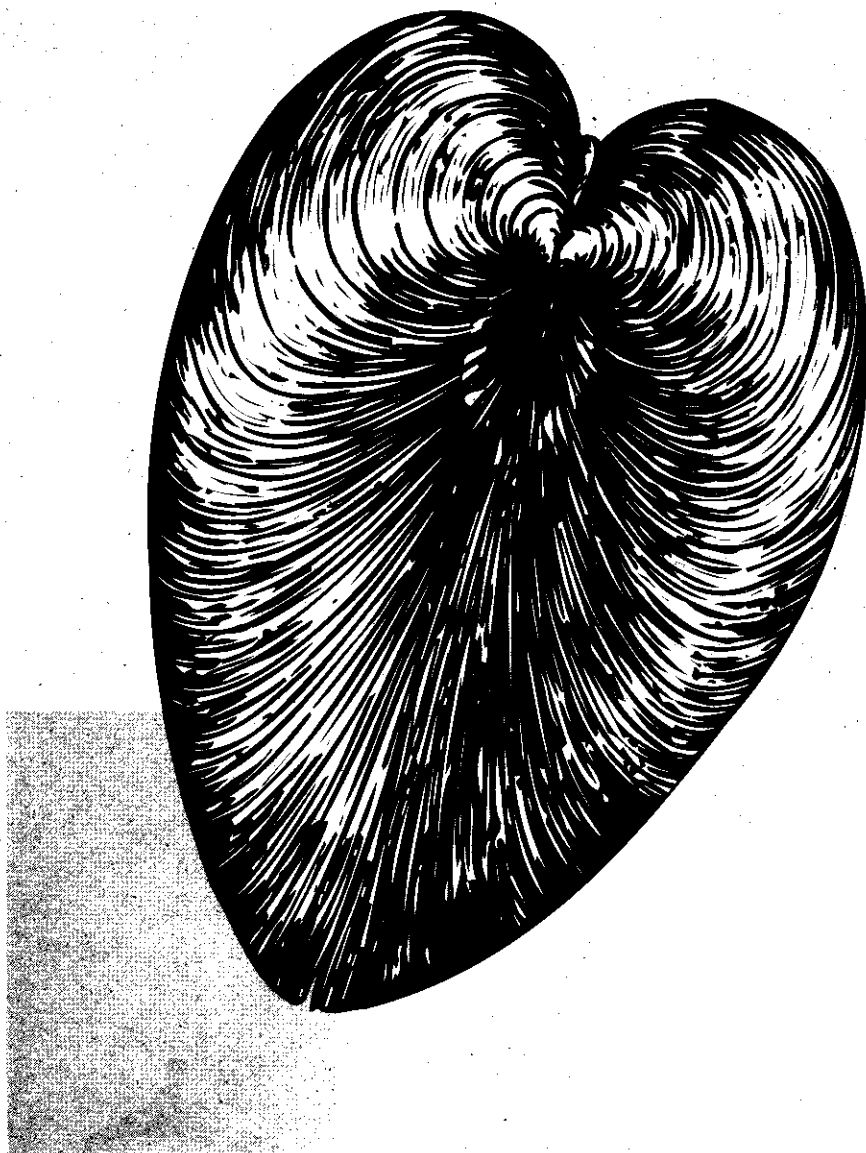
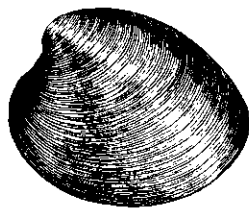


# A MANUAL FOR THE CULTURE OF THE HARD CLAM *MERCENARIA* SPP. IN SOUTH CAROLINA



by

Nancy H. Hadley, John J. Manzi, Arnold G. Eversole, Robert T. Dillon, Colden E. Battey, Nancy M. Peacock



A MANUAL FOR THE  
CULTURE OF THE HARD CLAM  
*MERCENARIA* SPP.  
IN SOUTH CAROLINA

by

Nancy H. Hadley

John J. Manzi

Arnold G. Eversole

Robert T. Dillon

Colden E. Battey

Nancy M. Peacock

---

---

# ABOUT THE AUTHORS

***Nancy H. Hadley***

S.C. Department of Natural Resources, Marine Resources Research Institute, PO Box 12559, Charleston, SC 29422-2559

***John J. Manzi, Ph.D.***

Aquaculture Technology Transfer, Inc., PO Box 62, Folly Beach, SC 29493

***Arnold G. Eversole, Ph.D.***

Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson SC 29634

***Robert T. Dillon, Ph.D.***

Department of Biology, University of Charleston, Charleston, SC 29424

***Colden E. Battey***

Atlantic LittleNeck ClamFarms, Inc., PO Box 12139, Charleston, SC 29422-2139

***Nancy M. Peacock***

Formerly with S.C. Department of Natural Resources



---

# CONTENTS

Foreword .....	vii
A note on units of measure and abbreviations .....	ix
List of Tables .....	xi
List of Figures .....	xiii
 <b>Part I. Introduction</b> .....	 1
Biology of the hard clam .....	1
Hard clam fishery and aquaculture industry .....	4
 <b>Part II. Hard clam aquaculture</b> .....	 5
Hatchery culture .....	5
Nursery culture .....	10
Field grow-out .....	18
Procuring seed clams .....	22
Shipping and storage of clams .....	25
Permits and licenses .....	27
 <b>Part III. Design and operation of a hard clam hatchery</b> .....	 31
Physical Plant .....	31
Procuring broodstock .....	36
Broodstock maintenance and conditioning .....	37
Spawning .....	40
Larval culture .....	50
Post-set culture .....	56
Algal culture .....	63
Genetic considerations for hatchery managers .....	79
Problems/Troubleshooting .....	81
Record keeping .....	86
Hatchery maintenance .....	86
Safety .....	88

---

<b><i>Part IV. Design and operation of an upwelling nursery system</i></b> . . . . .	91
System design . . . . .	91
System operation . . . . .	95
Monitoring seed stocks . . . . .	100
Problem solving . . . . .	101
Record keeping . . . . .	104
 Literature cited and further reading . . . . .	107
Glossary . . . . .	113
Appendix A. Agency/technical assistance contacts . . . . .	117
Appendix B. Seed suppliers . . . . .	121
Appendix C. Vendors and product directory . . . . .	123
Appendix D. Commonly cultured phytoplankton species and sources for starter cultures . . . . .	131
Appendix E. Units of measurement . . . . .	133

# FOREWORD

This manual is the result of 15 years of research on clam culture technology development and broodstock improvement. The research was initiated to encourage and support development of a clam culture industry in South Carolina.

This manual provides an overview of current clam culture technology and includes detailed operating manuals for a hatchery and nursery. The same level of detail has not been provided for field grow-out because grow-out technology is very site-specific and is also rapidly changing as more growers enter the field.

Funding for this work was partially provided by NOAA, Office of Sea Grant, under grant numbers NA80AA-D-00105, NA81AA-D-00093, NA83AA-D-00057, NA84AA-D-00058, NA85AA-D-SG121, NA89AA-D-SG093, NA90AA-D-SG790 and the S.C. Sea Grant Consortium. The U.S. Government is authorized to produce and distribute reprints. This work is Contribution No. 365 from the South Carolina Marine Resources Center.

January 1997.

## ***Acknowledgements***

The authors gratefully acknowledge the contributions of:

Marc Carter

H. Clawson and staff of  
Trident Seafarms, Inc.

Steve Hopkins and staff of Waddell  
Mariculture Center, Bluffton, S.C.

Staff of Atlantic LittleNeck ClamFarm

Joy Goodsell

Rich Knaub

Bill Buzzi

David McLean

S.C. Sea Grant Consortium staff

Mike Castagna, Skip Kemp, Bob Baldwin,  
David Vaughan, Dennis Hedgecock, Victor  
Burrell, Bill Anderson, Jack Whetstone,  
Steve Hopkins, Rick DeVoe and Nancy  
O'Connor reviewed the manuscript and  
offered many constructive criticisms.

Illustrations: © 1996 John Norton



Published by the S.C. Sea Grant Consortium, Charleston, S.C.. Copies of this manual may be obtained from the S.C. Sea Grant Consortium, 287 Meeting Street, Charleston, SC 29401.





---

# A NOTE ON UNITS OF MEASURE AND ABBREVIATIONS

The manual, for the most part, uses standard scientific measurements based on the metric system, decimals rather than fractions, and scientific notation for large numbers. Common US equivalents are usually given in parentheses. For those unfamiliar with the metric system, a conversion table is provided in Appendix E, which also includes a guide to using scientific notation.

We have made an exception for products which are sold in US measurements (e.g. PVC pipe and fittings) and for commercial clam categories which are legally or conventionally designated in inches. Since inches are most commonly referred to in fractions rather than decimals, we have followed this convention when using inches.

A confusion with which clam culturists and readers of the manual must live is that commercial categories are defined by shell

thickness in inches, while seed clams are more commonly described by shell length in millimeters.

The following abbreviations are used in the manual.

"	inch
C	Celsius (=centigrade)
cm	centimeter
F	Fahrenheit
L	liter
L/min	liters per minute
m	meter
m <sup>2</sup>	square meter
ml	milliliter
mm	millimeter
ppt	parts per thousand
SL	shell length
μm	micrometer (=micron)



---

# LIST OF TABLES

<b>Table 1.</b> .....	4
Characteristics of <i>Mercenaria</i> spp. which make it a suitable aquaculture candidate	
<b>Table 2.</b> .....	9
Estimated phytoplankton consumption rates of hatchery cultured clams and recommended feeding concentrations	
<b>Table 3.</b> .....	11
Length (mm), count (#/ml), and approximate age (under average culture conditions) of larval and juvenile clams retained on standard sieves	
<b>Table 4.</b> .....	37
Culture requirements for broodstock maintenance and conditioning	
<b>Table 5.</b> .....	41
Calculating daily ration for broodstock	
<b>Table 6.</b> .....	49
Production of viable gametes (%) after gamete storage of varying duration at two different temperatures	
<b>Table 7.</b> .....	52
Larval culture requirements	
<b>Table 8.</b> .....	57
Post-set culture requirements	
<b>Table 9.</b> .....	61
Stocking densities for post-set clams in 15 cm (6") or 30 cm (12") diameter silos	
<b>Table 10.</b> .....	63
Approximate size/volume relationships of post-set and juvenile clams	
<b>Table 11.</b> .....	78
Sources of algal culture problems and corrective measures	

<b>Table 12.</b> .....	79
Effect of unequal sex ratio on effective parental number ( $N_e$ )	
<b>Table 13.</b> .....	93
Calculation of intake screen surface area needed to meet entrainment restrictions	
<b>Table 14.</b> .....	97
Flow requirements and stocking densities for different size seed clams in an upwelling nursery	

---

# LIST OF FIGURES

<b>Figure 1.</b> .....	1
Commercial clam categories and how they are measured	
<b>Figure 2.</b> .....	2
Larval stages of the hard clam	
<b>Figure 3.</b> .....	8
Recirculating downwelling and upwelling units	
<b>Figure 4.</b> .....	13
Land-based upwelling nursery system	
<b>Figure 5.</b> .....	14
Floating upwelling nursery system	
<b>Figure 6.</b> .....	15
Tidal-powered upwelling nursery system	
<b>Figure 7.</b> .....	17
Soft bags and pens for field-based nursery culture	
<b>Figure 8.</b> .....	23
Clam seed prices, 1993	
<b>Figure 9.</b> .....	42
Internal anatomy of the hard clam	
<b>Figure 10.</b> .....	46
Sedgewick-Rafter chamber for counting larvae	
<b>Figure 11.</b> .....	47
Regression for determination of sperm concentration using a spectrophotometer	
<b>Figure 12.</b> .....	51
Veliger and pediveliger larvae	
<b>Figure 13.</b> .....	58
Downwelling and upwelling post-set culture cylinders	

<b>Figure 14.</b> .....	64
Sequential phytoplankton culture schematic	
<b>Figure 15.</b> .....	69
Vacuum filtration apparatus for cold sterilization of seawater	
<b>Figure 16.</b> .....	76
Hemocytometer slide for counting phytoplankton	
<b>Figure 17.</b> .....	91
Redundant seawater distribution system for a land-based nursery	
<b>Figure 18.</b> .....	94
Flow-through upwelling nursery system	

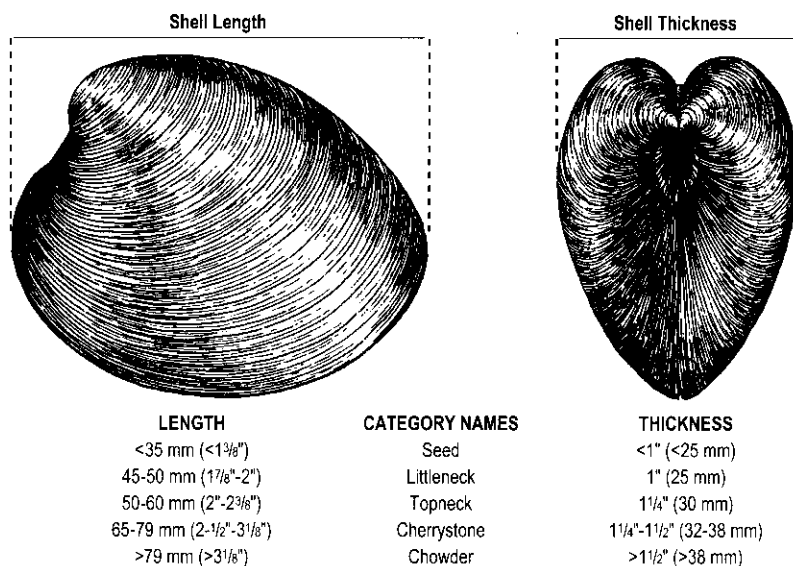
# PART I. INTRODUCTION

## BIOLOGY OF THE HARD CLAM

Hard clams of the genus *Mercenaria* are found from the Gulf of St. Lawrence to the Gulf of Mexico and have been introduced to other areas of the United States, notably the coasts of California and Washington, to Puerto Rico, and to Great Britain. There are two species, *Mercenaria mercenaria* and *M. campechiensis*. *M. mercenaria* is distributed primarily in the more northerly latitudes while *M. campechiensis* is the more southerly species. On the west coast of Florida and in Georgia, South Carolina and North Carolina, both species may be found and some hybridization between the two may occur. A subspecies *M. c. texana* is found in the western Gulf of Mexico. Most of this

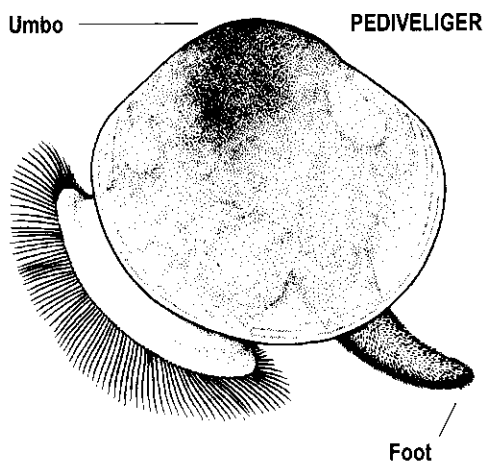
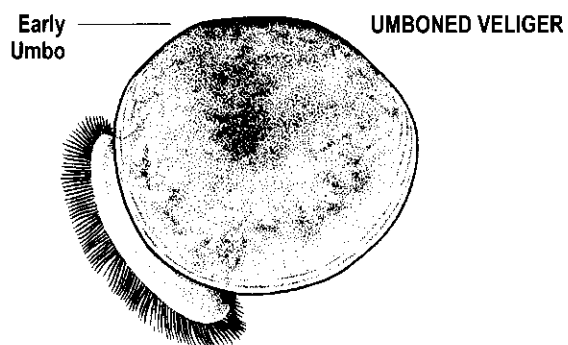
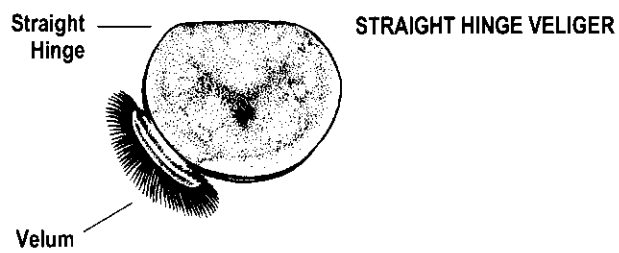
manual will be referring specifically to *Mercenaria mercenaria*, although some of the generalizations may apply to all hard clams.

The hard clam requires relatively high salinities and is rarely found where salinities average below 20 parts per thousand (ppt). Hard clams occupy intertidal as well as subtidal habitats, burrowing into the substrate to various depths (normally less than 20 cm or 8"). They are found in a variety of substrates including sand, mud, shell and mixtures of these. Clams are filter feeders, removing food particles, primarily small phytoplankters (single-celled algae), from the water. A large adult clam filters an average of 7-8 liters ( $\approx$ 2 gallons) per hour.



**Figure 1.**

*Commercial clam categories and how they are measured*



**Figure 2.**  
*Larval stages of the hard clam*



Hard clams usually reach sexual maturity at a size of about 35 mm ( $1\frac{3}{8}$ " shell length (SL) (**Fig. 1**). The sexes are separate but are externally indistinguishable. Clams are protandric, maturing as males at an early age and changing sex in subsequent years to spawn as females. When stimulated by appropriate environmental conditions (normally high water temperatures) clams release gametes (sperm or eggs) to the surrounding waters. The presence of gametes in the water stimulates other clams in the immediate vicinity to begin spawning. Fertilization occurs in the water column. In South Carolina, clams usually spawn intermittently from May through October. Clams are capable of producing prodigious quantities of young. A female clam can release several million eggs in a single spawning, but only an extremely small percentage survive the larval period to become juvenile clams.

Fertilized eggs (zygotes) undergo rapid cell division and within twelve hours develop into free-swimming trochophore larvae. Within another 12 hours, bivalve shells have formed and the larvae are in the veliger, or straight-hinge stage. Veligers are often referred to as "D" larvae because their shape resembles a capital letter "D" (**Fig. 2**). Prior to the veliger stage, the larva is sustained on lipids stored in the egg and dissolved organic matter (DOM) which is absorbed from the surrounding water. The veliger feeds on small phytoplankton, bacteria and DOM. The ciliated velum, which gives the veliger its name, is used for both locomotion and feeding. The length of the larval period is largely dependent on temperature and food supply. After 7–21

days the veliger larva develops a foot and is called a pediveliger (**Fig. 2**). This brief stage is soon followed by complete loss of the swimming organ (velum) and development of siphons. This is referred to as settlement, and newly settled clams may be called "set" or "post-set". Post-set clams assume the sedentary life-style of the adult. The term post-set is informal and usually refers to clams which have completed the larval stage but are still housed in a hatchery.

Juvenile clams, less than 35 mm ( $\approx 1\frac{3}{8}$ " SL), are called seed (**Fig. 1**). As seed grow to market size they are classified into commercial categories. These are arbitrary classifications which may vary from state to state and dealer to dealer. In most states the smallest legal size for wild-caught clams is a littleneck, usually defined as 1 inch ( $\approx 25$  mm) thick. This generally corresponds to a shell length of 45–50 mm ( $1\frac{7}{8}$ –2"). Slightly larger clams ( $1\frac{1}{4}$ " or 30 mm thick, 50–60 mm or  $2\text{--}2\frac{3}{8}$ " SL) are referred to as topnecks. Cherrystones are approximately  $1\frac{1}{4}$ – $1\frac{1}{2}$  inches (32–38 mm) thick and 65–79 mm ( $2\frac{1}{2}$ – $3\frac{1}{8}$ " long. Anything larger than that is a chowder. Littlenecks command the highest price of the commercial categories. In many states, maricultured clams may be sold at smaller sizes and some companies have created their own names for these size classes. In South Carolina, wild clams grow to littleneck size in 3–4 years, while maricultured clams may reach this size in about 2 years.

## HARD CLAM FISHERY AND AQUACULTURE INDUSTRY

Fourteen species of clams are harvested commercially in the United States, but the bulk of the landings (98% in 1991) is comprised of four species: surf clams (*Spisula solidissima*), soft-shell clams (*Mya arenaria*), ocean quahogs (*Arctica islandica*) and hard clams (*Mercenaria* spp.). In 1991, hard clams represented less than 10% of the total clam harvest, but accounted for almost 50% of the value (NMFS 1992).

Hard clams are harvested commercially in most of the Atlantic states, but a large proportion of the harvest comes from the northeast, primarily Long Island Sound. Reported landings have decreased steadily since the turn of the century. Decreased harvests may be attributed to overfishing,

habitat deterioration, and closing of shellfish grounds because of pollution. Whatever the causes, decreased landings have resulted in excess of demand over supply, creating conditions favorable for development of a clam culture industry.

The hard clam has several characteristics which make it an excellent aquaculture candidate (**Table 1**). Culture technology has been developed and private and/or public hard clam culture activities are ongoing in almost all coastal states except Alaska and Hawaii. Over the period 1980–1989, the production of hard clams from culture activities increased from 140,000 bushels to about 500,000 bushels (Adams et al. 1991). In 1989, culture activities accounted for more than 40% of the total hard clam landings in the United States (Adams et al. 1991).

**Table 1.**  
**Characteristics of *Mercenaria* spp.**  
**which make it a suitable aquaculture candidate**

### ***Economic***

High market demand  
High unit value  
Smallest size (littleneck) is also most valuable  
Good shelf life

### ***Biological***

Tolerance to wide range of environmental conditions  
High fecundity (egg production)  
External fertilization (ease of genetic manipulation)  
Spawnable year-round  
Ease of larval culture  
High survival under culture conditions  
Few diseases

---

## PART II. HARD CLAM AQUACULTURE

The culture of the hard clam can be divided into three stages: hatchery culture, nursery culture, and field grow-out. In the hatchery, broodstock clams are induced to spawn and the offspring are reared for 1–2 months to a size of about 1 mm SL. Beyond this size it becomes uneconomical to continue to rear the clams under controlled hatchery conditions. If seed clams were transferred to field culture at this point, survival would be extremely low. To overcome this problem, young clams are grown under semi-controlled conditions in a nursery for 3–6 months until they reach a size of 8–10 mm SL. Seed are then transferred to field culture. Grow-out to market size requires an additional 1.5–2.5 years in South Carolina.

A commercial clam venture may be totally integrated, including hatchery, nursery and grow-out. However, many ventures are limited to only one or two of the culture stages. There are commercial hatcheries and nurseries from which seed clams, anywhere from 300  $\mu$ m up to planting size (>8 mm), may be purchased. Thus, a commercial enterprise could purchase its young clams, bypassing either the hatchery or both the hatchery and nursery stages.

In this section we will present an overview of all aspects of clam culture, with special reference to implementation in South Carolina. There are many different methodologies and technologies in use and under development for hard clam culture,

particularly for the grow-out phase. It would be impossible to describe all of these in detail. We have concentrated on those systems which have actually been used in South Carolina and have attempted to describe basic concepts. Parts III and IV are detailed manuals for hatchery and nursery culture, based on an integrated culture system developed at the Marine Resources Research Institute in Charleston.

### HATCHERY CULTURE

A hatchery allows for the controlled production of larval clams. There are five main processes in hatchery culture of the hard clam: (1) maintenance and conditioning of broodstock; (2) spawning; (3) larval culture; (4) post-set culture; and (5) food production (algal culture). These activities are supported by a water distribution and treatment system, air distribution system, freshwater washdown facilities, supplemental lighting for algal culture, and support test equipment and instrumentation. This section provides a brief overview of hatchery processes. Details of operation are provided in Part III.

#### Maintenance and conditioning of broodstock

Adult clams are brought into the hatchery several weeks prior to spawning for “conditioning”. Conditioning is the process of inducing gametogenesis, or the

ripening of gonads, to bring the clams into spawning readiness. In South Carolina, clams become naturally ripe in the spring and remain intermittently ripe into the fall. To get them to ripen at any other time of year, one must simulate early spring conditions, i.e. cool temperature ( $\approx 18\text{--}20^{\circ}\text{C}$ ) and ample food. It generally takes 2–8 weeks for a clam to condition, but this varies depending on the time of year and the physiological condition of the clams. A clam which is already ripe can be maintained in this condition for a long period (up to 6 months) by keeping it in cool water ( $18\text{--}20^{\circ}\text{C}$ ) and providing an abundance of food. Partially ripe clams will become spawnable in 2–4 weeks under these same conditions. It is much more difficult to condition clams which have recently spawned. Therefore, for year-round operation, it may be advisable to collect naturally ripe clams in early spring and keep them in conditioning tanks in order to maintain a population of readily spawnable individuals. Naturally ripe clams may also be procured in the fall, although the portion of the wild population which is ripe at this time is lower than in the spring.

## Spawning

When clams spawn they release eggs or sperm into the water column where fertilization takes place. Spawning is induced by alternately heating and cooling the clams in a water bath. Sperm or eggs from a sacrificed clam may also be used to stimulate spawning. If controlled breeding is desired, the clams are spawned in

individual beakers, thus allowing control of fertilization. If it is only desired to produce larvae, and the parentage or breeding scheme is unimportant, it is easier to “mass spawn” the clams in a common container. Sperm from spawning males will stimulate other clams to spawn also and the eggs will be fertilized in the common spawning container.

## Larviculture

Zygotes (fertilized eggs) are maintained in clean, filtered seawater at relatively high densities (30 or more per milliliter of culture water) for 24 hours or until the veliger stage is reached. At that point the larvae are thinned to 5–10/ml and provided with food (algae). The veliger stage lasts from 7 to 21 days, the length depending largely on temperature and food quality. Over this time period the larvae grow from an initial size of  $\approx 100\text{ }\mu\text{m}$  to a size of 180–250  $\mu\text{m}$  and are gradually thinned to a final density of 1/ml. During this period, larvae are kept in static cultures, the water is changed frequently (daily if possible), and food is added daily. Some hatcheries aerate larval cultures but this is not usually necessary. Water for larval culture must be filtered (1–10  $\mu\text{m}$ ) to remove silt and native plankton. Depending on quality of the seawater, it may also need to be treated with charcoal to remove dissolved organics and sterilized (usually with ultraviolet light) to kill bacteria.

Suitable larval culture containers may be made of fiberglass or plastic, usually no more than a meter deep ( $\approx 3\text{ ft}$ ). Container

---

size is dictated by desired larval production. Plastic buckets (20 L  $\approx$  5 gal) can be used to grow small quantities of larvae ( $\approx$  20,000). Large hatcheries have fiberglass tanks which may hold several thousand liters of water and millions of larvae. Containers may be flat-bottomed or conical-shaped. Conical bottoms facilitate draining and cleaning. Tanks may have valved drains at the bottom or may be drained with siphon hoses. When the water is drained from the containers, the larvae are captured on a fine-mesh nylon or polyester screen.

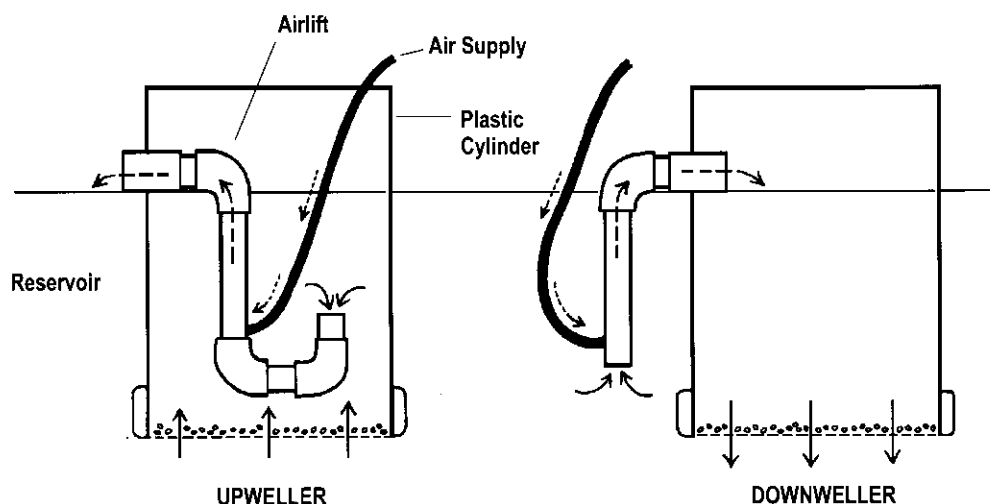
Towards the end of the larval period, usually at a size of 200–250  $\mu$ m, the larvae begin the metamorphosis to the juvenile form. The first indication of this metamorphosis is the appearance of a foot. This stage is called “pediveliger” because the larva has both a velum and a foot. The pediveliger stage is fairly short and within a few days the velum disappears entirely and siphons are formed. This metamorphosis is known as “setting” and the young clams are referred to as “set” or “post-set”.

### **Post-set culture**

Pediveligers are often removed from the larval culture system and maintained in a separate post-set culture system until metamorphosis is completed. This improves survival because pediveligers, although still capable of swimming, spend most of the time crawling on the bottom of the tanks where waste products and dead larvae are concentrated. Post-set may be cultured in a variety of systems. Many

hatcheries utilize shallow trays provided with a gentle flow of seawater, augmented with cultured algae. These trays may be stacked in tiers to save floor space. At MRRI, we maintain post-set in a recirculating culture system employing downwellers and/or upwellers. This system is easier to clean than a tray system, allows better control of water quality and feeding rations, and supports a large quantity of clams in a small space.

Downwellers (also known as “silos”) are open-ended cylinders, usually constructed of plastic pipe, suspended in a reservoir. The bottom of the cylinder is covered with a fine mesh which supports the clams. Before they can be moved to silos, the clams should be large enough to stay on a 150  $\mu$ m mesh. Smaller mesh will clog too readily, obstructing the flow of water. Water (and food) is circulated through the silos with airlifts (**Fig. 3**). In a downweller, the airlift is positioned outside of the cylinder and moves water from the reservoir into the silo. Downwellers are used for early post-set which might be sucked up the airlift. When the post-set reach a size of about 0.5 mm SL, the silos may be converted to upwellers by moving the airlift to the inside of the cylinder. Water is then pumped out of the silo, drawing water up through the clams. The silos are suspended in a reservoir (a container accommodating one or more silos). The reservoir volume should provide at least 0.5 ml water for each clam. Food is added to the reservoir either in batches or by continuous delivery. All the water in the reservoir is changed regularly,



**Figure 3.**

*Recirculating downwelling and upwelling units*

daily if possible but at least three times a week.

Post-set clams are usually maintained in a hatchery until they have reached a minimum size of 1 mm or can be retained on a 710  $\mu$ m mesh. At that point, the clams may be transferred to a nursery culture system.

### Food production

Broodstock, larvae and post-set are fed phytoplankton which is produced in the hatchery. There are three basic methods of algal production: the "Wells-Glancy" method, the "brown water method" (Ogle 1982), and the "Milford" method. The Wells-Glancy method is described in Castagna and Kraeuter (1981) and Castagna and Manzi (1989). This method and the brown water method are relatively low-tech and inexpensive, but are not as

- reliable as the Milford method for producing consistently high quality food. They are most often employed in seasonally operated, low-budget hatcheries or as a supplement to the Milford method.

The Wells-Glancy and brown water methods both rely on native phytoplankton available in the seawater supply. The water is filtered (5–15  $\mu$ m) to remove zooplankters and used immediately as culture water for larvae (the brown water method) or allowed to bloom for 24–48 hours before being used. Fertilizer may be added to encourage a denser bloom. Tanks used for this method are usually fairly shallow to allow light penetration. They should be aerated gently. This type of culture is usually performed in a greenhouse or solarium although artificial lighting may also be used.

The Milford method of algal culture is a sequential process in which single species of phytoplankters are grown in batch cultures. Phytoplankters for culture can be obtained by isolating native species, but more commonly are purchased from a laboratory specializing in isolation and production of phytoplankton clones, or from another hatchery (see Appendix D). Most hatcheries grow two or more different species of phytoplankters in order to provide the varied rations required by the larvae, post-set and broodstock. A detailed description of the Milford method is presented in Part III of this manual.

The quantity of phytoplankton needed is dependent on the seed production goals of the hatchery. Algal consumption rates for different size clams is presented in **Table 2**. Fifty broodstock (a minimum for a spawning) will require approximately  $1.5 \times 10^{11}$  algal cells a day, which will be 30–50 liters of dense algal culture ( $\sim 5 \times 10^6$  cells/ml). One million veliger larvae will consume  $1 \times 10^9$  cells/day (1 liter), setting size larvae 50 times as much. A million post-set will require 3–4 times as much algae as the broodstock (200 liters/day). Algal culture is probably the most time-consuming element of the hatchery but is essential to its success.

**Table 2.**  
**Estimated phytoplankton consumption rates of hatchery cultured clams and recommended feeding concentrations**

Life Stage	Age or Size	Consumption range* (algal cells/clam/day)	Food concentration (algal cells/ml of culture water)	
			Minimum	Maximum
Early veliger	1-2 days	1,000-5,000	10,000-25,000	50,000-75,000
	3-5 days	5,000-10,000		
Mid veliger	5-8 days	10,000-15,000	25,000-50,000	75,000-100,000
Late veliger	8-14 days	15,000-30,000		
Pediveliger	14-21 days	30,000-50,000		
Early post-set	300-400 $\mu$ m	50,000-75,000	50,000-100,000	150,000-200,000
Mid post-set	400-600 $\mu$ m	75,000-150,000		
Late post-set	600-1000 $\mu$ m	150,000-500,000		
Broodstock	>35 mm	$1 \times 10^9$ - $3 \times 10^9$	50,000-100,000	150,000-200,000

\* Based on feeding *Isochrysis galbana*

## NURSERY CULTURE

When post-set reach a size of  $\approx 1$  mm SL (retained on a 710  $\mu$ m screen) they may be transferred to a nursery system. The purpose of a nursery is to provide a protected environment for small seed until they reach a size (8–10 mm) suitable for field grow-out. Studies have shown that survival in field grow-out increases with seed size (Manzi et al. 1980; Kraeuter and Castagna 1985). However, it is difficult to maintain clams in a nursery beyond a size of 10 mm because of space and water flow limitations. Some growers plant their seed at smaller sizes (6–7 mm) but survival will be variable and generally much lower than for larger seed (Kraeuter and Castagna 1989).

There are many types of nursery systems in use. These can be roughly divided into land-based and field-based systems. Land-based systems in common use are raceways and upwelling systems. Field-based systems include a variety of on-bottom and off-bottom containers, floating rafts, and floating upwellers. Also included under field-based systems are nurseries located in protected areas such as impoundments and ponds.

Field-based systems are relatively inexpensive to construct and operate but have high maintenance requirements, offer limited predator protection, may be subject to environmental damage, and have unreliable production. Land-based systems are more expensive to construct and operate but provide almost complete predator control, ease of access, and near-optimal

conditions for growth and survival of seed clams. In this section we will briefly describe a variety of nursery systems which may be suitable for use in South Carolina and general concepts of nursery culture. Part IV of this manual is a detailed description of a land-based upwelling nursery.

### Land-based systems

A land-based nursery provides a semi-controlled environment for culture of juvenile clams. Water is usually not treated except for gross ( $\sim 200$   $\mu$ m) filtration to reduce the influx of fouling organisms (e.g. barnacles and oysters) and the natural food supply is unsupplemented. However, predators are excluded, fouling organisms are controlled, and conditions for rapid growth are provided. Clams can be introduced into a nursery at a size of 1 mm or larger and are usually grown to a size of at least 8 mm before transfer to field grow-out. During the rapid growing season (in South Carolina, March through October minus a few weeks in July-August), clams will grow approximately 2 mm per month. Therefore, it will take 3–4 months (at least) for a 1 mm clam to reach 8 mm, and another month for each 2 mm above that size (see Table 3).

There are two basic types of onshore or land-based nursery systems, raceways and upwelling systems. Both are energy-intensive, requiring continuous pumping of large volumes of high quality estuarine water. Both require considerable capital



investment for waterfront property and infrastructure. Both are labor-intensive requiring daily cleaning and frequent monitoring of the seed. Despite all these negatives, onshore nurseries are popular because they provide conditions for rapid growth with high seed survival.

### ***Raceway systems***

Raceways are shallow rectangular trays which may be stacked in tiers. Water is introduced at one end, flows over the seed clams, and exits at the other end through a

drain. Ten to 20 liters of water per minute (L/min) should be supplied for each liter of large seed clams (8 mm). Although raceways produce rapid growth and high survival (Hadley and Manzi 1984), they are not well-suited to South Carolina because of the high load of fine silt present in our estuarine waters. This silt settles in the raceways, creating labor-intensive maintenance problems. If not cleaned daily, small seed clams suffocate in this fine silt. Cleaning is particularly difficult if the seed are very small (<4 mm). Another drawback with raceways is that clams near

**Table 3.**  
**Length(mm), count(#/ml), and approximate age (under average culture conditions) of larval and juvenile clams retained on standard sieves**

Stage	US Standard Sieve Size	Mesh Opening	Length (mm)	Count (# clams/ml)	Age (days)
Veliger	—	55 $\mu$ m	0.1	—	1
Pediveliger	No. 100	150 $\mu$ m	0.2	—	14-21
Post-set	No. 70	212 $\mu$ m	0.3	99,000	18-24
	No. 45	335 $\mu$ m	0.5	21,000	30-45
Seed	No. 25	710 $\mu$ m	1	3,000	60
	No. 14	1.4 mm	2	300	80
	No. 10	2.0 mm	3	100	100
	No. 7	2.8 mm	4	40	120
	No. 6	3.35 mm	5	20	135
	No. 5	4.0 mm	6	12	150
	No. 4	4.75 mm	7-8	7	165
	1/4"	6.3 mm	9-10	3	195
	0.265"	6.7 mm	10-11	2	210
	5/16"	8.0 mm	12-13	1.5	240
	3/8"	9.5 mm	14-15	1	270-290

the drain end receive only water which has already passed over all the other seed and is largely devoid of useable food. Thus the seed must be rotated in the raceway to achieve uniform growth. Finally, raceways cannot support high biomasses of seed. A typical raceway can support about 0.2 L of large seed per 0.1 m<sup>2</sup> ( $\approx 1$  ft<sup>2</sup>). This is about 1400 seed at 8 mm SL or 700 at 10–12 mm SL. Raceways probably should not be used for seed smaller than 4 mm SL because of the cleaning difficulties. Seed require approximately 2 months to grow from 4 to 8 mm in raceways.

### ***Upwelling systems***

Upwelling systems are probably the most widely-used land-based nursery systems. Although they share the drawbacks of high capital cost and energy expense, upwellers provide rapid uniform growth of seed, are easier to maintain than raceways, and make more efficient use of space. An upwelling system consists of a reservoir, usually rectangular and about 2 feet deep, containing multiple culture units (**Fig. 4**). Each culture unit ("silo") has a screen bottom which supports the seed clams. The silos are placed so that their sides project above the water level and the bottom screen is several centimeters above the reservoir bottom. A drainpipe near the top of the silo extends through the silo wall and the reservoir wall, emptying into an external common drain. Water is pumped into the reservoir and rises up through the screens and the seed clams, exiting out the drains near the top. The vertical water flow supports much higher biomasses of clams

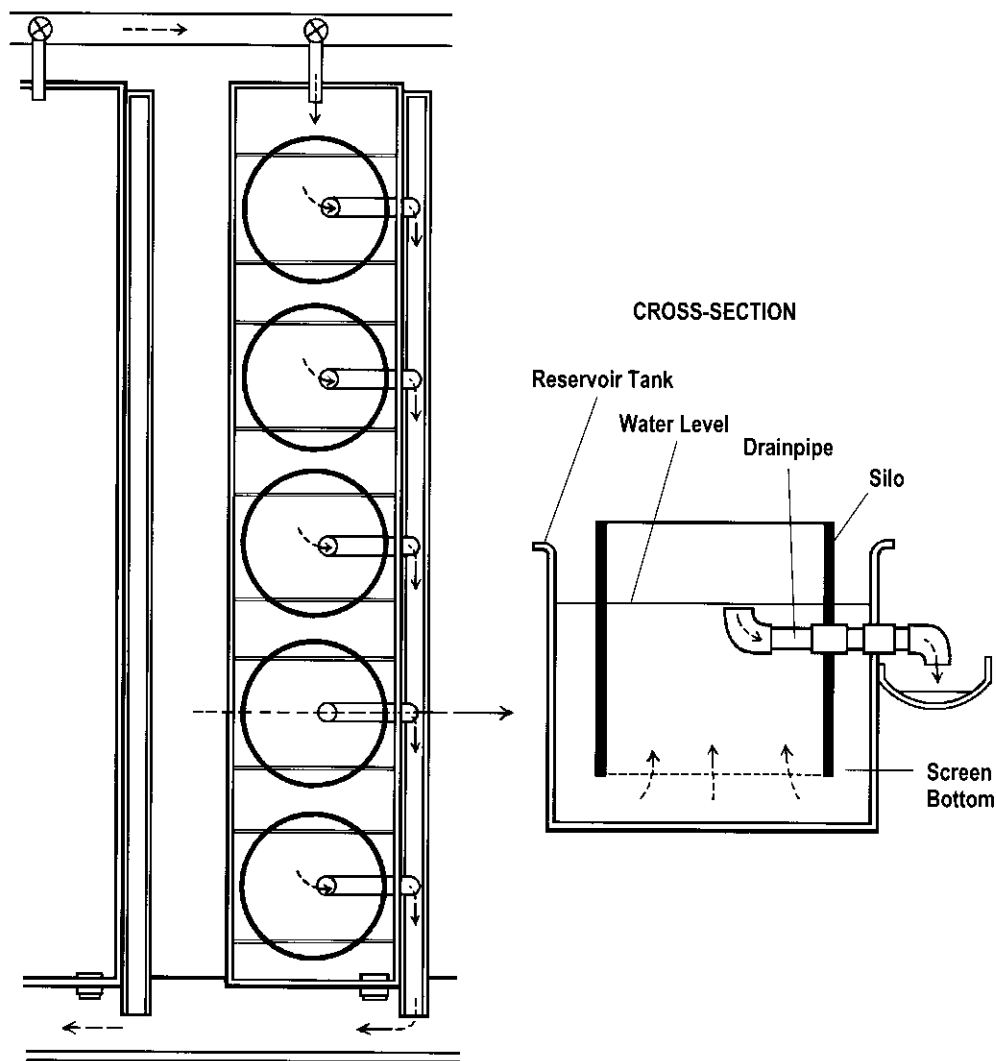
than the horizontal raceway flow. Each 0.1 m<sup>2</sup> of upweller screen can support 1.0–1.5 liters of planting size seed ( $\sim 7000$ – $10,000$  seed at 8 mm SL). The water flow requirements for upwellers are similar to those for raceways. Each liter of large (7 mm) seed requires 10–20 L/min flow and very small seed ( $<3$  mm) should receive 10 times that much. Details of operating an upwelling nursery are given in Part IV.

### **Field-based systems**

Field-based nurseries are not as reliable as land-based systems, but require less capital investment and are less expensive to operate. They do not provide as complete predator protection and are more susceptible to environmental damage (e.g. from storms). They are less accessible and consequently more difficult to maintain, but the maintenance is done less frequently. The water flow (and thus food supply) to the seed may be variable resulting in less uniform growth. However, some of the recently developed floating systems rival land-based nurseries in production capability and are relatively inexpensive to build and operate.

### ***Floating upwelling systems***

Three floating upwelling systems have been used to some extent in South Carolina. Two of these are powered by airlifts, and one is tidal-powered. Operation and maintenance is similar to that for a land-based system. Floating upwellers have been used with very small seed ( $\sim 1$  mm), but success is dependent on proper site selection.

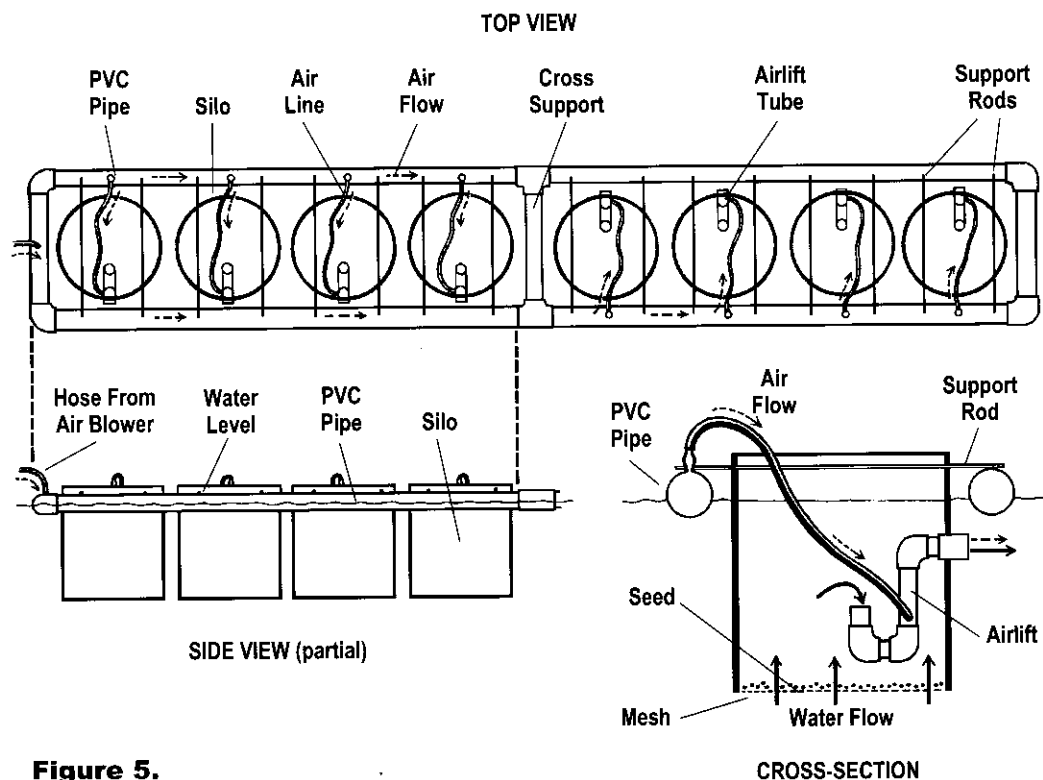


**Figure 4.**  
*Land-based upwelling nursery system*

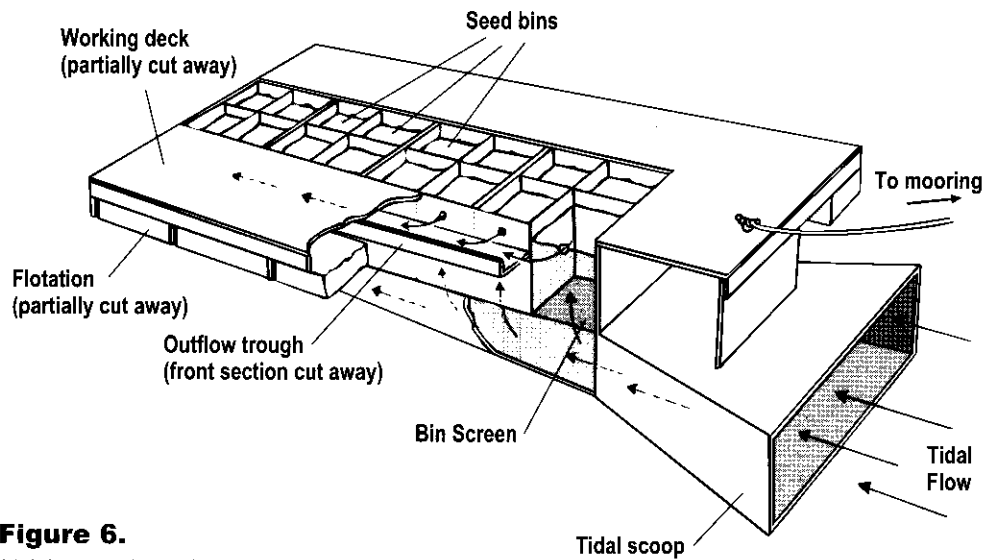
Both airlift systems are designed to be used in protected waters, such as salt-water ponds or impoundments. They can also be moored to a dock in a tidal creek if they will not be exposed to heavy wave action. One airlift system is based on a system developed in Florida. A raft of 4-inch PVC pipe serves as both the silo support system and an air manifold. The manifold is pressurized with a small air blower. Silos are suspended within the raft and water is circulated through the silos with airlifts (**Fig. 5**). The second airlift system consists of a reservoir (tank) which is floated in a

pond or adjacent to a dock. Water is pumped into the reservoir with a large airlift or with a submersible pump and flows out the silo drains just as in a land-based system.

Recently a tidal-powered upwelling nursery, modified from one developed in Maine (Mook 1988; Mook and Johnson 1988), has been tested in South Carolina. The nursery consists of a raft with a scoop on one end and an interior chamber where upwellers are housed (**Fig. 6**). The raft is tethered from the scoop end so that it



**Figure 5.**  
*Floating upwelling nursery system*



**Figure 6.**  
*Tidal-powered upwelling nursery system*

swings to point the scoop into the current. Water is directed by the scoop into the silo chamber and flows out through sideports at the water level. In pilot testing, this system appeared to foster more rapid growth than a land-based nursery. It offers the obvious advantage of low operating costs (maintenance only), but is vulnerable to storm action. A construction and operations manual (Baldwin et al. 1995) is available from S.C. Sea Grant Consortium (see Appendix A).

### **Bottom culture**

A number of bottom culture nursery systems have been tested and some are in use in South Carolina. These include trays supported off the bottom on racks in subtidal areas, trays planted on or just

above the bottom in intertidal and shallow subtidal areas, and "soft" bags or pens. All bottom culture systems require frequent maintenance to remove silt, fouling organisms, and predators.

The subtidal rack and tray system must be deployed in deep water in order not to represent a navigation hazard. Therefore it requires a large boat to deploy, maintain and harvest the trays. Trays are a wood frame with screening top and bottom. Several trays are supported on one rack in a tiered arrangement with space between the trays for water circulation. A rack and tray system is labor-intensive to maintain but, if properly sited, provides good growth and survival even for very small seed (1 mm and up). Small seed (1–2 mm) may be planted at very high densities (50,000-

150,000/m<sup>2</sup>  $\approx$  5,000-15,000/ft<sup>2</sup>) initially, but must be thinned as the seed grow. The fine mesh which must be used to retain very small seed requires weekly cleaning to remove fouling organisms and silt.

Smaller trays may be deployed in intertidal or shallow subtidal areas if substrate is firm (sand or shell). The trays are buried so that their tops are level with the surrounding substrate, filled with sand, and covered with screening ( $\sim$ 6 mm or 1/4" openings). If the trays are buried too deep they may be covered over by shifting sand or silt deposition. If they are not buried deep enough, they may create scouring currents which will wash out the tray. Screens must be checked frequently and any silt removed from the top screen. There are a limited number of sites in South Carolina where this method will work, because in most places the silt load is too heavy. Banks which have sandy substrate are often subject to strong currents which may wash substrate out of the trays. These trays are probably not suitable for very small seed (<2 mm).

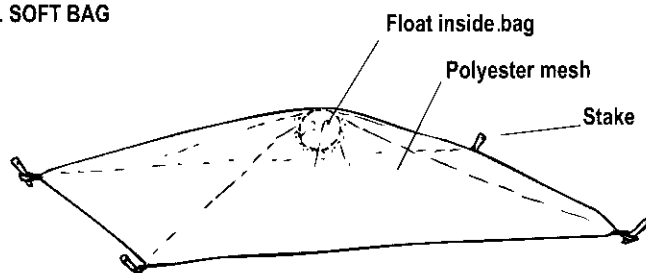
Trays or polyethylene mesh bags may be placed on racks in intertidal or shallow subtidal areas. Racks may be constructed of wood, rebar or PVC. Trays may have a solid bottom and be filled with sand or similar substrate. Bags will not retain substrate. These will only work in well-protected sites with no waves or strong currents and are not well-suited to seed <4 mm.

"Soft" bags are used extensively in Florida for nursery culture (Harbor Branch 1989;

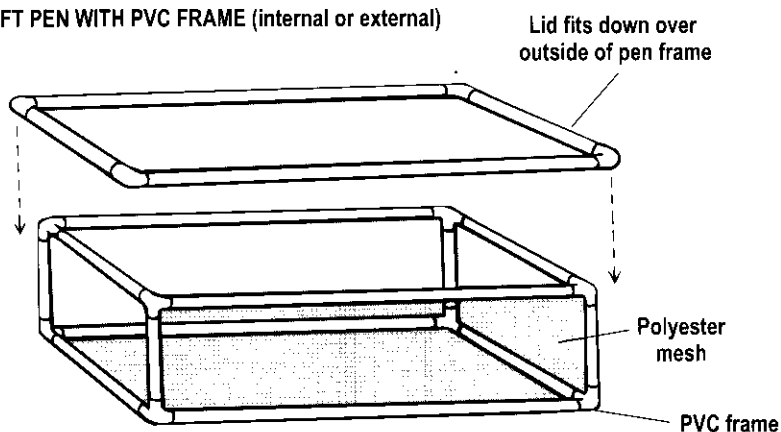
Vaughan and Creswell 1990). They have recently been tried in South Carolina with mixed success. The bags are made of woven polyester (e.g. Fablok<sup>®</sup>) in an appropriate mesh size to contain the seed (**Fig. 7a**). Bags are anchored to the bottom with a weighted rope, rebar, or stakes. A float placed inside the bag holds the upper side above the seed. In Florida, cages with several tiers of bags have also been tried.

In South Carolina, the float is often not adequate to prevent silt from collecting on the top of the bag. Therefore, a modification, referred to as a "soft pen" has been developed (**Fig. 7b**). These pens are constructed of the same soft woven material but have a rigid frame which supports the upper edge 25–36 cm (12–18") above the substrate. The frame may be made of PVC pipe placed either on the inside or outside of the mesh. A recent development is the use of a vinyl covered wire (14 gauge, 1/2" openings) skirt to support the soft bag (**Fig. 7c**). The wire fencing provides additional predator protection. Pens are constructed with a removable top which may be fabricated of the same soft mesh or may be made of lighter weight plastic netting. The lid must have a mesh size small enough to exclude most crabs and must fit securely over the edge of the bag so that there is no gap where crabs can enter. These systems are not suitable for small seed (<4 mm) because the mesh size required to retain such little seed would foul rapidly and restrict water flow.

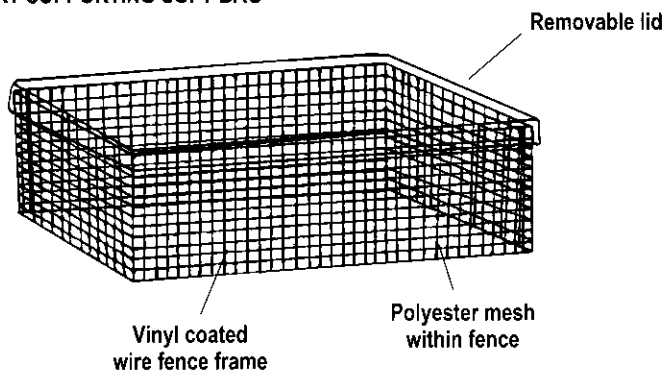
**A. SOFT BAG**



**B. SOFT PEN WITH PVC FRAME (internal or external)**



**C. WIRE SKIRT SUPPORTING SOFT BAG**



**Figure 7.**

*Soft bags and pens for field-based nursery culture*

---

### **Pond culture**

Ponds and impoundments may be used as nurseries to some advantage. They provide some protection from environmental perturbations and predation and are usually more accessible than field sites while being less expensive to operate than land-based nurseries. However, the cost of constructing a pond is considerable, so this is probably only an option for those who have access to an existing pond.

Pond culture offers the possibility of enhancing the natural food supply by managing for algal blooms. However, pond management is complex and poorly understood, particularly as relates to bivalve culture requirements. In addition to the food supply, the pond manager must consider many other factors which will affect clam growth and survival, including dissolved oxygen, pH, temperature, and waste-products.

Pond culture research is being conducted at the Waddell Mariculture Center in Bluffton, SC. Various sizes of seed have been planted in a variety of containment systems including airlift-driven upwellers, trays and bags supported on racks, trays and bags placed on the bottom, and pond-side upwellers to which water was pumped. Seed have also been planted on window-screening placed directly on the pond bottom. For pond culture, containers are used primarily to facilitate handling and harvesting the seed, as predators are usually physically excluded from the pond. Results of these studies have been variable

and no system tested to date has consistently provided high survival and rapid growth.

### **FIELD GROW-OUT**

The final and longest stage in clam culture is grow-out to market size, which may require 18–30 months, depending in part on the seed size at planting. Grow-out is almost always done in natural bodies of water (e.g. tidal creeks), because providing enough space and water flow in an onshore system would be prohibitively expensive. Grow-out methodology is less well-defined than either hatchery or nursery methodology. Many different methods are in use and new ones are continuously being developed. In this overview, factors affecting field culture success will be discussed and several systems described. There are several chapters with relevance to field culture in *Clam Culture in North America* (Manzi and Castagna 1989).

### **Site selection**

Clams require a relatively low energy environment. Sites subject to large waves, heavy boat traffic or strong tidal currents should be avoided. Also to be avoided are sources of bacterial and chemical pollution (marinas, sewage treatment plants, industrial sites, agriculture sites, golf courses, etc.). Accessibility may also be a factor, although there are probably not very many sites in South Carolina which can be reached without a boat.



Basic site requirements include high salinity (averaging >24 ppt) and sufficient tidal flushing. Avoid areas which are subject to freshets after heavy rainfall, such as rivers draining large watersheds. Tidal flow is requisite for delivering food and oxygen and removing wastes, as well as for maintaining the needed salinity. Too much flow may wash clams away or smother them in waves of sand. Too little flow not only may provide inadequate food but also may result in silt accumulation which necessitates frequent cleaning in order to avoid smothering of the clams.

Most substrates are suitable for clam culture, but really soft mud is not only difficult to work in but is indicative of a high siltation rate which will necessitate more frequent cleaning. A mixture of sand and mud, firm enough to support your weight but loose enough for raking, is perfect. However, there are a limited number of sites in South Carolina which meet this criteria and most growers will have to make do with somewhat less-than-perfect substrates.

One way to choose a good site is to look for native clams. Try to find topneck or smaller clams (<60 mm long and 30 mm thick). Clams of this size should be actively growing, as evidenced by a light-colored margin. (Do your survey anytime other than the winter as little growth occurs from December through February). Distance between rings on the shell is also some indication of growth rate. The farther apart the rings, the faster the clam has been growing. If native clams do not show

signs of rapid growth, that particular site may have inadequate food supply. If no clams are present on the site, there may be other problems which make the site biologically unsuitable.

Although clams can be grown subtidally, there are many advantages to intertidal culture. Aerial exposure will dry out macroalgae and prevent or retard growth of most fouling organisms. Exposure provides partial protection from some predators and parasites (e.g. boring sponge). The clams are visible for inspection at low tide. Drawbacks to intertidal planting are possibly slower growth (less feeding time); visibility (which may lead to vandalism, poaching or complaints from nearby residents or other waterway users); and limitation of working time due to tidal cycle. Also, many intertidal areas are characterized by soft soupy substrates, making maintenance more difficult. Planting in shallow subtidal areas (where the clams are visible but still slightly submerged at low tide) circumvents some of these problems.

Deep subtidal planting will require equipment (e.g. large boat, mechanical hoist) which is not needed for intertidal or shallow planting. Subtidal cages may be rapidly covered with surface fouling such as macroalgae, tunicates, bryozoans, and hydroids. Heavy fouling greatly reduces the water flow through the cages, resulting in poor clam growth. Therefore subtidal cages require frequent cleaning, perhaps even weekly.

## Predator protection

Seed may be field-planted for grow-out at a size of 7–8 mm. Survival of field-planted seed increases dramatically with size. Therefore, it may be more profitable to plant larger seed, even though initial costs will be increased. Also, the smaller the seed initially, the greater the size variation at harvest.

Even at larger sizes (e.g. 10–12 mm), seed must be protected from predators. Without protection, predation would claim virtually 100%. The major predator of clams in South Carolina is the blue crab, but other crabs (mud crabs, stone crabs, etc.), whelks, oyster drills, and moon snails also prey on clams. Blue crabs can prey on clams with shell lengths up to about 40 mm (1½") and may eat as many as 300 clams per day. Mud crabs are major predators of small seed (up to about 15 mm) and may eat more than 100 seed a day.

The most effective way to control predation is to completely enclose the clams with predator-excluding mesh. Enclosures may be box-like structures built of rigid plastic or vinyl-coated wire mesh, pens like those described for field nurseries, or wooden trays with mesh over the top. A 12 mm (½") opening provides adequate water flow and excludes most predators. However, seed <18 mm SL will not stay on this size mesh. Therefore, you must either start with a smaller mesh size, increasing it later as the clams grow, or line the container with a smaller mesh which can be removed

later. For wooden trays, window screening or lightweight flexible netting (6–12 mm openings) may be used as the top covering.

Clams grow better in substrate, preferably sand or sandy mud. Any space between the substrate and the top covering will promote crab growth. Small crabs, which may enter as larvae, are protected from predators and have a captive food supply. This must be considered when designing trays or cages, selecting initial seed size, and determining inspection frequency.

For subtidal culture and intertidal culture on firm bottom, wooden trays with a top covering are suitable containers. These should be filled with sand or sandy mud prior to planting the seed. Trays may be supported on racks, as described for field nursery culture, or placed directly on the bottom. Small trays may be buried in firm substrate if they are checked frequently to remove silt from the surface.

In soft, muddy bottoms, cages built entirely of mesh and "soft" pens, like those described for nursery culture, are used. These are allowed to accumulate silt prior to planting the seed, but must be checked frequently to prevent silt from covering the top of the cage and to remove predators. Because of the heavy silt load, the cages are usually at least 15 cm (6 inches) deep, with 10–12 cm (4–5 inches) of silt inside. Unfortunately, this leaves room for crabs to grow in the space between the silt and the top cover, necessitating frequent predator removal. However, if the cages are filled completely with silt, they must be cleaned

frequently (perhaps even daily!) to remove overlying silt which will smother the clams.

In other states, clams are often planted with only a top covering. This may be suitable for some firm bottom sites in South Carolina. The substrate is raked to loosen it and aggregate (gravel or crushed shell) may be placed on the bed to provide some protection from crabs. Seed clams are then scattered on the surface at a rate of 500–1000/m<sup>2</sup> (50–100/ft<sup>2</sup>) and additional substrate (sand or shell-sand mixture) may be sprinkled on top of the seed. A light mesh (usually 6–12 mm or 1/4–1/2" openings) is then placed over the seed and anchored around the edges, using leadline, rebar or sandbags. Floats may be placed under the netting at intervals to reduce silt buildup on the covers. This planting method does not work on soft, muddy sites because the top mesh silts over too quickly, smothering the clams.

In addition to exclusion methods of predator control, some growers place crab traps in or around their culture areas. These are only lightly baited, in order not to attract crabs from any distance. If you are placing more than 2 traps, you may need a commercial boat license (\$20–25) and a license for each pot. These are issued by the S.C. Department of Natural Resources, Commercial License Office. Another method of predator control which some growers have found effective is to place oyster toadfish in the culture units. These fish will eat crabs and their very presence may keep crabs away.

Regardless of the type of predator protection, frequent inspections are mandatory to remove crabs which may have entered as larvae and to repair tears in the mesh. Clams inspected weekly may have survival rates twice as high as those inspected only monthly. Outlook is poor for any seed not inspected at least monthly.

### Planting density

Seed clams (7–8 mm) may be planted at very high densities (up to 5000/m<sup>2</sup>, ≈500/ft<sup>2</sup>). However, these densities are too high for the entire grow-out cycle, necessitating labor-intensive thinning. A suitable density for the complete grow-out cycle is 500–650/m<sup>2</sup> (=50–65/ft<sup>2</sup>). Small seed (<15 mm) may be planted at almost 10 times this density (4000–5000/m<sup>2</sup>) and thinned to the lower density after 6–12 months, or when the average size is about 20 mm. There is some evidence that survival of small seed is improved by planting at high densities (Eldridge et al. 1979; Manzi et al. 1980). However, if allowed to remain at high densities beyond a size of about 20 mm, the clams will be stunted and survival may be poor.

### Growth rate

Growth rates vary seasonally as well as between individual clams. They also vary between sites. An annual average of 1.5–2.0 mm/month can probably be expected in South Carolina, although some sites may consistently produce better growth than this. Thus it may require 18–30 months to grow a seed clam from 10 mm

to 45 mm SL. Clams should be examined regularly in conjunction with cleaning and predator removal and harvested when about 80% of the population reaches the target size. After grading, the sub-market clams can be returned to the field for additional growth. Some culturists have established markets for these smaller clams. Although the market value will be lower, the labor of replanting and harvesting will be avoided. (South Carolina does not have a minimum size limit for aquaculture-produced clams. Regulations may differ in other states.)

## PROCURING SEED CLAMS

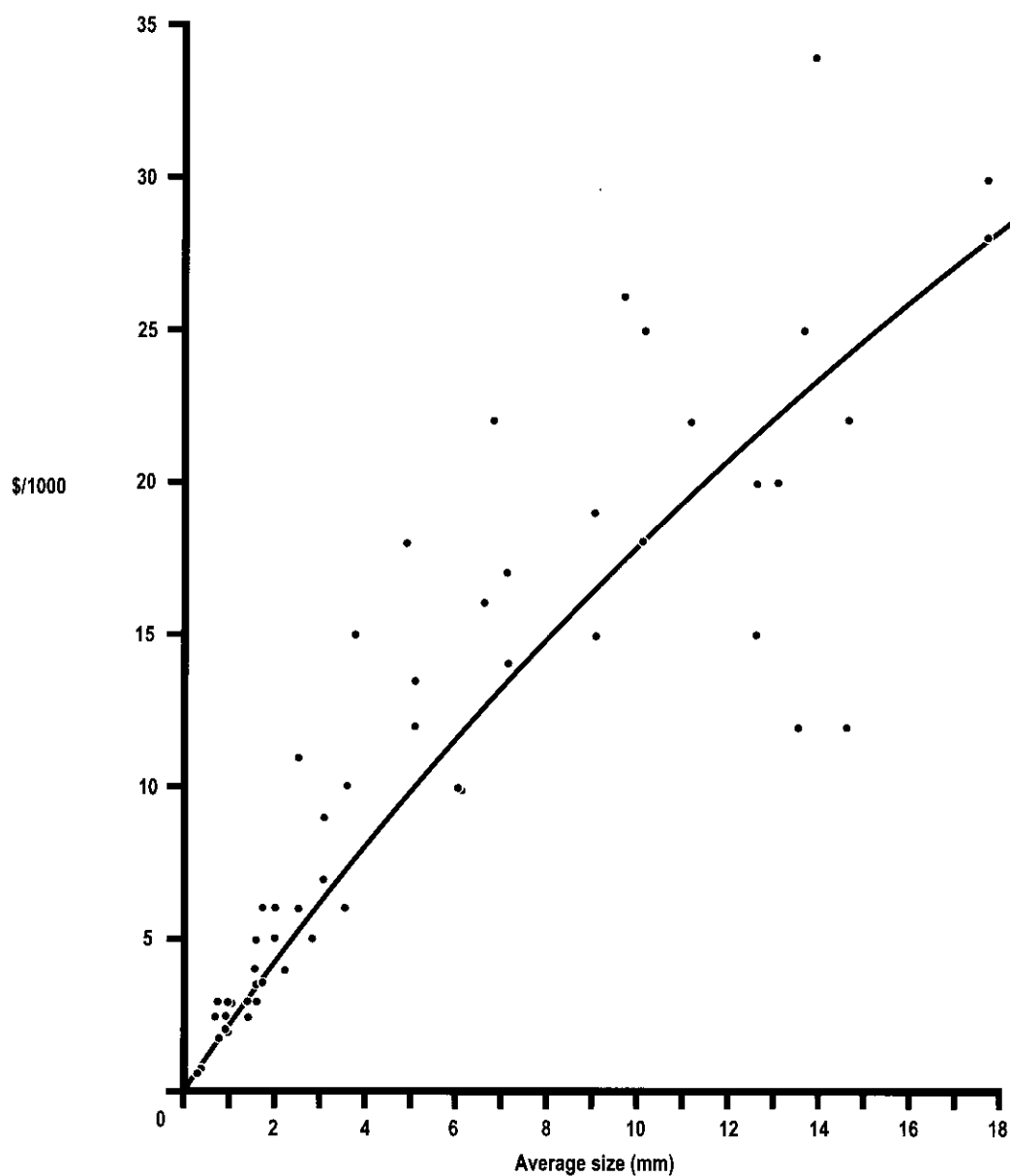
One of the first decisions to be made in starting a clam culture business is whether to include a hatchery. There are advantages to producing your own seed. These include the ability to control production to match your planting needs, the elimination of potential problems associated with importing seed from other states, and the ability to develop a breeding program to produce seed tailored to your operation. However, hatcheries are expensive to build and operate and require a degree of technical expertise.

If you have no prior experience it might be advisable to start with only a grow-out operation. Later, once you have some cash flow, a nursery could be added. Finally, once you have experience in handling seed in the nursery, a hatchery might be included. This decision will depend on many factors, including your experience

level and your financial situation. Before making this decision, we recommend that you read "Investing in Commercial Hard Clam Culture" (Adams et al. 1991), a guide to the economics of clam farming which is available from most state Sea Grant offices.

There are a number of commercial hatcheries and nurseries which sell clam seed in a range of sizes from less than 1 mm up to planting size (>8 mm). A list of seed sources is provided in Appendix B. The list includes hatcheries and nurseries, so not all sizes may be available from all suppliers. Some of the companies deal primarily in oysters, but will produce clam seed if there is a demand. Many of the sources listed produce seed primarily for their own use and only sell to other parties if they have excess. This is as complete a list as we could obtain but new enterprises seem to appear each year. You may be able to obtain additional information on seed sources from the information resources listed in Appendix A or from other growers. We do not imply endorsement of any particular hatchery or nursery.

Seed prices increase with size and vary from year to year. **Figure 8** shows 1993 prices for various size categories. Some suppliers will provide a discount for large orders, and end of season discounts are often available in the fall, particularly from the more northern hatcheries. If you are not going to include a nursery, you must purchase seed that are at least 8 mm SL. If you plan to use a field nursery, purchase 5–6 mm seed. For a land-based nursery or



**Figure 8.**  
*Clam seed prices, 1993*

---

floating upwellers you can purchase seed as small as 1 mm. Call well in advance so the grower can schedule production of your seed. Many hatcheries require orders and prepayments several months in advance of shipment.

In deciding how many seed to order, count on 50% survival in the field grow-out stage, 75% survival in a land-based nursery or floating upwellers, and 50% survival in other field nurseries. Thus, if you wish to harvest 50,000 clams, you will need to plant 100,000 seed. To produce that many planting size seed in a land-based nursery, you should start with 135,000. A field nursery should be stocked with 150,000.

When selecting a seed supplier there are a number of important considerations. These include the ability of the supplier to meet your demand, both in terms of quantity and schedule; the quality of the seed; and the suitability of the seed for your particular operation. If possible, obtain names of other customers from your area and check these references. Before purchasing seed from an out-of-state hatchery, check with the S.C. Department of Natural Resources, Office of Fisheries Management, concerning regulations on importation of seed. There are risks involved with importing seed and you may be required to have the seed tested for certain diseases.

One factor affecting the suitability of the seed for your operation is the geographic origin of the broodstock used to produce the seed. Clams produced from northern

broodstock may be unable to tolerate high water temperatures while those produced from Florida stock may be intolerant of cold winters. If the parental stock are from Florida, you will want to inquire whether they are *M. mercenaria*, *M. campechiensis* or a hybrid. *M. campechiensis* is reputed to have a poor shelf life and therefore is difficult to market. Hybrids have performed well for some growers and usually grow faster than *M. mercenaria* but have not been extensively tested in South Carolina. Stock from your own geographic area are most likely to be well-adapted to the growing conditions they will encounter in your operation.

It would also be desirable to know the history of the broodstock used to produce your seed. If the stock has been bred over many generations in the hatchery, it may have improved performance (if the hatchery has used a good breeding program) or it may actually have poorer performance than wildstock. You need to know what characters (if any) the hatchery has targeted in its selection program (e.g. fast growth; high survival; shell markings) and when the selection was performed. Then you can determine whether those characters are ones you consider important. For example, if a breeder has selected for fastest growth in the hatchery (by discarding the slower growers), that may be of no interest to you (since you are buying the seed after the hatchery phase). Fast growth in the field is the character which will have the most bearing on your operation. Bear in mind that a clam selected to grow rapidly in Florida or Maine may not grow rapidly in South Carolina.

Determine when the seed were spawned. By referring to **Table 3** you can tell whether growth has been reasonable. You would prefer not to receive the slowest growing clams from a particular spawn, unless you are getting a cut-rate price. Also, ask whether the clams have any unusual characteristics of which you should be aware. We once purchased seed which could not tolerate being rinsed with freshwater. You would certainly want to know that.

## SHIPPING AND STORAGE OF CLAMS

One of the advantages of clams as an aquaculture species is that they survive extended periods of time without water. This is very convenient when shipping clam seed and broodstock and when transferring clams from the nursery to the field. This section addresses methods of storing, packing and shipping larval, juvenile and broodstock clams. These are not methods for storing and shipping clams to market, which are specified by FDA guidelines. The South Carolina Department of Health and Environmental Control (SCDHEC) regulates transport of shellfish. Interstate shipment of clams may be restricted in some cases. Make sure proper permits are obtained before making shipping arrangements. In South Carolina, contact SCDHEC for assistance (see Appendix A).

## Storing clams out of water

Seed clams may be held out of water for several days if they are kept cool and moist. They should never be allowed to stand in water, such as ice melt, but neither should they be allowed to dehydrate. It is usually sufficient to place them in an ice chest with damp newspaper on top. The ice chest may be refrigerated or one or two frozen gel packs may be placed on top. Do not let the frozen gel packs contact the clams directly. Survival of clams under refrigeration will depend in large part on the physiological condition of the clams when removed from water and the water temperature to which they are acclimated. Survival time will be shorter in the summer and any time the clams have been under stress. Small clams (<3 mm) can survive a few days of refrigeration, while adult clams may survive 10 days or more. Allow the clams to warm to water temperature before returning them to water.

## Shipping clams

Late-stage larvae and post-set clams may be shipped by methods developed for remote setting of oysters. The clams are shipped damp in a nylon mesh of appropriate pore size. The clams are concentrated in the center of a small square of mesh and the corners of the mesh are folded up and tied. This damp ball is wrapped in several layers of wet paper and may be placed in a plastic bag (not sealed) to retain moisture. The package is placed in an insulated shipping carton with a frozen gel pack and shipped by overnight express. Younger

---

larvae may be packed and shipped like tropical fish.

Small seed clams and small quantities of larger clams are best shipped by air, using commercial carriers such as UPS, Federal Express, or airline package express services. They should be packed in heavy-duty insulated (e.g. styrofoam) cartons with frozen gel packs. Line the box with damp newspaper. (If the box is not watertight, line with a large plastic bag first). You want the paper wet enough to keep the clams moist but not so wet that water will drain out of it. Add the clams and shake gently to settle them. If the clams don't fill the carton, use additional crumpled paper, styrofoam nuggets, or damp seaweed to fill up the extra space. Cover with damp newspaper. Wrap a frozen gel pack (or several, depending on carton size) in dry newspaper and place it on top. Do not use regular ice because it will melt sooner and the clams will be sitting in water. The gel packs should be separated from the clams by dry newspaper to avoid freezing the animals themselves. Limit the time in transit to a day or two.

It may be more economical to send large shipments by refrigerated truck. However, there is a risk in shipping clams by that method as trucks are routinely inspected for compliance with interstate shipping regulations. There is often no recognition of the fact that conditions for delivering a cultured clam with the least stress may not be the same as conditions for preventing human health problems. If the truck temperature does not meet the standards

set for human health purposes, your valuable broodstock or seed may be destroyed along with consignments which were actually destined for the market. Also, seed clams have sometimes been confiscated by inspection officials because they were below the size limit for wild-caught clams. If you are going to ship by truck make sure all your packages are clearly labelled as culture-derived stock. Contact appropriate agencies in all states the truck will pass through (in South Carolina, SCDNR and SCDHEC) for current regulations on shipping seed, as these may be subject to change. For refrigerated truck shipment, clams are usually packaged in onion or grapefruit bags.

Clams that are being transported to planting sites in the field, even though the time in transit is usually much shorter, should be treated in much the same way. Clams should be removed from the culture containers, rinsed with freshwater, and loosely packed into coolers. Place damp newspaper on top to prevent dehydration. If they will be subjected to heat in transit, add frozen gel packs wrapped in dry newspaper. If weather is mild and the trip is short, just let them sit at ambient temperature. However, be sure not to let them sit in direct sun.

### **Handling clams after shipment**

When a shipment of clams is received, it should be processed immediately. Open the box and remove the gel pack(s). Examine the clams to determine their condition, removing any obvious dead



ones. For larger seed and broodstock, examine them for the presence of fouling organisms, associated species, etc. and remove these if possible. Common nuisance species which could arrive with clams include small mussels, sea squirts, and oyster drills. Clams could be also be harboring bacteria, fungi, parasites or any of a number of other potential problems. Soaking the clams in freshwater or a dilute chlorine bath (1 ml household bleach in 1 liter freshwater) will eliminate many of these and is a good precaution. Freshwater soaks may be used for clams as small as 250  $\mu\text{m}$ . Small seed (1–2 mm) can tolerate a 5-minute chlorine soak; larger seed can be soaked for an hour or more as long as the water is not warm. Chlorine soaks should not be used on clams <1 mm. If the clams are still cold, let them warm up to the temperature of the water before placing them in the culture system. Otherwise, put them in water immediately or, if that is not possible, refrigerate. (However, avoid repetitive warming up and cooling down as this will badly stress the clams). It is advisable to examine the clams 24 hours after introduction to your system to determine if any additional mortality has occurred. If possible, keep the new arrivals segregated from any clams already in your system until you are sure they are healthy.

## PERMITS AND LICENSES

### Mariculture permit

Clam mariculture is regulated by the South Carolina Department of Natural Resources (DNR), Office of Fisheries Management (OFM). Grow-out operations will require a mariculture permit from this office. Permits are granted for five years at an annual rental rate of \$5.00/acre and may be renewed. Tidelands in the state are considered public trust properties and are, for the most part, available for shellfish culture. Exceptions are intertidal grounds ceded to individuals or estates as king's or legislative grants. Also, no mariculture permits are allowed in areas where water quality prohibits shellfish harvesting. Mariculture pens or containers are not allowed to cover or encroach on natural shellfish populations, inhibit navigation, or impede normal commercial and recreational uses of the tidelands.

Mariculture permits are different from shellfish culture permits, which used to be called oyster leases. Shellfish culture permits are issued to individuals or businesses for commercial harvest of native shellfish (in most cases, oysters) in a specific area. Mariculture permits may be allowed within the boundaries of existing shellfish culture permits if the uses are compatible. If the area proposed for a mariculture permit is already designated as a shellfish culture permit area, you should obtain a memorandum of understanding from the shellfish culture permit holder indicating that party's willingness to allow

---

dual use of the permit area. The Shellfish Management Program office at DNR will assist you in identifying existing shellfish culture permit areas.

Prior to applying for a mariculture permit, you should contact the DNR's Shellfish Management Program (see Appendix A) to discuss your plans and potential permit sites. Detailed maps of state shellfish grounds are available and any conflicting uses can be identified. After a potential site is selected, biologists will perform a preliminary site investigation. This will save time and money by preventing an application that has little chance of approval for biological or other reasons. It is also advisable to obtain a copy of Marine Fisheries and Related Laws, available from DNR. Of specific interest are the sections pertaining to shellfish (Chapter 17, Article 3) and aquaculture (Chapter 18).

After a potential site for the grow-out operation is selected, an application for a mariculture permit is filed with the DNR, Office of Fisheries Management. There is a \$25.00 non-refundable application fee. Supporting documentation in the form of maps of the desired permit area and a detailed operations plan are required. An outline of a generic mariculture operations plan may be obtained from OFM, Shellfish Management Program. This office will also assist in map preparation.

Conditional approval is granted to the applicant if all permit criteria are met and a sound operations plan is presented. At

this point, the applicant must advertise in the local newspaper for three weeks, during which time written objections may be submitted to DNR's Shellfish Permit Committee. This is also the appropriate time to apply for other needed permits (see next section). The committee will usually be able to render a final decision within a month after the advertising period. However, this final approval will not be granted until all other required permits are received. The mariculture permit process requires approximately three to six months, if the applicant meets requirements in a timely fashion and no objections are received.

No planting or harvest requirements are placed on mariculture permits. However, an annual operations plan and activities report must be submitted to the Shellfish Management Program. Once harvest begins, monthly production reports are required by the DNR's Fisheries Statistics Program.

### **Critical zone and discharge permits**

Grow-out operations may require permits from the Office of Ocean and Coastal Resources Management (OCRM), a division of the South Carolina Department of Health and Environmental Control (SCDHEC), and the U.S. Army Corps of Engineers to conduct activities in the "critical zone". These agencies use a joint application and permitting process. Specific requirements or conditions may be placed on the permit to avoid environmental degradation, prevent use conflicts, and

---

avoid navigational interference. Hatcheries, nurseries and ponds or impoundments which will discharge into natural waterways will require discharge (NPDES) permits from the SCDHEC.

Even if the above permits are not necessary for a specific operation, written exemptions from these agencies must be obtained before the DNR will issue a mariculture permit.

### **Purchasing, harvesting and selling clams**

In order to import seed clams or to be in possession of seed clams, you will need an additional permit from DNR. This no-cost permit is only issued to persons who are already in possession of a mariculture permit and therefore have a legitimate reason to be in possession of undersized clams (<1" thick). You must carry this permit whenever you are in possession of seed clams (e.g. in transporting them to field grow-out areas). This will protect you from seizure of your clams should a law enforcement officer challenge you. If you ship seed clams, a copy of the permit should be enclosed with the shipment.

Before harvesting your clams, you must obtain a harvest permit from DNR. These annual no-cost permits are primarily for your protection. The permit specifies the area from which you are authorized to harvest. Anyone harvesting clams in your mariculture permit area without a harvest permit could be subject to legal action. Any illegal activities on your permit area

(e.g. poaching, vandalism) should be reported to DNR's District 9 law enforcement office (see Appendix A). Harvesting of cultured clams is permitted year-round.

Before marketing your clams you will need a land and sell license, which allows you to sell to wholesale dealers. If you wish to sell directly to the public you will also need a wholesale dealer's license. Both of these licenses are obtained from the commercial license office of SCDNR.

A wholesale dealer must have a certified shellfish holding facility. Holding facilities are certified by SCDHEC, which will perform periodic inspections to insure adherence to FDA guidelines, including cleanliness and refrigeration requirements. SCDHEC also inspects vehicles used for the transport of shellfish. All shellfish must be bagged and tagged, identifying area and date of harvest. Accurate records must be kept to substantiate harvests and shipments. Contact SCDHEC for details on storage, shipping and tagging requirements.

### **Other permits**

It is difficult to specify all permits and licenses which you may need, because this will vary depending on the type and location of the culture operation (e.g. see DeVoe and Whetstone, 1987). For instance, you may need local zoning permits, building permits, etc. Also, regulations may change, so it is imperative to check on current permit and license requirements. Assistance in identifying permit requirements may be obtained from the SCDNR

---

Shellfish Management Program, S.C.  
Department of Agriculture Aquaculture  
Permit Assistance Office, and the S.C. Sea  
Grant Consortium (see Appendix A).

---

## PART III. DESIGN AND OPERATION OF A HARD CLAM HATCHERY

This section is a detailed description of the design and operation of a year-round hatchery for hard clam production. If you are considering including a hatchery, this section will give you a feel for the complexity of such an undertaking. If you have already decided to include a hatchery, this can serve as a training and reference manual. Please read the general description of a hatchery in PART II of this manual before reading this section.

### PHYSICAL PLANT

The size of a hatchery will depend on the level of seed production desired. A typical hatchery contains the following components: phytoplankton culture area; main hatchery area; and support facilities (laboratory, stock algal culture room). Most of the physical space will be devoted to the main hatchery. This area will be kept at a temperature of 24–28°C (75–82°F) year-round. It will contain tanks for broodstock conditioning, larval culture, post-set culture and water storage. Some portion of the building must be devoted to algal culture. It is preferable to have this in a separate air-conditioned room. Intense lighting must be supplied to achieve rapid growth of the algal cultures. Air-conditioning compensates for the heat generated by the lights, and maintaining the cultures at

cooler temperatures helps control bacterial contamination. A separate, smaller highly-controlled room is desirable for maintenance of stock cultures. This can be an incubator within the support lab. The support lab provides space for microscopes and other instruments which are best kept in a dryer environment than the main hatchery. The hatchery will require various types of support equipment including a seawater procurement and distribution system, seawater filters, seawater chillers (and possibly heaters) for the broodstock tanks, and an air supply system. A system of floor drains is essential. The floor should be graded to direct all water to the floor drains. It is desirable to provide a non-slip surface by overlaying the floor with a special coating of grit or by incorporating raised wooden slats which will allow the water to drain through.

### Seawater supply and distribution

A hatchery must have a dependable source of clean, high-salinity (>24ppt) seawater. Ideally, the hatchery is situated close enough to a natural body of water to allow pumping seawater into the building. An alternative for a small-scale hatchery might be a portable water reservoir which could be trucked to a seawater source for filling. Many hatcheries operate in conjunction with a land-based nursery and share the

---

same water source. Continuously flowing seawater is not needed for hatchery operations, but water must be available on demand.

This manual will make no attempt to describe a seawater pumping system, since the possible configurations are endless. (There is more discussion on this in Part IV). However, bear in mind the following rules: all wettable parts of the pumping and distribution system for the hatchery should be constructed of non-toxic materials. Most hatcheries use PVC plumbing and valves and pumps made of plastic, cast iron, stainless steel, or fiberglass. The size and style of the pumps is dependent on the hatchery's water flow requirements. Some hatcheries have duplicated plumbing systems. This allows one set of piping to be cleaned and sterilized without interfering with water distribution capabilities.

## **Water treatment**

In most locations in South Carolina, it will be necessary to make some provision for silt-removal from your incoming seawater. This can be done with flow-through filtration or by use of a settling tank. Large filters filled with diatomaceous earth and sand allow for continuous water flow, but the filters require frequent back-flushing. Since continuous flow is not required for the hatchery, it is often simpler and cheaper to use settling tanks. A settling tank should be shallow relative to its capacity. Our experience is that it takes at least 24 hours (and often longer) for most of the silt to settle out of seawater, longer if

the water is particularly silty. You may wish to have two settling tanks to assure a continuous supply of settled water. The incoming water should be grossly filtered (e.g. 25  $\mu\text{m}$ ) to remove zooplankters before filling the settling tank. The settling tank must be accessible for regular cleaning to remove the silt build-up. Settling tanks may be located outside the hatchery building, but if they can be placed inside they will serve the additional function of adjusting the water to room temperature. This will eliminate the need for heating or chilling water for larval and algal culture.

Even after filtration or settling for 24–48 hours, the water will still not be clean enough for many hatchery requirements. Some provision must be made for fine filtration immediately prior to use for both larval and algal culture. Some possibilities are polyester felt bags (available in pore sizes as small as 1  $\mu\text{m}$ ) and cartridge filters (usually wound polyester, available in 1  $\mu\text{m}$  to 200  $\mu\text{m}$ ). Bags are easily cleaned and may be reused hundreds of times. Cartridges cannot be cleaned as easily and must be replaced frequently as they become clogged.

Fine filtration is often the only water treatment necessary. Many hatcheries, however, employ additional treatment such as activated charcoal treatment or ultraviolet (UV) sterilization. Passing the water through activated charcoal will remove dissolved organic compounds but does not remove bacteria. The pore size of the charcoal affects its efficiency and longevity. Charcoal made from coconut shell is very

inefficient compared to that made from coal. As water passes through the filter bed, organic compounds are attracted to the charged surfaces on the charcoal. Thus, charcoal with more surface area is more effective. When all the charged surfaces are loaded, the charcoal is no longer active. Since it is not possible to see when the charcoal is fully loaded, it should be changed frequently. A pound of charcoal can treat about 1,000 gallons of water before it is no longer effective. Charcoal cartridges are available for in-line applications, or you can make your own by filling a length of pipe with activated charcoal and placing a cloth pad and a hose adaptor fitting on each end. Charcoal is effective even on poorly filtered water, but the charcoal will need more frequent replenishment and the cloth pads may clog frequently, reducing the flow rate.

Ultraviolet sterilization kills bacteria in the water. Water must be crystal clear for effective UV sterilization, so fine filtration must precede UV irradiation. There are some indications that UV sterilization can be counterproductive. One possible drawback to its use is that beneficial, as well as deleterious, bacteria are killed. If pathogenic bacteria subsequently are introduced from some other source, they are able to multiply rapidly with no competition. Another problem with UV irradiation is that organic compounds in the seawater may be oxidized, resulting in various compounds which may be toxic to larvae. If your water has a high dissolved organic load, it should be treated with activated charcoal prior to UV sterilization.

## **Temperature control**

For year round operation, a hatchery will need the capability of heating and chilling seawater. Depending on the size of the hatchery, a "central" system might be provided, or the water temperature might be modified as needed by several smaller units. This depends somewhat on the water distribution system. If water is held in settling tanks prior to use, it will assume room temperature and be suitable for larval, post-set and algal culture. If, however, the distribution system draws directly from the estuary, water will be at ambient temperatures. This will be too warm for larvae in summer and too cool in winter, and a central heating/chilling system will be needed.

Broodstock conditioning tanks are kept at the same temperature year-round, which is cooler than average room temperature. Therefore, they will need their own temperature control units. If possible, locate the units outside of the hatchery building so that the waste heat produced will not heat up the building. In selecting seawater heating and chilling units, remember to consider the possible toxicity of any metal parts, and the potential longevity (or lack thereof) in seawater. Titanium heat exchangers are available at an affordable cost and have a life expectancy of several years.

## **Freshwater**

A hatchery uses a large volume of hot and cold freshwater to maintain the rigorously

clean conditions necessary for reliable production. Tanks will be washed daily with hot water, and algal culture containers, and occasionally tanks, will be filled with cold water for sterilization. Hot and cold water may also be needed for spawning. To avoid the hazard (and frustration) of garden hoses trailing long distances over the floor, there should be several water taps within the hatchery.

## Drains

A hatchery produces a lot of "waste" water. This is primarily seawater from culture tanks, but also includes the freshwater used for cleaning. The hatchery must have a floor drain system to remove all this water. If the water is to be drained back into the natural waterway, it may be necessary to procure a point-source discharge (NPDES) permit from the SCDHEC (see Appendix A). Other possibilities are to connect the drains to a sanitary sewer system or to provide a lagoon for the discharge water. Lagoon construction will probably require a permit and in some areas may be contraindicated because salt might leach into the groundwater.

## Air supply

The hatchery must have an air supply and distribution system. Depending on the hatchery size, an air pump may be adequate. Air pumps are available which can provide 2 cubic feet per minute (cfm) at water depths of 4 feet, enough to operate 50 airstones. For greater needs, regenera-

tive blowers are more economical choices.

Air is needed for algal culture and for post-set culture. Algal culture containers are usually deep (>1 m) while post-set culture utilizes airlifts which are usually no more than 25 cm (10") below water. It may be easiest to supply these different air needs with separate pumps or blowers. The algal culture needs high pressure, while the post-set do not.

The air distribution system can be constructed of PVC pipe with airline valves threaded directly into it. The pipe should be at least Schedule 40 PVC in order for the pipe wall to be thick enough to hold the airline valves which will be screwed into it. The diameter of the main distribution line should be as great as practical (usually 1½") but sidelines off of this manifold may be smaller. The smallest diameter which should be used is ¾". Airline valves should be screwed directly into the distribution pipe. In the algal culture and post-set culture areas, valves should be spaced at 1 foot intervals. The best type of valve is chrome-plated brass with a lever. Brass valves corrode rapidly in the seawater environment, and plastic valves do not last well. The lever-type valve will operate trouble free for a longer period than screw-types.

Air systems are often the main source of contamination in algal cultures. A fine-pore filter should be installed in the distribution line to remove air-borne bacteria and other contaminants. In-line disposable filters may also be used at each



tap where air is drawn off. Moisture in the air distribution system contributes to proliferation of bacteria and protozoans. A condensation trap may be installed to reduce this moisture.

Large-scale algal culture requires CO<sub>2</sub> addition to control pH. Most hatcheries inject this CO<sub>2</sub> into the air distribution system, using a timer to pulse CO<sub>2</sub> into the system as needed.

## Lighting

The main hatchery requires no special lighting arrangements, as long as visibility is adequate to provide safe working conditions. The algal culture area will require special lighting, usually provided with banks of fluorescent bulbs. Two fluorescent bulbs should be provided for each Kalwall®. Some hatcheries use high-intensity metal halide lamps for algal culture. Dense algal cultures may be light-limited and we have found that the cultures grow faster and reach higher densities with metal halide lamps. However, these lamps generate a lot of heat. If the algal culture area has natural illumination (e.g. solarium or greenhouse), auxiliary lighting does not need to be as intense.

## Electrical requirements

The hatchery should be fitted with ground fault circuits throughout. Many hatchery operations may require portable electric devices (e.g. submersible pumps). In order to avoid the danger of using extension cords on the wet floor area, it is advisable

to install overhead electrical outlets with pull-down cords.

## Instrumentation

A number of instruments are needed for efficient hatchery operation. Minimum instrumentation includes a light microscope for examination of algal and larval culture, a dissecting scope for examination of post-set, and instruments for measuring pH, salinity, and temperature. A number of probes, some very inexpensive, are available for measuring pH, or you can use paper pH strips. Salinity is most often measured with a refractometer. Mercury thermometers are adequate for measuring water temperature. It is also desirable, but not essential, to have a balance for weighing chemicals. A fluorometer, spectrophotometer, or Coulter® counter would be useful for determining algal concentrations. You might want to consider instruments or kits for measuring ammonia, nitrate, nitrite, and dissolved oxygen.

The efficiency of the hatchery operation can be improved by automating various functions. For example, automatic systems are available to monitor water temperature and turn on/off heating and chilling systems, an improvement over typical thermostats. It is possible to integrate a number of monitoring functions, such as temperature and PH, in one unit. An ideal system might use a computer to accumulate and store the information, providing a continuous record of hatchery conditions. Such systems can also be programmed to provide various alarm signals if something

---

is wrong and an automatic dialer can be included which will call staff at home to report malfunctions.

## PROCURING BROODSTOCK

Many hatcheries obtain their original broodstock from the wild. Wild clams are most likely to be well adapted to environmental conditions in your area. However, there are now a number of hatcheries from which stock may be obtained. These may be the product of various breeding efforts to improve growth, survival, or some other desirable trait. Spawnable adults may be purchased or borrowed, or you may buy seed and grow it to reproductive size. If obtaining broodstock from a hatchery (or seed that you intend to raise to use as broodstock in the future) there are a number of factors which you must consider.

The potential advantage to using a hatchery-produced broodstock is that it may outperform wildstock. However, this will depend in large part on how the broodstock was produced. It is important to know what characters the hatchery has been targeting (growth rate, appearance, survival) in its breeding program, how many generations of selection have been performed (each generation increasing the level of inbreeding), where the founder population was procured, how many individuals were used to establish the broodstock line (the more the better), and whether the stock has been outcrossed at any point (eliminating inbreeding). If the hatchery has been using a careful breeding

regime, the stock may be improved. Even so, that may not make it suitable for your particular culture needs.

We have already mentioned the importance of geographic origin when purchasing seed. This may be even more critical when obtaining broodstock. Stocks from different geographic areas may mature at different times of year or under different conditioning regimes. This may affect your ability to spawn the stocks year-round and to condition two stocks simultaneously for interbreeding. Be sure to ask the supplier how to condition the stock (temperature, time of year, salinity), how long it takes to ripen, how long it can be held in a ripe state, whether it can be conditioned and spawned year round, and whether individuals can be expected to spawn multiple times. If possible, determine when the stock naturally matures.

Another consideration in bringing broodstock from another hatchery or a different geographic area is the potential for introducing diseases or exotic species. Adult clams may harbor bacteria, fungi, protozoans, internal or external parasites, and fouling organisms. Check with the Office of Fisheries Management at DNR prior to importing broodstock. There may be restrictions on bringing clams from certain areas. Any imported broodstock should be thoroughly washed to remove any external parasites and fouling organisms and should be isolated under quarantined conditions until their health can be assured. Infected broodstock may release harmful bacteria during spawning which

will cause larval mortalities. Therefore, broodstock should not be considered safe until their ability to produce healthy larvae has been demonstrated.

Eventually, a hatchery may wish to establish its own broodstock line. This is more complicated than simply selecting your largest clams to spawn. We recommend that you read the references on genetics and breeding listed in the back of this manual. It would also be advisable to seek expert help in designing a breeding program. You may be able to locate a geneticist by contacting local colleges, universities, or research facilities. While some of these will charge for their advice (e.g. college faculty will probably expect an honorarium), others may be able to assist you for free. Check with state or federal

facilities such as research laboratories and extension services. Qualified staff members may be available to assist you.

## BROODSTOCK MAINTENANCE AND CONDITIONING

Broodstock are brought into the hatchery for conditioning 2–8 weeks prior to the desired spawning. At some times of year, the clams are naturally ripe and can be spawned immediately. More often they will require at least a few weeks of conditioning. At other times, when the clams have recently spawned, a lengthy conditioning period may be required. A supply of spawnable broodstock may be kept on hand at all times by procuring ripe individuals in the early spring and maintaining

**Table 4.**  
**Culture requirements for broodstock maintenance and conditioning**

<b>Culture Method:</b>	batch culture in raceways
<b>Temperature:</b>	18–20°C for maintenance and gradual conditioning 22–23°C for rapid conditioning/priming immediately before spawning
<b>Water treatment:</b>	coarsely filtered (25µm) seawater pretreatment with charcoal filtration or UV if water quality is suboptimal, complete exchange at least 3 times a week
<b>Salinity:</b>	25ppt or higher
<b>Stocking rate:</b>	4 liters water/clam
<b>Feed type:</b>	mixed phytoplankters including diatoms and flagellates
<b>Feed rate:</b>	1–3 x 10 <sup>9</sup> (1–3 billion) algal cells/clam/day
<b>Feed method:</b>	batch, continuous drip, or discontinuous metering
<b>Records:</b>	temperature, salinity, feed rate/type
<b>Duration:</b>	2–8 weeks for conditioning up to 6 months for maintaining ripe clams

them in conditioning tanks. Each spawning will require a minimum of 50 broodstock to insure that enough individuals of each sex spawn and produce an adequate quantity of gametes. (Also see other cautions about the number of spawners in the section on genetics of hatchery management).

At any one time, several different stocks might be in a conditioning tank simultaneously. The stocks should be held in individual trays which are carefully labelled. The labels should identify the origin of the clams and the date they were introduced to the conditioning tank. This information should also be recorded in a broodstock ledger. In some cases the clams themselves are individually labelled to identify them, particularly if they have been spawned previously or are part of a broodstock selection program. Individual clams may be labelled with indelible felt-tip markers, enamel paint, or engraving. Engraving can be done with a small drill similar to a dentist's drill, using a carbide tip. Individual clam identification codes should also be recorded in the broodstock ledger.

### **Tank and water requirements**

Conditioning tanks are usually long rectangular raceways, often manufactured of fiberglass. A raceway 6 meters ( $\approx 20$  ft) long, 0.6 m ( $\approx 2$  ft) wide and 0.6 m deep will hold about 2,000 liters ( $\approx 500$  gallons) when almost full of water and can accommodate up to 500 broodstock (4 liters per clam). Conditioning tanks are operated on

a batch culture basis. That is, they are filled with seawater which is then recirculated for 24–48 hours, after which a total water exchange is performed. When the water is exchanged, the temperature and salinity of the new water must be the same. The seawater should be filtered (25  $\mu\text{m}$ ) to remove large particles and zooplankters. Broodstock tanks should be supplied with slight aeration, 1–2 airstones for a tank of the size described.

Conditioning tanks must be maintained at a steady temperature. This is accomplished by circulating the water continuously through a seawater chiller, using a small pump such as a swimming pool or spa pump. A 1/2 hp chilling unit can cool 1200 liters (300 gallons) of water from 30°C to 20°C in about 24 hours and then maintain that temperature in a room as warm as 28°C. For a greater volume of water, a larger temperature differential, or a warmer room temperature a larger unit will be needed. Chillers should be designed for seawater use or they will quickly corrode. Titanium heat exchanger coils have the longest life under seawater exposure. The temperature in the conditioning tanks will be more easily maintained if the raceways and the plumbing for the recirculating system are insulated. The chilling units themselves should be located outside the hatchery if at all possible since they generate heat and noise.

For long-term maintenance of already ripe individuals or for gradual conditioning (over 6–8 weeks), the temperature should be maintained at 18–20°C, with no more

than a 1°C change over an hourly period. For rapid conditioning (2–4 weeks) a slightly higher temperature (22–23°C) can be used. This higher temperature is also useful for “priming” broodstock for a few days immediately prior to spawning. However, temperature control is much more critical at this higher level, since the clams may spawn spontaneously. (When very ripe, clams may spawn spontaneously at temperatures as low as 19°C.)

At most times of the year, exchange water for the conditioning tanks will need to be pre-chilled or pre-heated prior to use in order to avoid a temperature shock.

Pre-chilling requires a separate raceway or dedicated reservoir tank and another chiller. Pre-heating can usually be accomplished by bringing the water into the hatchery 24–48 hours ahead of time to let it warm to room temperature, using an auxiliary chiller, if necessary, to prevent its getting warmer than desired.

When the water in the conditioning tank is exchanged (at least 3 times a week), the trays of clams should be thoroughly washed with cool tapwater to remove silt, feces, and pseudofeces. This also helps to prevent any fouling organisms from growing on the clams or trays. If possible, the clams should be moved to a different tank at this time. That will allow the dirty tank to be thoroughly washed and air-dried. If a spare tank is not available, remove the clams from the tank long enough to wash it with hot water. Refill the tank with the exchange water which has been brought to the proper temperature.

Handle the clams gently during this entire process. Any shock may cause ripe clams to spawn spontaneously when returned to water. Do not hold the broodstock out of water longer than 15–30 minutes and keep them cool during this time (~20–22°C). If there is a problem necessitating that the clams be out of water for longer than that, or if your ambient temperature is >22°C, they may be refrigerated. Make sure to cover the clams with damp newspaper or equivalent in the refrigerator to prevent dehydration. It is preferable to have a clean tank ready and transfer the clams directly without holding them out of water.

Once a week, the conditioning tank should be scrubbed and washed with dilute bleach (e.g. 10 ml of domestic bleach such as Clorox, diluted in 4 liters of water). After bleach treatment, the tank must be thoroughly rinsed to remove all traces of chlorine. If necessary, sodium thiosulfate may be used to neutralize the chlorine.

Once a month the recirculating lines to the chiller should be sterilized by flushing with bleach. Fill the tank with freshwater, add bleach (0.5-1 ml/liter of water), and circulate this mixture through the system for at least 30 minutes. The bleach should then be neutralized (0.5-1 g sodium thiosulfate per 10 liters water). Continue to recirculate the water for 30 minutes to insure that there is no residual chlorine before draining the lines. The tank and the plumbing should be flushed with seawater prior to use.

## Food requirements

There are many algal species which may be cultured for feeding to broodstock (see Appendix D). It is best to feed more than one type of algae as a single species may not be a nutritionally complete diet for the clams. At MRRRI we commonly culture the Tahitian strain of the naked flagellate *Isochrysis galbana* (T-Iso) and diatoms (*Chaetoceros* spp). We have found these to grow reliably under the light and temperature conditions we are able to maintain.

Broodstock clams require large quantities of algae in order to produce and maintain ripe gonads. Depending on physiological and environmental conditions, the requirements may be  $1-3 \times 10^9$  (1-3 billion) algal cells/clam/day. If this entire ration were fed at one time it would result in very high food concentrations (up to 750,000 cells/ml). Algal cells in such high concentrations may actually interfere with feeding. The clams produce copious pseudofeces until the concentration is decreased to a manageable level. Thus, much of the algae is wasted, and the clams may not receive enough food. To avoid this, the daily ration must be fed in smaller doses. This can be accomplished by dividing the daily ration into several smaller "batches", by continuous addition of food at a very slow rate (e.g., with a peristaltic pump), or by discontinuous metering with a larger volume pump. **Table 5** illustrates calculation of daily rations for broodstock and feeding volumes for the batch and continuous methods.

When more than three batches per day would be required, it is more convenient to use some sort of automatic food addition. An initial batch feeding should be fed to bring the concentration in the brood tank to 100,000 cells/ml. Algae is then added slowly to maintain this level and provide the daily ration over a 24 hour period (**Table 5**). There are a variety of metering pumps on the market, or you could fill a head tank with the algae and let it drip in through a small valve. An experienced operator can judge whether the algal addition rate is appropriate from the color of the water. If available, a fluorometer provides a quick means of checking the algal level in the conditioning tank. Counts may also be made with a hemocytometer (refer to section on Algal culture for details).

The broodstock clams should be observed several times a day to determine if they are feeding (siphons extended, feces present). If the clams are not feeding, there may be insufficient food, excess food, or poor water quality.

## SPAWNING

### Determination of ripeness

When broodstock have been adequately conditioned (2-8 weeks depending on time of year and initial state of the broodstock) they may be induced to spawn. To determine if the clams are ready to spawn, a few individuals are sacrificed and dissected to observe gonadal state. Carefully open the

clam, taking pains not to tear the body. Peel back the mantle (**Fig. 9**). A ripe clam will have a creamy white gonadal mass covering the gut area. A ripening, but not yet ready, clam will have a network of white tissue in this area, looking rather like

white veins. An unripe clam is tan or grayish in color and the dark gut may be visible through the watery outer tissue. If the clam is very ripe, puncturing or slicing the gonad area will cause gametes to ooze out.

**Table 5.**  
**Calculating daily ration for broodstock**

**Variables (examples given):**

- A. # of broodstock = 50
- B. Size of tank = 200 liters (4 liters per clam)
- C. Density of algal culture =  $5 \times 10^6$  cells/ml

**Constants (from Table 2):**

- D. Consumption per clam = 3,000,000,000 ( $3 \times 10^9$ ) algal cells/day
- E. Minimum desired concentration in tank = 100,000 cells/ml = 100,000,000 cells/ liter =  $1 \times 10^8$ /L
- F. Maximum desired concentration in tank = 200,000 cells/ml =  $2 \times 10^8$ /L

**Calculate daily ration:**

- G. Total daily ration =  $A \times D = 50 \times (3 \times 10^9) = 150 \times 10^9 = 1.5 \times 10^{11}$

**Calculate batch feeding:**

- H. Maximum single batch =  $B \times F = 200 \times 200,000,000 = 40,000,000,000 = 4 \times 10^{10}$
- I. Number of batches needed =  $G \div H = \text{Total ration} \div \text{batch size} = (1.5 \times 10^{11}) \div (4 \times 10^{10}) = 3.75$  (round to 4)
- J. Batch volume =  $H \div C = (4 \times 10^{10}) \div (5 \times 10^6) = 8 \times 10^3 \text{ ml} = 8,000 \text{ ml} = 8 \text{ liters}$

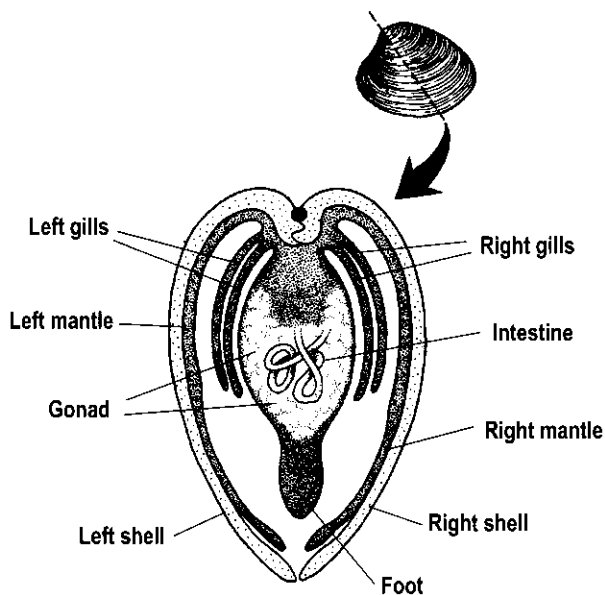
**Calculate automatic (metered) feeding:**

- K. Background amount (initial batch) =  $B \times E = 200 \times (1 \times 10^8) = 2 \times 10^{10}$
- L. Initial batch volume =  $K \div C = (2 \times 10^{10}) \div (5 \times 10^6) = 4 \times 10^3 \text{ ml} = 4 \text{ L}$
- M. Remaining ration =  $G - K = (1.5 \times 10^{11}) - (2 \times 10^{10}) = 1.3 \times 10^{11}$
- N. Hourly ration =  $M \div 24 = (1.3 \times 10^{11}) \div 24 \approx 5 \times 10^9$
- O. Hourly volume to feed =  $N \div C = (5 \times 10^9) \div (5 \times 10^6) = 1,000 \text{ ml} = 1 \text{ L}$

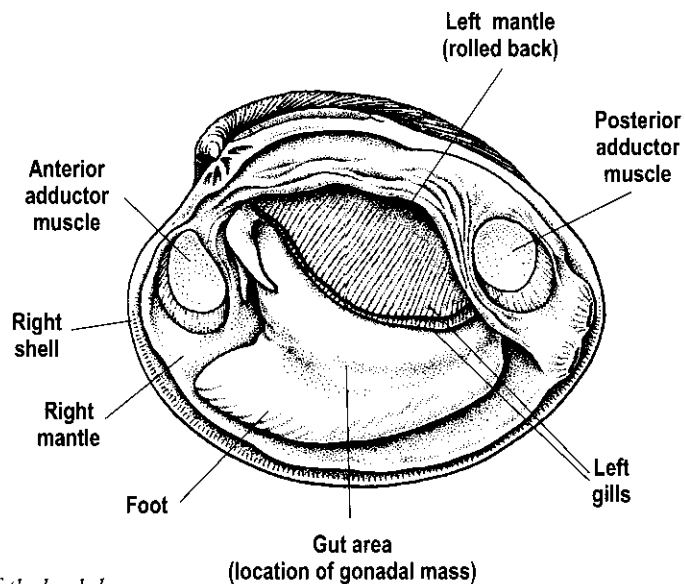
**The hourly ration may be added all at once with a timed metering pump or continuously with a peristaltic or other low-volume metering pump. For continuous addition, calculate the flow rate per minute:**

- P. Continuous flow rate =  $O \div 60 = 1,000 \div 60 \approx 17 \text{ ml/minute}$

A.  
GENERALIZED  
CROSS SECTION  
THROUGH MIDDLE  
OF CLAM



B.  
VIEW OF CLAM  
WITH LEFT SHELL  
REMOVED



**Figure 9.**  
*Internal anatomy of the hard clam*



Gonadal smears should be examined microscopically to determine whether sperm are active and eggs are fully developed. To make a smear, slice the surface of the gonad with a sharp knife, and rinse the oozing gametes into a small beaker using a washbottle filled with filtered seawater. Place a small drop of this solution on a microscope slide and observe at 100X (10X ocular, 10X objective). Mature ova will be well rounded and uniformly opaque. Less mature ova may be tear-drop shaped, stalked, and/or irregularly shaped with a transparent area. The larger the transparent area the less mature the egg. Mature sperm should be actively swimming. Sperm often need to be diluted with seawater and/or warmed slightly before they appear active. It is desirable to examine at least one female, as males tend to become ripe sooner.

### **Spawning preparations**

Preparations for spawning should be made a day in advance. The broodstock to be used are removed from the conditioning tank, scrubbed and rinsed with freshwater to remove any algae or fouling organisms. They may also be dipped for a short period (5–10 minutes) in a dilute chlorine bath (1 ml clorox/liter tap water, NOT seawater) and rinsed well. After cleaning, the broodstock are stored overnight in a refrigerator or insulated cooler. The clams should be covered with damp newspaper or something similar to prevent dehydration. If they are stored in a cooler, 1–2 frozen gel packs, wrapped in dry newspaper, may be placed on top if necessary to maintain a cool temperature.

If more than one stock is to be spawned, make sure that each group is clearly labelled. It is a good idea to measure the potential spawners and record any identifying codes. This information is essential if you are trying to develop your own improved broodstock. After spawning you will want to update the information to show which clams actually spawned, their sex, and possibly their production of gametes.

### **Spawning induction**

Clams are induced to spawn by varying the water temperature. If you have a source of heated and chilled seawater, the clams are simply placed in a shallow trough (referred to as a spawning table) which can be flooded with seawater of the desired temperature. A simple way to preheat and prechill the seawater is to use plastic trash cans or similar reservoirs with either immersion heaters or frozen gel packs. The spawning table is alternately filled with this pre-heated or pre-chilled water to accomplish the temperature changes needed to induce spawning. A submersible pump may be used for transferring the water.

An alternative method of heating and chilling the seawater is to use a water bath. This will also be necessary if the clams need to be isolated in order to control fertilization. The clams are placed in small containers (e.g. 1 liter plastic beakers or glass bread pans) of finely filtered seawater (1  $\mu$ m). The containers may hold one or more clams depending on the breeding

requirements of the spawn. Glass containers will transfer heat better, but plastic will also work. Never use metal containers for spawning. The containers are placed on the spawning table, which is essentially a table with raised sides and a standpipe at one end for adjusting water depth and draining the table. A mesh or slats under the containers will allow water to circulate under the containers, speeding heat transfer, and can also be used to adjust depth of the containers to prevent floating. The table is then filled with hot or cold tap water. The temperature of the bath will need to be higher (or, for chilling, lower) than the desired seawater temperature. Monitor the temperature in the spawning containers closely. When heating, care must be taken to drain the bath water before the temperature in the seawater containers reaches the target temperature because the water may drain more slowly than the heat transfers to the seawater.

Fill the spawning table or individual containers initially with cool water (~20°C) that has been finely filtered. Allow the clams to acclimate and begin siphoning before starting to manipulate the temperature. After the clams begin siphoning, water temperature should be gradually increased to 28–30°C (max 32°C) over a period of 20–30 minutes by running hot water on the table. Let the water remain warm for 20–30 minutes unless the clams appear stressed. (Stressed clams usually stop pumping and close their valves.) At the end of this period, rapidly cool the clams to 20–22°C. This process is repeated until spawning occurs. Very ripe clams

may spawn on the first temperature increase. Less ripe clams may require several shocks before spawning. If necessary, clams may be further stimulated by introduction of sperm or egg wash. These are obtained by sacrificing a clam, scoring the gonad, and rinsing the gametes into a beaker with seawater. If the sacrificed clam is a male, the sperm may be used directly, or, if control of fertilization is desired, may be pasteurized by heating to 60°C for 10 minutes. The pasteurized sperm should be cooled and observed microscopically to verify deactivation (no movement) before using. If the sacrificed clam is female, pour the eggs through a 20 µm screen, catching the water (egg wash) in a second beaker. To use the sperm or egg wash as a stimulus, use a small pipette or eye dropper to slowly add a small stream of the solution near the incurrent siphon of each clam.

As the clams spawn, their sex may be visually determined. Sperm disperses rapidly in the water, looking a lot like a trace of smoke. The eggs disperse more slowly and have a granular appearance, somewhat like salt or sugar crystals. Eggs often settle on the bottom in mounds. Samples may also be removed and examined microscopically if there is any question of sex.

### **Handling of gametes/ Fertilization**

When an individual begins spawning it should be removed from the spawning table. If you are using individual containers, simply remove the container from the

water bath. Otherwise, place the spawning clam in a beaker of clean water. The individual may continue to spawn for up to an hour. During this time, it may be necessary to transfer the spawner to another clean container. When the clam is no longer releasing gametes, it should be removed from its container, marked in some fashion, and placed aside.

After the spawner is removed, wash the gametes to remove any contaminants (feces, etc.). Eggs are approximately 70 µm in diameter. Sperm is much smaller. Rinse the gametes on a screen which will allow the gametes to easily pass through but will retain larger particles. A 150 µm mesh will work equally well for sperm or eggs.

If the clams have been spawning in a common container, the eggs are probably already fertilized. However, if the spawners have been isolated in individual containers, it will be necessary to fertilize the eggs by adding some sperm. Depending on the desired outcome, there may be different protocols for pooling eggs and sperm prior to fertilization. In the simplest scenario, all eggs are pooled in one container and all sperm in another. A suitable container may be a 20 liter bucket. Add additional filtered seawater to reduce the density of the eggs. Although fertilization is most rapid if the eggs are concentrated (densities up to 2,000/ml), they should not be held at such high densities for longer than 30 minutes. If the eggs are to be held for an extended period of time prior to fertilization they should be diluted to less than 200/ml. If eggs must be held longer than an hour,

they should be gently aerated or collected on a 20 µm sieve and transferred to a new container of filtered seawater once an hour.

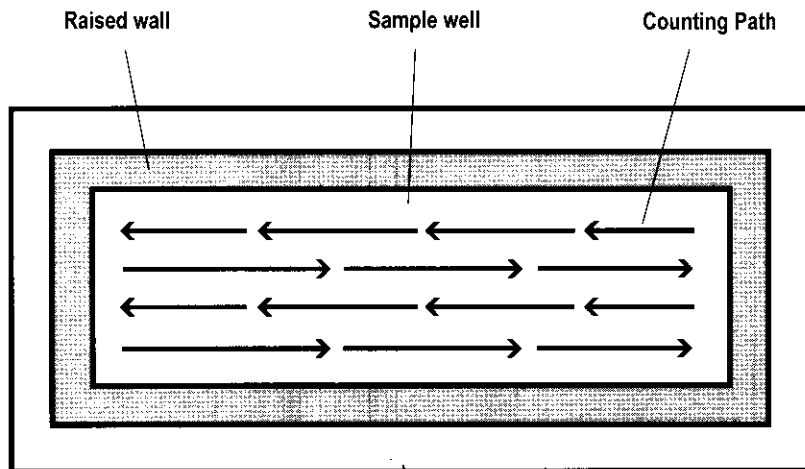
Fertilization is a simple matter of mixing sperm and eggs together. However, it is important not to use too much sperm, which might result in polyspermy with resultant abnormal development. It is therefore advisable to quantify the gametes (eggs and sperm) in order to determine an appropriate amount of sperm suspension to use for fertilization.

The quantity of eggs may be determined with a Coulter® counter, if available. However, most often it will be necessary to count subsamples of the eggs, using a light microscope. If the egg concentration is greater than 200/ml, make a diluted sample prior to counting. Estimate your number of eggs to determine if dilution will be necessary, using the formula:

$$\frac{(\# \text{ females spawned} \times 1 \text{ million})}{\text{volume of water in egg container}} = \# \text{ eggs/ml}$$

1 million is a conservative estimate for a single female's production. The actual density may be considerably higher.

If your estimated density is less than 200/ml, take a sample directly from your container of pooled eggs. Otherwise, dilute the eggs prior to sampling, as described below. Thoroughly mix the eggs with a specially made plunger. The plunger is a flat plastic disk with holes drilled in it, attached to a cylindrical handle. We have found no vendor for this product and



**Figure 10.**

*Sedgewick-Rafter chamber for counting larvae*

manufacture our own. Different sized plungers should be on hand for different sized containers. The plunger must be large enough to thoroughly mix the entire container, but must also be able to move up and down freely in the container without causing splashing. Mix thoroughly by moving the plunger up and down for 15–20 strokes. For sampling consistency, it is a good idea to always use the same number of mixing strokes each time. As soon as you make the last stroke, immediately remove a 1 ml sample using a pipette. For greatest repeatability, use a 1 ml autopipet. You will need to make at least three counts. If enough counting chambers are available, you may take all three samples at once.

If the eggs need to be diluted, mix as described above and remove an aliquot and

place it in a graduated container. Fill the container with filtered seawater to the desired level. A graduated cylinder will be most accurate, but a graduated beaker will also work. The size of the aliquot and the size of the graduated container depend on the dilution needed and the availability of sampling equipment. For instance, if you estimate your egg density to be 1000/ml, you need to make at least a five-fold dilution (in order to count ~200 per sample). You might accomplish this in several ways:

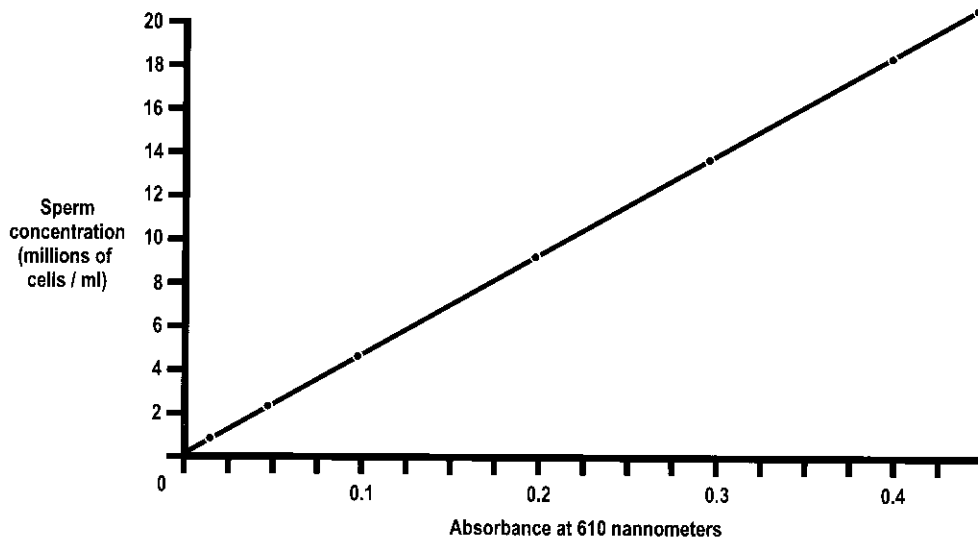
5 ml sample + 20 ml water in  
25 ml container  
50 ml sample + 200 ml water in  
250 ml container  
200 ml sample + 800 ml water in  
1000 ml container

depending on the sizes of containers you had available. The diluted sample is mixed as described previously, but with a smaller plunger, and 1 ml samples are removed for counting. After counting, multiply by the dilution factor (5 in the example above) to find the original concentration.

The samples are counted with a compound microscope and a Sedgewick-Rafter counting chamber (**Fig. 10**). This is a special microscope slide with raised walls which create a well which holds exactly 1 ml with a coverslip in place. Some models have a built-in grid which greatly facilitates counting. Count the number of eggs using a compound microscope at the lowest magnification. Starting at the lower left corner, move the slide to the right until an entire strip the width of the slide has been counted. Then carefully move the

slide down and count the next strip, continuing in this fashion until the entire sample has been counted. Count three samples and average them to determine the concentration of eggs (#/ml). If a dilution was made, multiply your average count by the dilution factor to get the original concentration. For example, if a five-fold dilution resulted in counts of 90, 95, and 100, the density in your dilution chamber is 95/ml and the density in your original container is 475/ml ( $95 \times 5$ ).

Sperm may be quantified with a spectrophotometer, if available. Place a small quantity of sperm suspension in a sample cuvette. Set the spectrophotometer on 640nm and read the absorbance. **Figure 11** shows the relationship of absorbance to sperm concentration (from Bricelj, 1979). Add sperm to the pooled eggs at a rate of



**Figure 11.**

*Regression for determination of sperm concentration using a spectrophotometer*

1000–2000 sperm for each egg. If the sperm concentration is unknown, add sperm in small aliquots and microscopically observe a sample from the egg pool to determine if enough sperm have been added. If the eggs are in a relatively small volume of water, use aliquots of 5–10 ml of sperm. If the eggs have been diluted in a larger container, you will need to add proportionally more sperm. For example, in a 1 liter container, 5 ml is a good aliquot to try. In a 4 liter container, use 20 ml, in a 10 liter container 50 ml. Mix gently with a plunger to speed contact between sperm and eggs. After 5–10 minutes, remove a 1 ml sample, place on a microscope slide, and examine at 100X. If eggs are fertilized, one or more sperm may be observed clinging to the gelatinous sheath surrounding the egg. If fertilization occurred more than 15 minutes prior to examination, the first polar body may be apparent as a small bump protruding from the side of the egg. The first cell divisions occur approximately 30 minutes after fertilization. If fertilization is not apparent within 15–20 minutes, add another aliquot of sperm and re-observe.

Shortly after the eggs are fertilized, the sperm should be removed by straining the eggs on a 20  $\mu\text{m}$  sieve. The sieve will retain the eggs (~70  $\mu\text{m}$  in size) but allow the smaller sperm to pass through. Rinse the eggs gently with seawater and transfer to a container of clean filtered seawater.

Unfertilized gametes deteriorate fairly rapidly. It is most desirable to have the eggs fertilized within an hour after the female completes spawning. If you are

fortunate, enough broodstock spawn in a relatively short period of time and fertilization may be accomplished within that hour. This is often not the case, however, and gametes from early spawners must be held for some period of time before fertilization. Eggs are best held at room temperature, while sperm should be chilled. The longer the gametes are held, the lower the chances of producing viable zygotes. **Table 6** illustrates the effect of storage on eggs and sperm. To derive the data in **Table 6**, newly spawned eggs were challenged with stored sperm and stored eggs with newly spawned sperm. If both eggs and sperm have been stored, survival would be expected to be lower. If at all possible, fertilization should be accomplished within 1–2 hours of spawning. After that, production of viable larvae will be less than 50%. If gametes must be held longer than 1–2 hours, it is advisable to increase the quantity of sperm used for fertilization and to keep more eggs than you would otherwise (to offset lowered viability).

### Stocking Larval Containers

As soon as possible after fertilization, the quantity of zygotes is determined. If the eggs were counted previously and the concentration has not been changed by dilution, the egg count is adequate. Otherwise, the fertilized zygotes should be counted in the same manner as used for counting eggs.

The zygotes are now ready to be placed in culture containers. Culture containers may range from 1 liter beakers to 12,000 liter

**Table 6.**  
**Fertilization success (%) after gamete storage of**  
**varying duration at two different temperatures**

Hours	Eggs		Sperm	
	4 °C	22 °C	4 °C	22 °C
0.5	90	90	90	90
1	50	60	60	50
2	30	50	40	55
3	25	45	50	35
4	15	40	40	15
5	0	35	35	0

tanks, depending on the quantity of larvae you wish to raise. A universal requirement for culture containers is that they be of non-toxic, inert materials. It is desirable for the containers to have a very smooth inner surface, which will facilitate removal of the larvae and cleaning. Larval culture containers may be plumbed with a drain valve at the bottom. Conical bottomed containers with a central drain are easiest to drain and clean. If not plumbed, water and larvae will have to be removed by siphoning.

The culture containers should have been previously cleaned and stored dry. If they have been stored for more than 24 hours, rinse with hot water followed by seawater prior to use. Fill the containers with finely filtered seawater at room temperature. The zygotes are stocked in the culture containers at a density of 20-30/ml. Thus, a 100 liter culture container can be stocked with 2 million to 3 million zygotes.

Using the previously determined density in the zygote container, calculate the quantity to add to each culture container:

$$\frac{\text{vol. of culture container (ml)} \times \text{desired density (\#/ml)}}{\text{density of zygotes (\#/ml)}} = \text{volume to add (ml)}$$

The zygotes should be mixed thoroughly with the plunger before removing the appropriate aliquot, which is transferred immediately to the already filled culture container. Culture containers should be labelled with the spawning date, stock identification, stocking density, and total number of zygotes in the container. Covers may be placed loosely on the cultures to avoid airborne contamination. No aeration is necessary and no food is added since the developing larvae do not feed until the veliger stage is reached.

---

## Handling of broodstock after spawning

Update your records to indicate which clams spawned and their sex. If desired, label individual clams. If they were not previously measured, do so now. Record all information in the broodstock ledger and in a separate log for this particular larval group. This information will be needed later for determining if the individuals are particularly desirable as broodstock.

None of the broodstock, regardless of whether they spawned or not, should be returned to the conditioning tank at this time, because they might spawn and cause all other clams in the tank to spawn. If the broodstock are to be used again, they may be held in a tank by themselves at cool temperature for at least 24 hours to assure that they are not continuing to spawn, prior to returning them to the conditioning tank. If nursery space is available, the broodstock may be temporarily placed there, if temperatures are appropriate. From there, they may be returned to conditioning or transferred to field culture units as the schedule permits. If a nursery or quarantine tank is not available, the clams should be refrigerated until they can be replanted in labelled field cages.

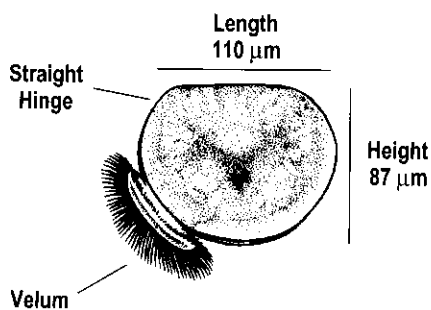
## LARVAL CULTURE

The day after spawning, the larval cultures should be checked for the presence of veligers. Veligers can be expected 18–24

hours after fertilization. Veligers are recognized by their distinctive “D” shaped shells (**Fig. 12**). Healthy veligers will actively swim and spend a large proportion of the time near the surface of the culture container. A small sample removed from the surface without disturbing the culture should be adequate to determine if veliger stage has been reached. (Such a sample, however, will not provide any indication of survival or density in the culture.) As soon as veligers are present, the larvae must be fed. Enough algae should be added to the cultures to give a density of 25,000 algal cells/ml. Since the cultures were stocked with zygotes at 20–30/ml, this will yield about 1000 algal cells per larva. This is sufficient if the water is changed later that day. Otherwise, another batch of algae may need to be added towards the end of the day.

The veligers should be allowed to “harden up” for at least 6 hours before handling. During this time the survival can be determined. Counts may be taken directly from the containers after thorough mixing with a plunger. Three counts per culture should be made. The larvae are examined at low power (4X objective) for general condition—activity level, shape, etc. Any unusually shaped larvae should be noted, as this is often a sign of contamination or disease. Also note the presence of any ciliates or other contaminating organisms, and any pre-veliger larvae (trochophores or earlier larval stages). Presence of contaminating organisms means that your culture water needs to be more carefully filtered. Presence of pre-veliger stages is an indication that development is delayed. This

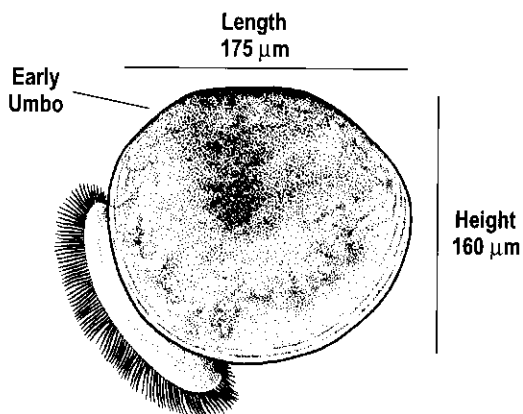




#### STRAIGHT HINGE VELIGER

Age range: 1 – 5 days

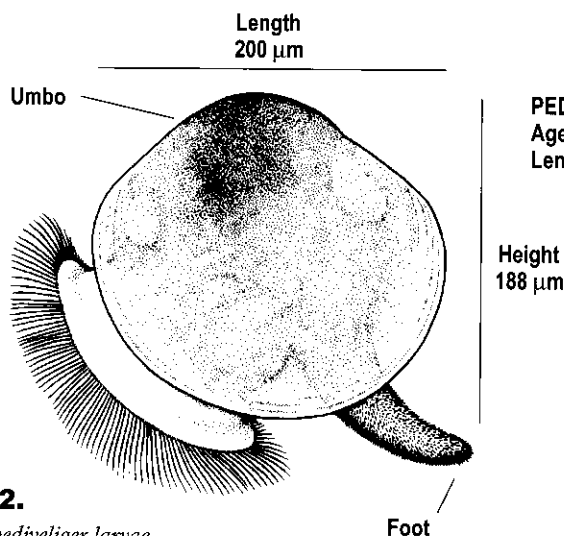
Length range: 90 – 140  $\mu\text{m}$



#### UMBONED VELIGER

Age range: 3 – 15 days

Length range: 140 – 220  $\mu\text{m}$



#### PEDIVELIGER

Age range: 8 – 20 days

Length range: 170 – 230  $\mu\text{m}$

**Figure 12.**

*Veliger and pediveliger larvae*

could be due to low temperature, but may also be a sign of poor quality zygotes, contamination or disease. After making live observations, add a drop or two of 10% formalin (4% formaldehyde) to the slide to kill the larvae so they may be counted. The counting process is identical to that already described for egg enumeration in the previous section. Survival rates may vary from 0–100% but are usually between 25% and 90%. If larval survival in repetitive runs is consistently less than 50% there may be a water quality problem. All observations and measurements should be recorded in a log book.

After the larvae have been allowed to harden up, the water should be changed and the larval density reduced if necessary. The culture water is drained through a 45–55  $\mu\text{m}$  plastic mesh sieve which will retain the larvae. Most hatcheries construct their own sieves of varying mesh sizes and diameters. A typical sieve consists of a short length of PVC pipe, 4–12 inches (10–30 cm) in diameter with a nylon or polyester mesh stretched across one end. Polyester is preferable as nylon tends to absorb water and swell, thus reducing the size of the pores. Sieves should be constructed in a range of sizes from 20  $\mu\text{m}$  to

**Table 7.**  
**Larval culture requirements**

<b>Culture method:</b>	batch culture
<b>Temperature:</b>	24–28°C optimal; 20–30°C acceptable
<b>Water:</b>	finely filtered (1 $\mu\text{m}$ ) seawater pretreatment with charcoal or UV if water quality is suboptimal complete exchange at least 3 times a week, daily if possible
<b>Salinity:</b>	25ppt or higher
<b>Stocking rate:</b>	zygotes: 20–30/ml early veligers: 5–10/ml late veligers: 1–2/ml
<b>Feed type:</b>	early veligers: small flagellates (e.g. <i>Isochrysis</i> , <i>Monochrysis</i> ) mid-late veligers: small flagellates + diatoms (e.g. <i>Chaetoceros</i> , <i>Skeletonema</i> )
<b>Feed rate:</b>	10,000–25,000 cells/ml of culture water (background) plus: early veliger: 1000–5000 cells/larva/day mid-veliger: 5,000–10,000 cells/larva/day late veliger: 15,000–30,000 cells/larva/day pediveliger: 30,000–50,000 cells/larva/day
<b>Feed method:</b>	batch, continuous drip, or discontinuous metering
<b>Records:</b>	temperature, salinity, feed rate/type; larval counts/measurements at least weekly
<b>Duration:</b>	7–21 days

500  $\mu\text{m}$ . Above 500  $\mu\text{m}$ , commercially available stainless steel or brass sieves may be used.

The sieve should be sitting in a pan of seawater to avoid impinging larvae on the mesh during the draining process. If previous observations indicated poor survival or presence of contaminants, it may be desirable to avoid complete draining of the culture. Since healthy larvae should be actively swimming, it is possible to siphon off most of the culture, with the healthy larvae, and leave the last few centimeters of water which will contain most of the dead or dying animals.

The screen containing the veligers is gently rinsed with seawater and the clams are transferred to a beaker or other appropriate calibrated container filled with filtered seawater. If the original counts were very low, additional counts can be made from this container to determine the survival. Twenty-four hour old larvae may be cultured at 10/ml; however, by 72 hours they should be at a density of only 5/ml, so it may be desirable to thin them to 5/ml at this point in order to avoid having to thin again in 2 days. An appropriate aliquot (to yield the desired new density of 5–10/ml) is transferred to a clean culture vessel already filled with seawater. The remaining veligers may be cultured in additional containers if space permits; otherwise they are discarded.

## Larval culture routine

The larval stage may last 7–21 days, but 10–12 days is probably average. During this time, the water in the culture containers should be changed daily if possible, but at least three times a week. The seawater used for changing must be the same salinity and temperature as the current culture water and should be finely filtered (1  $\mu\text{m}$ ). A convenient way to accomplish this is to have a large indoor reservoir for settling/holding seawater. If this tank is kept full, there will always be pre-settled, room temperature seawater available for changing the larval cultures. The water from the settling tank should be finely filtered immediately before use. Depending on the quality of the source water, it may be necessary to further treat it prior to use for larval culture, as described previously.

As the larvae grow, the mesh size of the sieve used for water changes may be increased. This will reduce the tendency for the sieve to clog and overflow and will remove dead larvae and associated bacteria. Separate screens should be used for each culture container to avoid cross-contamination. Alternatively, screens should be rinsed with hot tap water between cultures or washed with detergent and rinsed. Rinse the clams with a gentle stream of seawater to remove excess food and transfer them to a beaker or other calibrated container for microscopic examination, counts and measurements. The veligers should not be kept at high densities for extended periods of time. Only a few larval containers should be

---

drained at a time so that no groups are out of the culture vessels for a long time.

The larvae should be examined microscopically to determine general condition, survival and size. Take 1 ml samples as described previously. Make notes of gut color, shell shape, shell state (clean or fouled), activity level, and proportion of empty shells. Healthy larvae should be actively swimming and have dark gut color. The presence of ciliates in the culture is a strong indication of bacterial contamination and warrants paying more attention to cleanliness and water treatment. Ciliates are single celled animals which feed on bacteria and decaying tissue. The most common ciliates observed in clam cultures are oval-shaped and range in size up to about 20  $\mu\text{m}$ .

After making live observations, add a drop of 10% formalin to the slide to facilitate counting and measuring. After counting all individuals in the sample, measure 25 using an ocular micrometer. An ocular micrometer is a precision ruler which fits into the eyepiece of the microscope. Make sure to record the magnification at which the measurements are made. Healthy larvae grow at a rate of 10–20  $\mu\text{m}/\text{day}$ .

By the third day, cultures should be at densities no higher than 5/ml. If necessary, thin the cultures when the water is changed. When the larvae are a week old, the density should be reduced to 1–2/ml. If the veligers need to be thinned, make at least 3 counts to get a good estimate of density and then follow the procedure as

for day one thinning. If there is a large amount of shell which is smaller than the live animals, the clams may be rinsed through a series of screens to remove the shell. A rule of thumb is to multiply the nominal screen size by 1.5 to arrive at the minimum size larva which will be retained on that screen. For example, a 100  $\mu\text{m}$  screen will retain larvae that are at least 150  $\mu\text{m}$  long.

When the water is changed, the culture containers should be cleaned. If extra containers are available, larvae may be moved to new containers. Otherwise, the containers must be cleaned while the larvae are being examined. The containers should be scrubbed with hot water and a laboratory grade detergent (e.g. Alconox) which does not leave a residue. Rinse with filtered seawater prior to refilling. Containers should be sterilized with bleach once a week. If diseased larvae are observed in the cultures, daily chlorination may be warranted. Sterilization may be accomplished by filling the container with tap water and adding bleach or by scrubbing with a small amount of dilute bleach (10 ml/liter) and rinsing thoroughly with hot water and culture-grade seawater. Be particularly careful to remove all chlorine before reusing the containers. Always sterilize containers between larval runs and after long-term storage or disuse.

Cultures should be observed daily to assess the health of the larvae. Things to note are clearance rate (how rapidly is the algae removed from the water), activity level, and fouling of the containers. A reduction

in clearance rate may indicate poor health or mortality and warrants more careful examination of the larvae and possibly efforts to remove any dead or dying individuals by selective screening. Veligers often congregate near the surface of the water in tight clusters. Larvae may be observed trailing just below these clusters in a formation which resembles a tornado funnel. This behavior is known as "rafting" and is a sign of healthy larvae. Fouling of the culture containers is cause for immediate concern and warrants more attention to cleaning during water changes. Of particular concern are any pink areas on the walls or bottom of the container, which are evidence of bacterial colonies. Any affected containers should be thoroughly chlorinated and the holding tanks, transfer pumps and hoses should all be examined and cleaned to eliminate possible sources of the contamination. The seawater supply may need to be sterilized (e.g. with ultraviolet light). A slimy film on culture containers often indicates overfeeding or feeding of contaminated algae.

## Feeding

It is very important to give the larvae enough food. However, as previously noted for broodstock, excessive food concentrations are counter-productive. The consumption rate increases dramatically as the veligers grow, but this is offset by reductions in density due to mortality or intentional thinning. Estimated consumption rates are listed in Tables 2 and 7. There should always be a background level of 10,000–25,000 algal cells per ml of

culture water. If the algal concentration falls below 10,000 cells/ml, the larvae must expend too much energy finding food. In addition to the background level, feed an amount equal to the expected consumption rate of cells/larva/day.

Food may be added in discrete "batches" or on a continuous basis. If added in batches, each batch should result in a density in the culture of 25,000–50,000 cells/ml. If using a continuous feed, an initial batch is added to bring the density to 10,000 algal cells/ml and the remaining ration is added slowly over a 24 hour period, as described previously for broodstock conditioning. Batch feeding amounts are calculated in the same manner as for broodstock (see Table 5). Two or three batches a day will probably be needed. Algal cultures should be as dense as possible, preferably 5 million or more cells/ml. After the food has been added, culture water may be examined microscopically, using a hemacytometer, to verify the algal density. If a fluorometer is available, it provides a quick means of verifying algal concentrations.

For the first week to ten days, feed the larvae small flagellates such as *Isochrysis*. Towards the end of the larval period, diatoms (e.g. *Chaetoceros*, *Skeletonema*) can be fed in addition to the flagellates. Initially diatoms are included as 25% of the daily ration (by cell count), gradually increasing to 50%. Since the diatoms are larger than the flagellates, the total cell count needed may decrease. For example, *Chaetoceros* is approximately 1.5 times the

size of T-Iso. If the total ration in terms of T-Iso would be 60,000 cells, a 50:50 ration would be 30,000 T-Iso cells plus 20,000 *Chaetoceros* cells ( $30,000 \div 1.5$ ).

### Metamorphosis (setting)

At a size of 180–200  $\mu\text{m}$ , larvae begin the metamorphosis to the adult form (**Fig. 12**). For a short period (2–5 days), the larvae will have both a foot and a velum. While still capable of swimming, they will spend a large part of the time crawling on the bottom and sides of the culture container. This intermediate stage is known as “pediveliger”. Pediveligers secrete sticky byssal threads which help them to cling to surfaces. As a result they are more difficult to remove from the culture containers and are virtually impossible to count. Pediveligers may be rinsed with freshwater during water exchange. This will make removal of the sticky pediveligers easier, will help to reduce contamination by ciliates, other zooplankters, and surface-coating bacteria, and will facilitate removal of excess food, pseudofeces, etc. However, never leave the larvae sitting in freshwater. Make sure the water is cold and use a gentle spray from a nozzle. If freshwater is used to rinse the pediveligers from the sieve, it should be carefully decanted from the container immediately and replaced with seawater.

The short pediveliger stage is quickly followed by complete metamorphosis as the larvae lose their swimming capability and “set”. Metamorphosis is very stressful and often a large proportion of the popula-

tion may die during this period. Survival is improved if larvae are transferred to the post-set culture system when they reach the pediveliger stage. Sieve the larvae using a 118–150  $\mu\text{m}$  screen, at least as large as the screens in use in the post-set system (see description below). The clams retained on the screen are moved to the post-set system and the smaller ones are returned to larval culture.

## POST-SET CULTURE

### Culture system

A variety of different post-set culture systems can be used. The one described here is a recirculating system which uses downwelling cylinders initially and upwellers later. A typical cylinder (often referred to as a “silo”) is constructed from plastic pipe 4–12 inches (10–30 cm) diameter and 12–18 inches (30–45 cm) long with a plastic mesh screen glued to one end. Thus, a silo resembles the sieves previously described, but is usually deeper. Silos may be constructed with removable screens so that the mesh size can be increased as the clams grow (**Fig. 13a**).

The silos are designed to have an airlift attached (**Fig. 13**). At the upper end of the silo, a coupling or short length of pipe is installed through the wall of the cylinder. The distance from the top of the cylinder is not critical, but in operation the coupling should be at or just above the water level. All silos should have the coupling in the same position to eliminate the need for

**Table 8.**  
**Post-set culture requirements**

<b>Culture system:</b>	recirculating downweller/upweller
<b>Temperature:</b>	24-28°C ideal; anything below 30°C acceptable
<b>Water quality:</b>	for post-set in size range: 200-500µm: finely filtered (e.g. 1 µm bag); additional UV sterilization or charcoal filtering may be provided 500µm-1mm: slow flow ambient water may be added for gradual acclimation to non-hatchery conditions
<b>Salinity:</b>	>25ppt
<b>Feed type:</b>	mixed diatoms and flagellates, approximately 50% diatoms
<b>Feed rate:</b>	background level of 25,000-50,000 cells/ml plus allowance for consumption
<b>Record-keeping:</b>	temperature, salinity, feed type, feeding rate; weekly volumes, measurements; daily observations
<b>Duration:</b>	4-6 weeks

individually adjusting silo heights in the reservoir tank. An airlift attaches to the coupling and may be installed outside the cylinder (downweller) or inside (upweller). A typical airlift consists of an elbow and a length of PVC pipe (**Fig. 13**). For upwellers, the diameter of the cylinder must be great enough to accommodate the coupling and airlift. The minimum practical cylinder diameter is 4 inches (10 cm), but 6 inches (15 cm) is easier to handle. For small diameter cylinders, the airlift should be constructed of 1/2–3/4 inch plastic pipe. Larger diameter cylinders (e.g. 12") can have 1 1/2 inch diameter airlifts.

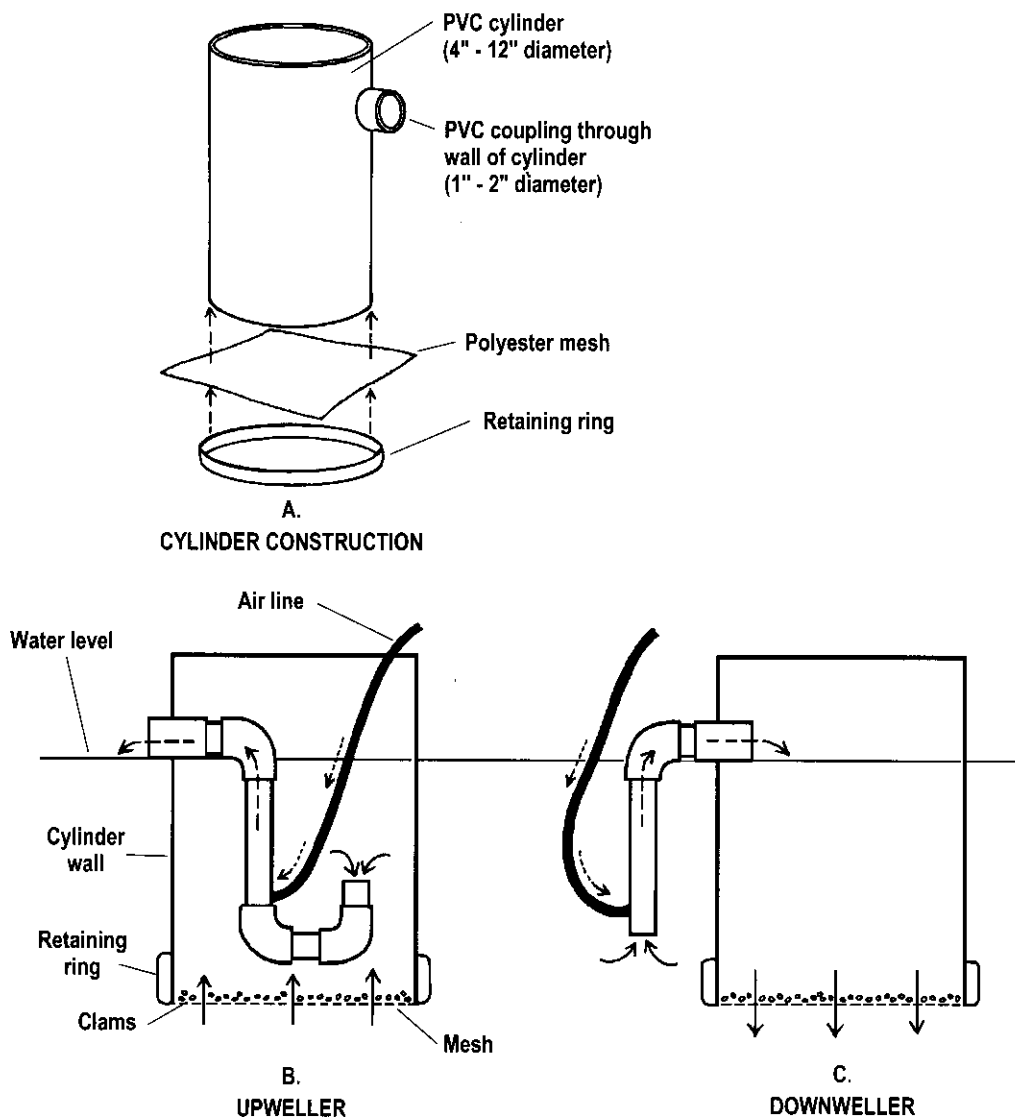
For pediveligers and early post-set, the airlift is installed on the outside of the cylinder, thus creating a "downweller" (**Fig. 13c**). Air injected into the lower end of the airlift will cause the water to rise up

the pipe and overflow into the silo. The flow of water into the silo assures that any still-swimming larvae remain in the culture cylinder.

One or more silos are suspended in a reservoir. A reservoir can be any container of appropriate size and material, from a trashcan to a large fiberglass tank. The size of the reservoirs and cylinder diameters may be varied according to the production requirements. Many silos may be run in a common reservoir, but the risk of disease transmission is reduced if multiple reservoirs with fewer silos are used.

### System operation

Reservoir water should be changed daily if possible but a minimum of three times a week. This will reduce the likelihood of



**Figure 13.**  
*Downwelling and upwelling post-set culture cylinders*



disease and prevent build-up of metabolites. Finely filtered seawater is used for small post-set. Exchange water should be at the temperature and salinity to which the post-set are acclimated. When the water is changed, the silos and clams should be rinsed with freshwater to remove accumulated silt, feces, algae, etc. At least three times a week the silos should be transferred to a clean reservoir. If this is not possible, the reservoir should at least be scrubbed with detergent and rinsed with hot water. If there are disease problems, the reservoir should be sterilized with dilute bleach.

When the reservoir is being filled, small post-set will have a tendency to float. It is important to break the surface tension of the water so that these floating clams will sink. Air may be trapped under the screen during the filling process. Such air pockets will interfere with water flow through the screen. They can be released by gently tipping the silo to one side, elevating one edge of the screen slightly. After the reservoir is filled, verify that all airlifts are functioning and flow is appropriate.

Optimal flow rates for recirculating downweller/upweller systems have not been established. Usually the only circulation in the reservoir is provided by the airlifts, so the combined flow of all airlifts must be high enough to keep food in suspension and provide good mixing throughout the reservoir. All silos should have approximately the same flow. In order to have maximum flow the airlift outflow should be even with the reservoir water

level but not be completely below the water surface. Flow will be reduced if the airlift is above the level of the reservoir water. However, if pediveligers have been placed in downwellers, the airlift should be positioned slightly above the water to prevent their swimming out of the silo.

Check flow rates several times a day to make sure water is running through all silos. A short time with low flow or no flow can be fatal to the young clams. In addition to inappropriate airlift height with respect to the reservoir level, other possible causes of reduced flow are (1) airline not properly positioned in the airlift, (2) airline constricted, (3) air valve improperly set or malfunctioning, or (4) screen fouled. If the screen is fouled on a downweller, the water level inside the silo will be higher than in the reservoir. On an upweller, the reverse is true. Gently bouncing the silo may resuspend the fouling material. Otherwise, the silo may have to be removed from the reservoir and washed with freshwater. Frequent screen clogging is a sign that the post-set need to be thinned, the screen size needs to be increased, or the clams are being chronically overfed.

At least once a week, all the animals should be removed and the silo scrubbed. Include all parts of the airlift system in this washing. Exercise care in cleaning the screen as the mesh tears very easily. Carefully examine the screen for holes or tears. If holes are present, mark and set aside that silo for repair. Small holes may be patched with silicon sealer. Larger holes or tears require replacing the mesh entirely.

If possible, fresh silos are used at this time and the old ones allowed to dry. Also replace the airline at this time.

While the clams are out of the silo, they may be measured and their volume determined (see details later in this section). During this time, do not leave the post-set sitting in seawater or they will quickly run out of oxygen. For short periods of time, carefully decant most of the water from the container, leaving the clams just barely damp. If the clams must be out of the reservoir for longer, they may be kept on a damp sieve. However, do not allow them to totally dry out—spray the screen gently with seawater occasionally to keep the clams damp or immerse occasionally in seawater for a few minutes.

After 1–2 weeks of culture, the downwellers may be converted to upwellers. In an upweller, the airlift is inside the silo and thus water is pumped out. Silos can be converted to the upwelling mode when the post-set reach about 500  $\mu\text{m}$ . Below this size, the clams could be sucked out of a silo by the airlift. Even with 500  $\mu\text{m}$  clams, it is advisable to put a U-shaped extension on the airlift so that water is not drawn from directly off the seed bed (**Fig. 13b**). The mesh for upwellers should be increased to reduce the likelihood of clogging at the higher feeding levels that will be employed. A mesh of 200–300  $\mu\text{m}$  may be used.

Post-set clams are cultured in the hatchery until they reach a size of 1 mm or will stay on a 710  $\mu\text{m}$  screen. This post-set period

may last 4–6 weeks, or even longer if temperatures are low. Towards the end of this period, it may be necessary to acclimate the clams to the ambient nursery conditions. A slow flow of ambient water may be introduced into the reservoir and gradually increased. After 3 weeks of post-set culture, some of the clams should be 1 mm, and it may be desirable to sieve off the largest clams and move them to the nursery. This will reduce crowding in the post-set system and allow the remaining post-set to grow more rapidly.

### Stocking density and feeding rates

In considering the density at which post-set clams are cultured, there are two factors to consider: the volume of water in the reservoir and the area provided by the cylinder screen. Approximately 0.25–1.0 ml of water should be provided for each post-set clam. A rule of thumb in determining reservoir volume is that the amount of algae added between water changes should not exceed 5–10% of the culture volume. Thus, if the reservoir is 100 liters, no more than 5–10 liters of concentrated algae should be added between water changes. If the calculated consumption rate requires addition of more than 5 liters of algae, the density in the reservoir should be reduced, or a larger reservoir should be used.

If the silo diameter is small, post-set may be physically crowded even though the reservoir volume is sufficient. The area of the silo screen should be large enough to accommodate the clams in a layer no more

than 1–2 clams deep. If the silo screens are clogging frequently, it probably means the density is too high. Either use a larger diameter silo, or divide the clams among multiple silos.

**Table 9** shows suggested stocking densities for post-set clams. These numbers have been determined empirically in the culture system at MRRI and may vary in other hatcheries. From this table you can see that the clams will require thinning several times between setting size and 1 mm. One way to thin the clams is to sieve off the larger individuals and move them to new silos with larger mesh screens or to the nursery. If no additional space is available, it will be necessary to discard some of the post-set in order not to overload the system. If it is necessary to discard animals, this should be done without regard to size; i.e., randomly remove clams representing all sizes, rather than removing only the smallest. There is more discussion

on this point in the section on Genetic Considerations.

Post-set clams are voracious eaters (**Tables 2 and 8**). They may be fed a mixture of phytoplankters. The food is added to the reservoir and circulated past the clams by the airlift. The food may be added in two or more batches a day, or continuously. The food concentration should be 25,000–100,000 cells/ml. If fed continuously, an initial batch should be used to bring the background level up to 25,000 algal cells/ml. The remaining ration can then added gradually either by continuous drip or intermittent metering, as previously described for broodstock feeding. For batch feeding, rations are calculated as for broodstock (**Table 5**).

## Measurements

Post-set should be observed at each water change by removing a small sample to a

**Table 9.**  
**Stocking densities for post-set clams in 15 cm (6")**  
**or 30 cm (12") diameter silos**

Size (mm)	Density (ml/cm <sup>2</sup> )	Volume (ml)		# of clams		Reservoir volume (liters per silo)	
		15 cm	30 cm	15 cm	30 cm	15 cm	30 cm
0.3	0.006	1	4	100,000	400,000	30	120
0.5	0.03	5	20	100,000	400,000		
0.7	0.03	6	25	50,000	200,000		
1.0	0.04	8	32	25,000	100,000		

petri dish and examining under a dissecting scope. Healthy clams should have dark guts and clean shells and should be extending their feet and crawling. If contaminants are obvious (e.g. zooplankters), sieve the clams on an appropriate screen to remove them. If a lot of shell is present, it may be desirable to sieve the clams on a series of screens to remove as much shell as possible. Empty shells provide a good surface for bacterial growth.

Volume determinations and shell length measurements should be made weekly when the clams are removed to scrub the silo. Post-set clams are too large to be successfully suspended in water for counting. Instead, the volume of the clams is determined and a subsample of this volume may be counted or the number estimated from an equation relating length to volume.

#### ***Determining packed volume***

Rinse clams from the silo into a dishpan or screen, using freshwater. Pour off most of the water and transfer the clams into a beaker of seawater. If they are to be left in the beaker long, remove as much water as possible. To determine the packed volume of post-set, rinse the clams into an appropriate graduated container, using a wash bottle of seawater and a funnel. Always work over a dishpan in case of spillage. Use as small a graduated cylinder as practical to improve precision of your measurement. Fill the cylinder, allowing at least 2 cm of water above the surface of the

clam layer. Pack the clams by tapping the cylinder on the palm of the hand or a countertop. The top of the column of clams is the packed volume.

#### ***Determining average size***

Post-set are measured on a dissecting scope fitted with an ocular micrometer, using the highest magnification for very small post-set and gradually reducing down to 10X as the clams grow. Make sure to record the magnification at which the clams were measured. The clams will be easier to measure if they are in freshwater—otherwise they will crawl around. Place the clams in a petri dish, add freshwater, and measure at least 25 from each culture. In calculating the average size, take into account the magnification factor.

#### ***Counting***

Post-set clams are very difficult to count. However, the number may be estimated from the size (shell length), volume and survival. **Table 10** gives approximate counts (#/ml) for different size post-set. The actual number will vary because not all the clams are the same size. Once you have determined the size, use **Table 10** to determine the number per ml and multiply that by the packed volume. If mortality is greater than 10%, the total number will be overestimated. This problem can be alleviated by sieving to remove as much of the dead shell as possible.

As post-set approach 1 mm, it may be feasible to perform actual counts. A very

**Table 10.**  
**Approximate size/volume**  
**relationships of post-set**  
**and juvenile clams**

Size (Length, mm)	#/ml
0.3	99,000
0.4	42,000
0.5	21,000
0.6	12,000
0.7	8,000
0.8	5,000
0.9	4,000
1.0	3,000
1.5	800
2.0	300

small volume of clams (e.g. 0.5 ml) is measured in a graduated, conical bottom centrifuge tube, using the same technique as for determination of packed volume. The clams are then transferred to a petri dish for counting under a dissecting scope. If clams are too numerous to count all of them, a gridded petri dish can be used. Disperse the clams as evenly on the petri dish as possible and count enough grids to total 1/10 of the total dish area. From this the total number is extrapolated. Alternatively, pile a known volume (e.g. 0.5 ml) of post-set on a small dish and divide in half. Remove one half and divide the remainder in half. Repeat this procedure until the number remaining is ~100–200. (Make sure to keep track of the number of divisions in order to calculate the total number). To facilitate counting, the clams

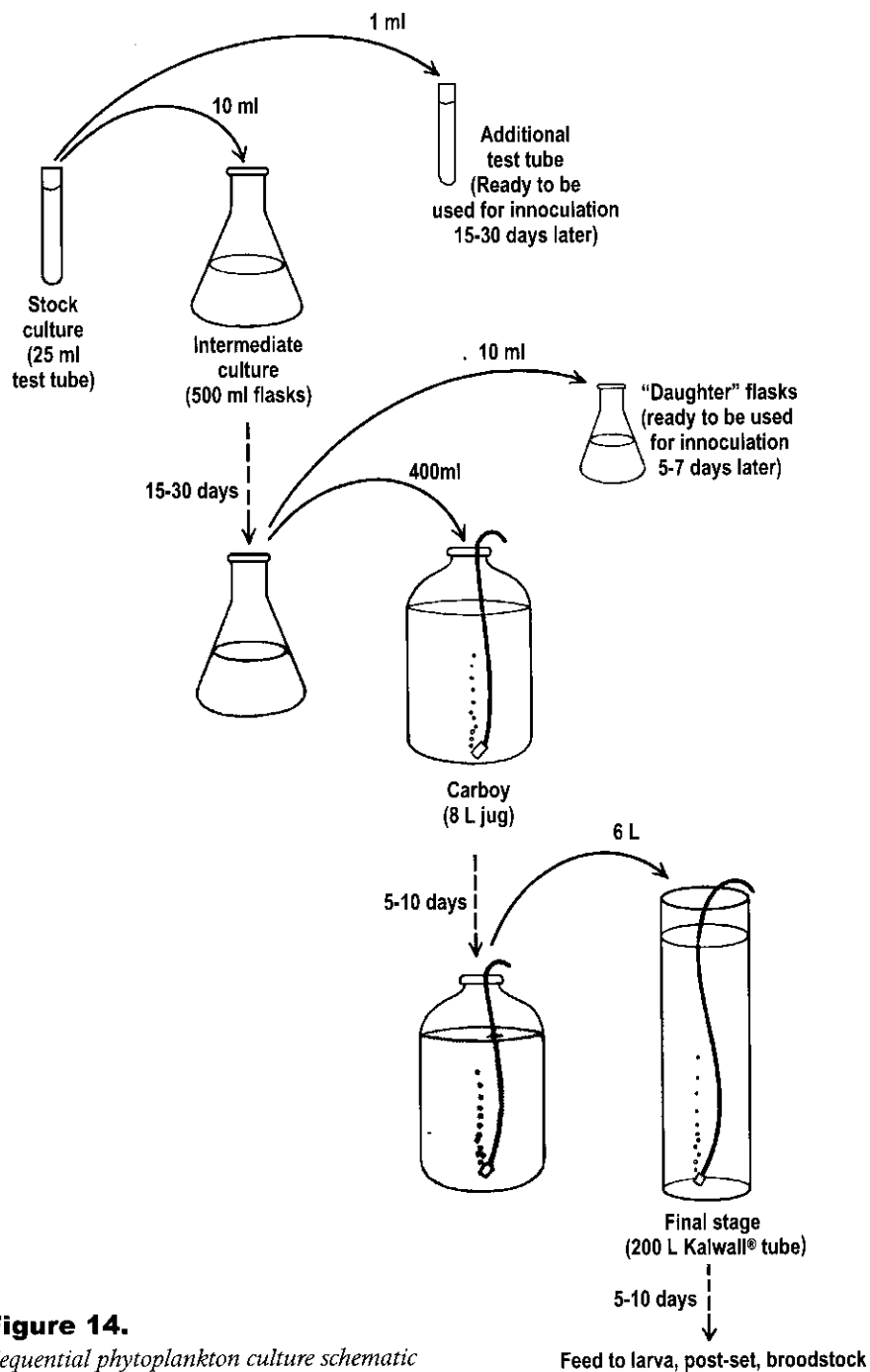
should be rinsed with freshwater and the water gently decanted until fairly dry. (In seawater the clams will crawl around the dish.) Clams will wash around rather easily if the water is not removed from the dish, making counting very difficult.

## ALGAL CULTURE

The final major process in the hatchery is food production. Although numerous substitutes have been tried, no adequate replacement for live phytoplankton as a larval and post-set diet has been found. Therefore, culture to produce large quantities of suitable phytoplankters is an integral part of a hatchery operation.

This section describes the “Milford” method of phytoplankton production. Most of the information given here is derived from Guillard (1983). Other methods of algal culture are described in Castagna and Kraeuter (1981), Ogle (1982) and Castagna and Manzi (1989).

The method described here is a sequential culture method utilizing small cultures to inoculate successively larger cultures until the final production stage is reached (**Fig. 14**). The MRRI system utilizes 4 levels of culture: stock cultures (25 ml test tubes); intermediate cultures (500 ml flasks); carboys (4–20 liter glass or polycarbonate jugs); and final stage production (200 liter Kalwall® tubes). Test tubes and flasks are kept free of bacteria and other contaminants and provide a long-term source of clean algal cells for inoculating larger



**Figure 14.**  
*Sequential phytoplankton culture schematic*

---

cultures. The larger cultures by their very nature are prone to contamination and are therefore grown for as short a time as possible prior to use. The entire process from the smallest culture to the harvest stage requires 15–30 days.

A typical culture cycle starts with inoculating one or more flasks from a stock test tube. Additional test tubes are inoculated at the same time. After 5–10 days, the flasks are used to inoculate daughter flasks and a carboy. The carboy is allowed to bloom for 5–10 days and is then used to inoculate a Kalwall®. After 5–10 days the Kalwall® is harvested and fed to the clams.

Sufficient algae must be cultured to meet the current feeding needs, inoculate for future needs, and guard against unexpected “crashes”. Depending on algal culture densities, broodstock conditioning may require 30–50 liters of algae a day for about 50 broodstock. One million larvae will require 1–10 liters a day, one million post-set 10–100 liters per day. There must always be enough carboys and Kalwalls® on line to accommodate this feeding rate and to provide backups in case of crashes. Cultures should be at high densities (3–5 million algal cells/ml) before feeding. This will allow you to add enough algal cells without adding excessive algal media.

Starter phytoplankton cultures are available from a number of sources (see Appendix D). Many institutions maintain cultures for research purposes and several private companies market some of the commonly used species. A list of algal

collections is contained in Miyachi et al (1989). Established hatcheries are often willing to share their stock cultures, although these may not always be pure. If you have the time, patience and necessary equipment, you can isolate your own algae from raw seawater, but most hatcheries just obtain starter cultures from other sources.

## **Culture environment**

A number of factors must be considered regarding the physical environment for cultures: types of culture vessels; light; temperature control; aeration and pH control.

### ***Culture vessels***

Culture vessels must be of appropriate size and material. The choice of vessel depends on several factors, such as size of autoclave, height of shelving, arrangement of lights, budget restrictions, etc. Dimensions of culture vessels will determine the arrangement of the algal culture areas (or the available space may determine the size vessel to purchase). Tubes, flasks and carboys should be autoclavable. Typical culture vessels are 15–50 ml glass test tubes, 500–2000 ml Erlenmeyer glass flasks, and 4–20 liter (1–5 gallon) clear glass or polycarbonate carboys or jugs. Stoppers for each type of vessel are also necessary: screw-caps for tubes, disposable plugs or screw-caps for flasks and cotton plugs for carboys. An ample supply of tubes, flasks, carboys and stoppers should always be available for transfers and to compensate for inevitable breakage.

Carboys are used to inoculate larger cultures, usually the final stage in the culture process. One of the easiest vessels to use for this purpose is a transparent fiberglass cylinder available commercially in a range of sizes and shapes. The transparent fiberglass may be purchased in sheets from solarium suppliers to custom-build your own containers. The cylinders were marketed for several years as Kalwalls® but are now called Sunlite® tubes. Because of its familiarity, the term Kalwall® will be used in this manual.

Large hatcheries may include another culture stage beyond Kalwalls®. This is usually large, shallow (<1 m) fiberglass tanks which hold 1000 or more liters. These are inoculated with one or more Kalwalls®.

### ***Light requirements***

Rapid growth of the phytoplankton cultures, particularly in the carboy and Kalwall® stages, is essential and can only be accomplished with supplemental lighting. Fluorescent tubes are the most widely used source of light for production of algae. These can be grow-light bulbs, but algae will grow well with less expensive bulbs, commonly described as “cool-white”, “daylight” or “natural”. Using a mixture of these (e.g. equal numbers of daylight and natural) works well. For Kalwalls® and particularly for fiberglass tank cultures, 250–400 watt metal halide lamps are widely used. These are generally marketed for warehouse or

outdoor lighting applications and are available in a variety of housings.

Not all marine algae tolerate continuous illumination, but most species commonly raised as bivalve food will. Although there is no use supplying more light energy to a culture than will result in increased yield, larger cultures (Kalwalls® and tanks) are often light-limited. The saturation level for yield will depend on the volume and density of the culture. Consult Guillard (1983) for more detailed information on saturation levels and light intensity.

### ***Temperature control***

Although marine algae will grow at room temperature (23–28°C = 73–82°F), cooler temperatures (19–20°C = 66–68°F) will help to inhibit bacterial contaminants. Because the artificial lighting produces a considerable amount of heat, the algal culture room will have to be air-conditioned most of the year. Most marine phytoplankton cannot tolerate temperatures above 35°C (95°F).

A smaller controlled environment chamber or incubator, with temperature and light control, is useful for stock cultures. Stock cultures should be kept in a separate area from carboys, to reduce the chance of contamination and to provide backup cultures in the event that carboys become contaminated or environmental controls in the main culture area fail. Stock cultures should be maintained at 15°C (59°F) to retard growth and inhibit bacteria.



### ***Aeration and CO<sub>2</sub> addition***

Aeration of cultures serves to keep algae in suspension, to supply the carbon needed for plant growth and pH control, and to strip O<sub>2</sub> from the culture media, preventing supersaturation. Carboy and Kalwall® cultures always require aeration, which should be gentle during the first day or two after inoculation and then increased in rate as the culture grows. Flasks and tubes, however, are never aerated, as the chance of contamination is too high. They should be shaken by hand once a day.

Optimum pH levels for phytoplankton are between 7.0 and 8.5. The pH of coastal seawater is usually between 7.0 and 8.0. As the phytoplankton grow, they utilize carbon in the seawater, causing the pH to increase. If no measures are taken to counteract this, the pH in a rapidly growing culture can easily exceed 9.0. High pH values inhibit phytoplankton production. Aeration has some ameliorating effect on pH, but air alone cannot effectively control pH in very dense cultures. Increasing the CO<sub>2</sub> content of the aerating gas makes pH control possible. Adding commercial-grade CO<sub>2</sub> for 30–45 seconds each hour through the air distribution system is usually adequate to maintain pH within the desired range. This can be accomplished with a cycle timer and a CO<sub>2</sub> cylinder equipped with a regulator. The output is sent directly into the air distribution line. The regulator pressure should be adjusted to a level which just exceeds the air pressure in the distribution line. If the regulator is set too high, the pH will be

driven too low. Only enough CO<sub>2</sub> should be added to reduce the pH to 7.0–7.5. At the end of an hour, it will have risen to about 8.0–8.5.

The most common cause of contamination in algal cultures is the air distribution system, which is typically moist with condensation and provides a good environment for bacterial growth. In-line bacterial filters placed on the airline feeding each individual culture container will greatly reduce the contamination rate. If your budget is tight, acceptable filters can be made by stuffing pipettes or plastic tubes with cotton. Most air pumps have a built-in filter which should be cleaned regularly.

### ***Routine for production of algae***

Production and maintenance of algal cultures demands a routine which depends on the species of algae grown and the quantity of algae required. The most important point is to make transfers (inoculations) before half the life span of the culture has passed. In most cases, transfers should be made at least once a week. Stock cultures should be transferred at least once a month. A weekly routine of transfers allows sufficient growth time for algae which is well within the life span limit.

### ***Preparation of culture medium***

#### ***Seawater***

Natural seawater of suitable salinity (>25 ppt) is the most satisfactory medium for growing phytoplankton in large quantities.

For very small quantities, it may be possible to use artificial seawater. Prior to use, seawater must be filtered to remove native phytoplankton, zooplankton, detritus and silt. A 25  $\mu\text{m}$  filter bag will remove most zooplankters. To remove phytoplankters and very fine silt, it is necessary to further filter the water through a 1  $\mu\text{m}$  filter bag or cartridge. It is often necessary to settle the water between the gross filtration (25  $\mu\text{m}$ ) and fine filtration (1  $\mu\text{m}$ ) steps. Otherwise the heavy silt load will cause frequent filter clogging. Most of the silt will settle out in 24–48 hours. For test tubes, flasks, and nutrient stock solutions, a final filtration step may be included to remove bacteria. This will require a vacuum source and a special vacuum filtering apparatus which holds a glass fiber filter disk (**Fig. 15**). Only small amounts of water (usually up to a liter) are filtered at a time and the filter may need to be changed between batches. A glass-fiber filter in the 0.2–0.45  $\mu\text{m}$  range removes all bacteria.

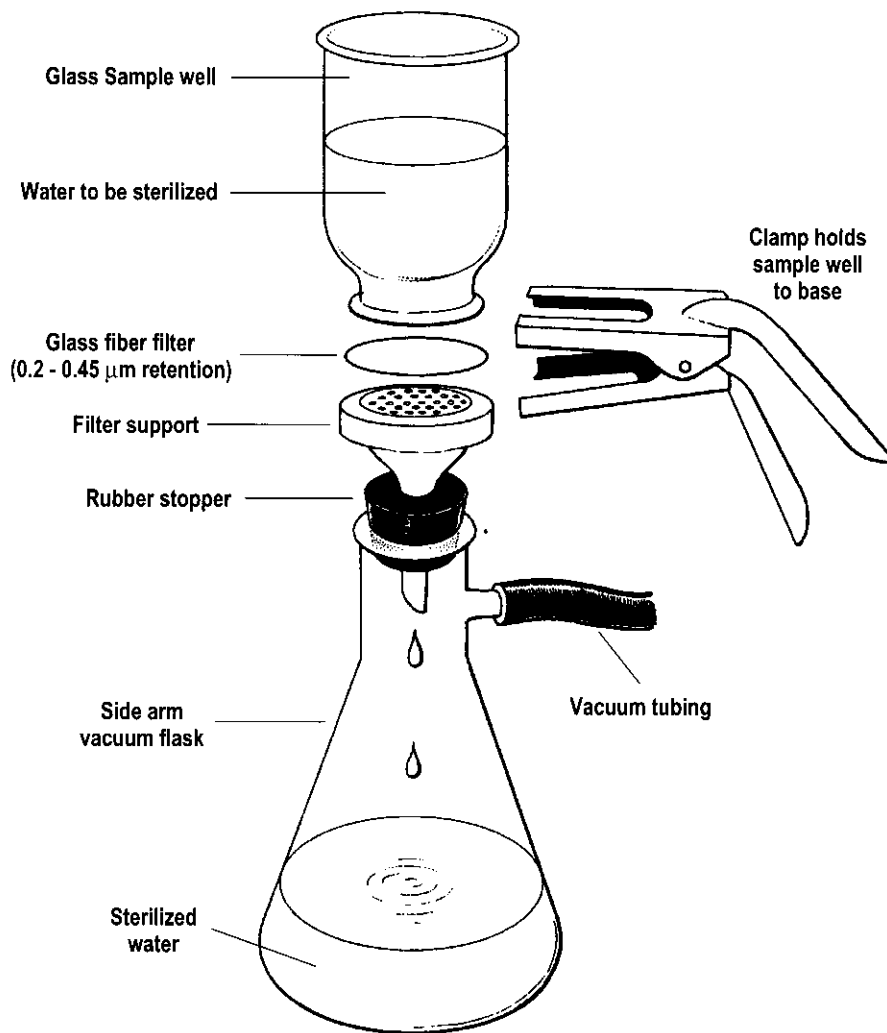
### **Sterilization**

The culture containers (tubes, flasks, carboys, Kalwalls®) and the culture water must be sterilized prior to introducing the algal inoculant. Improper or inadequate sterilization will result in contaminated cultures which are not useable in the hatchery. After inoculation, every effort is made to keep tubes and flasks sterile; carboys and Kalwalls® are kept as uncontaminated as practicable.

Tubes, flasks and small carboys are usually sterilized by heat and pressure, using an

autoclave, pressure cooker, or pressure canner. A microwave can also be used to sterilize small vessels. The culture water is sterilized simultaneously. Clean containers are partially filled ( $\frac{1}{2}$ – $\frac{3}{4}$  capacity) with seawater which has been filtered as described above. If the containers are too full they may boil over during the sterilization process. Nutrient media (see below) is added to test tubes prior to autoclaving. Sodium silicate, if needed, is added to test tubes and flasks prior to autoclaving. The prepared containers of seawater are closed with a screwcap (not completely tightened) or a foam or cotton plug covered with aluminum foil. They are then autoclaved for 15 minutes at 250°C or pressure-cooked following manufacturer's instructions for canning. After sterilizing, the culture media must be cooled to room temperature prior to use. Transfer pipets should be sterilized in this manner also. They should be wrapped in autoclavable plastic or aluminum foil or placed in a sealed autoclavable container.

Larger carboys and Kalwalls® may be sterilized with chlorine. After thorough cleaning, the containers are filled with 1  $\mu\text{m}$  bag- or cartridge-filtered seawater and household bleach is added at the rate of 0.5 ml/liter. Airlines and airstones are placed in the containers so that they are sterilized at the same time. The container is chlorinated for at least 30 minutes. After 30 minutes or more of sterilization, sodium thiosulfate (0.05 g/L) is added to neutralize the chlorine. Allow at least 30 minutes for the neutralization and check with a chlorine test strip or test kit before adding nutrient media.



**Figure 15.**

*Vacuum filtration apparatus for cold sterilization of seawater*

---

## **Nutrients**

Seawater alone does not have enough nutrients to support dense phytoplankton blooms and must be enriched with appropriate nutrients prior to inoculation. The most commonly used culture medium is referred to as "f/2". Some hatcheries mix their own nutrients "from scratch". If you wish to explore this alternative, consult Guillard (1983). Mixing your own allows you to vary the concentration of selected nutrients. However, many different stock solutions must be maintained and it is much easier to buy pre-mixed nutrient packages available from several vendors (see vendor list in Appendix C). These are available in liquid or dry form, with instructions for diluting to achieve f/2 strength. In addition to the basic nutrients, diatoms require silicate for growth, so this must also be added. It is usually mixed and stored separately from the other nutrients to avoid precipitation. For short-term culture purposes, household plant fertilizer is an adequate substitute for the more expensive specially formulated media.

Nutrients are stored as concentrated stock solutions. A small aliquot can then be added to a larger volume of seawater to produce the desired strength medium. Stock cultures (test tubes) are maintained in dilute media (e.g. f/4 = half the strength of f/2) to retard growth, while other stages are generally raised in f/2. It is desirable to make separate stock solutions for small and large cultures. Convenient concentrations might be 25X (25 times as concentrated as f/2) for tubes and flasks and

1,000X (1,000 times as concentrated as f/2) for carboys and Kalwalls®. To achieve f/2 strength in the final media, 1 ml of 25X solution would be used for each 25 ml in a flask. For tubes, which should be maintained in f/4, 0.5 ml of the 25X solution would be used for each 25 ml of media. One ml of 1,000X stock would be added to each liter in a carboy or Kalwall®. Any convenient stock concentration is acceptable, as long as the final media concentration is correct. Make up relatively small quantities of stock solution at a time, so that it will be consumed in about 30 days. Using a stock solution over a longer period of time may result in contamination. If you purchase a liquid formulation in a large container, it is advisable to transfer it to several smaller containers.

To prepare the stock solutions, select an appropriate storage container (autoclavable, glass or plastic, with screw cap) and clean with laboratory-grade detergent. If there are any precipitates on the glass, acid-washing may be needed. Choose a container which will be almost filled by a 30 day supply of your stock solution. For instance, for flasks and tubes a 1 liter container is appropriate, while a 4 liter container might be needed for carboys and larger cultures.

Fill the container with finely filtered seawater. If possible it should be vacuum filtered through a glass-fiber filter with retention capacity of 1.2  $\mu$ m or smaller. Cover the container and sterilize as described above. When cool, add the appropriate amount of pre-mixed nutrients

to obtain the desired stock solution. Pre-mixed nutrients which are in liquid form may be cold-sterilized prior to addition by vacuum filtering through a very fine filter, such as a 0.2  $\mu\text{m}$  membrane filter. Stock solutions made with dry-packed nutrients may be cold-sterilized after the solutions are made. We have experienced little trouble with nutrient contamination and do not routinely cold-sterilize our stock solutions.

Silicate stocks are prepared in distilled water, rather than seawater. The desired final concentration is 15–30 mg/liter of sodium metasilicate. It is convenient to make two stock solutions: 100X strength (1.5–3.0 g/liter) for flasks and tubes and 1000X strength (15–30 g/liter) for carboys and Kalwalls®. Use 1 ml of the 100X stock for each 100 ml of final media for flasks and tubes. Use 1 ml of 1,000X for each liter of final media for carboys and larger cultures. The silicate solution should be autoclaved.

Store the stock solutions in the refrigerator, tightly capped. Remove stock solution as needed with a sterile pipet, or by pouring. Open as seldom as possible to avoid airborne contamination. If a large quantity is prepared at one time, it may be advisable to store in several small containers to reduce the number of times a container is opened. Label and date the stock solutions and discard when a month old.

For stock cultures (test tubes), nutrients may be added prior to autoclaving the culture water. For other culture levels, add

the nutrients to the seawater after it is sterilized, immediately prior to inoculation. A precipitate forms in f/2 medium upon autoclaving, particularly in large volumes of water. This is avoided by adding the medium after autoclaving. Additionally, some of the vitamins may be denatured by the heat of autoclaving. Stock cultures, which do not need to grow rapidly, do not appear to be adversely affected by any loss of vitamins, but the production cultures will probably perform better if the vitamins are not autoclaved.

### *Inoculation and culture*

#### *Stock cultures*

Stock cultures (test tubes) are used for long-term maintenance of uncontaminated algal cells. Stock cultures should be kept isolated in a carefully controlled environment. Temperature should be maintained at  $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and appropriate lighting (preferably designed for plant growing) must be provided. Constant illumination may be provided, or lighting may be timed to simulate a diurnal cycle. Tubes should be agitated daily to keep the cells in suspension. Media for stock cultures is usually dilute ("f/4" or less) to retard growth. If full strength media is used, the stock cultures will grow too rapidly and will exhaust the nutrient supply unless more frequent transfers are made. Stock culture tubes are opened only when transfers are made.

At least once a month, new stock cultures should be inoculated from the old ones.

This assures that healthy stock cultures are always available in case other levels of production become contaminated. After inoculating “daughter” test tubes, the remainder of the stock culture is used to inoculate one or more flasks. This rejuvenates the flask culture level, which is more prone to contamination than the tubes. If possible, transfers should be made under a hood with an ultraviolet lamp, which kills bacteria. At the very least, they should be made in a clean, dust-free area, with no drafts.

Preparation of culture tubes has already been described. Remember to add nutrients (“f/4” or less) to the tubes prior to sterilizing. Several new tubes may be inoculated from one older tube. This will provide additional emergency stocks in case a catastrophe wipes out all your flasks. One or two flasks (depending on the size of the tubes and flasks) should also be inoculated at the same time.

To inoculate, gently agitate a stock culture tube to evenly distribute the algal cells. Open the stock culture tube and use a sterile pipet to transfer 1 ml to each new tube, immediately capping the inoculated tube. Pour the rest of the stock culture into a prepared flask(s). Use about 10 ml to inoculate a 500 ml capacity flask containing 250–350 ml of media. Label the newly inoculated cultures with the species identification and the date of inoculation. Add the appropriate amount of nutrients from the stock solution to the flask (nutrients are already in the tubes). Repeat this process for each stock culture tube to be transferred. Make sure to use a new sterile pipet for each tube.

### **Flask cultures**

Erlenmeyer flasks are commonly used as the first level of the production process. A 500 ml capacity flask containing 250–350 ml of media will produce enough inocula for a 4–8 liter (1–2 gal) carboy (also  $\frac{1}{2}$ – $\frac{3}{4}$  full). If larger carboys are used, you may wish to use larger flasks. Flasks up to 2 liter capacity are manageable for this stage. The flasks may have screw caps or may be stoppered with disposable foam plugs or homemade cotton plugs. Flask cultures are grown in a well-lighted area, preferably provided with special plant-growing lights. Although the algae will grow at room temperature (23–28°C), cooler temperatures (19–20°C) will inhibit bacterial growth. Flasks are not aerated but should be agitated daily to resuspend algal cells.

Glassware and seawater for flask culture are sterilized as described in the previous section. Flasks may be prepared in advance and stored (tightly covered) in a clean cabinet until use.

On a weekly basis, flasks are used to inoculate new flasks and carboys. A single flask can inoculate 1 or 2 new flasks and a carboy. If more flasks are available than needed for inoculations, they may be allowed to grow for a second week. Flasks more than two weeks old should be discarded. To inoculate, select a flask with a dense culture. Agitate the flask to evenly mix the algae. Use a sterile pipet to transfer 10–20 ml of algal culture to each new flask. Use the remaining amount to inoculate a carboy (see below). Add

nutrients to the new flasks, cap, and label. On a monthly basis, and whenever there are signs of contamination, flasks should be inoculated from stock culture tubes, as described previously. These will take longer to bloom than flasks inoculated from other flasks.

### **Carboy cultures**

Carboys are glass or clear plastic containers, usually jug-shaped, with a capacity of 4–20 liters (1–5 gal). Carboy cultures may be grown at cool room temperature (23–28°C) but bacterial contamination will be retarded if they can be kept cooler (19–20°C). They must be supplied with artificial lighting and aeration. They should be located in a low-traffic area to prevent breakage.

Carboys are sterilized prior to use by autoclaving or chlorination as described previously. After sterilization, nutrients and sodium silicate from stock solutions are added. The carboy is then inoculated with algal culture from a flask by simply pouring the contents of the flask into the carboy. The carboys will reach harvest density more rapidly if more inocula is used. If 250 ml is used to inoculate 2.5–3.5 liters of seawater, it will be ready for use in 3–5 days. If the same amount is inoculated into 5–7 liters, it may take 6–10 days to grow. The longer the culture must be grown, the more likely that it will become contaminated. For larger carboys, two flasks of the same species may be used as inocula.

Carboys require aeration. Use clean airlines and a clean, self-weighting airstone. Cover the top of the carboy with a sandwich bag, inverted plastic beaker, or other protective covering to prevent airborne contamination. Use an in-line bacterial filter or cotton-plugged tube between the air source and the carboy. Do not aerate too vigorously—a slight stream of bubbles is adequate. CO<sub>2</sub> addition is optional.

Observe carboys daily to make sure they are properly aerated and to assess the condition of the algae. If the culture is not noticeably denser after three days, it should be discarded. Cultures are usually ready to use as Kalwall® inocula after 5–7 days. If not used within 2 weeks they should be discarded.

### **Kalwall® cultures**

Kalwalls® are clear fiberglass cylinders available commercially in several sizes. They are currently marketed as Sunlite® tubes. The most commonly used sizes are 80 and 200 liter. These may be fitted with a valve for ease of draining. Cultures are grown at cool room temperature with intense artificial lighting, aeration and addition of CO<sub>2</sub> to control pH. Kalwall® cultures usually require 5–7 days to become dense enough to be useful as food. Cultures more than 14 days old should be discarded.

Kalwalls® are sterilized with bleach as previously described. After neutralization with sodium thiosulfate, stock nutrients are

added. The culture is then inoculated with the entire contents of a carboy. The amount and density of the inoculant will effect the growing time prior to harvesting the Kalwall®. A 150 L culture inoculated with 3 liters from a dense carboy will require 4–6 days to reach harvest density ( $3\text{--}5 \times 10^6$  cells/ml). The Kalwalls® must be well aerated and should be covered with a lid or plastic bag to exclude air-borne contaminants. An in-line bacterial filter should be used on the airline. The pH should be checked regularly, either with pH strips, a hand-held meter, or a pH probe suspended in a Kalwall® and connected to a remote meter.

Kalwall® cultures are more prone to contamination than the previous culture stages. Signs of contamination are foam on the surface of the water, abnormal color change (e.g. from brown to greenish), clumping of algal cells, slimy growth on the airline, or failure of the culture to bloom in a reasonable period of time. Suspect cultures should be examined microscopically. Contaminated cultures should be discarded immediately.

### **Cleaning practices**

All glassware and equipment used for algal culture should be cleaned with laboratory detergent and hot water before use. Additionally, glassware may need to be dipped in dilute muriatic acid occasionally to remove mineral deposits. Culture vessels and transfer pipets must be sterilized. Autoclaving equipment (e.g., vacuum hoses, filtering rigs, etc.) is also an excellent idea to prevent unwanted contamination.

After each harvest, Kalwalls® should be scrubbed with laboratory-grade detergent and hot water. The slightly irregular surface of the fiberglass allows deposits and growths to build up. To prevent this, the Kalwall® is soaked with muriatic acid (~50 ml in 200 liters of water). Make sure a cover is on the cylinder while it is soaking to contain fumes. After soaking, drain and rinse well with freshwater.

**CAUTION: Use gloves and eye protection when handling acid. Muriatic acid and chlorine bleach combine to release an extremely toxic gas. Be very cautious not to use these chemicals in the same area or discard them in a common drain!**

### **Determining algal cell concentrations**

Kalwalls® are ready to harvest when they reach a density of 3–6 million cells/ml. There are several ways to determine algal density, including a modified secchi disk, fluorometry, spectrophotometry, Coulter® counting, and counting with a hemocytometer. An experienced technician can usually estimate algal density fairly accurately by observing the color of the culture. However, all of these methods require that the algae be manually counted periodically for calibration. This is done with a hemocytometer and a compound microscope.

A hemocytometer is a special microscope slide originally used in the medical field for counting blood cells (**Fig. 16**). The slide is constructed to evenly disperse a



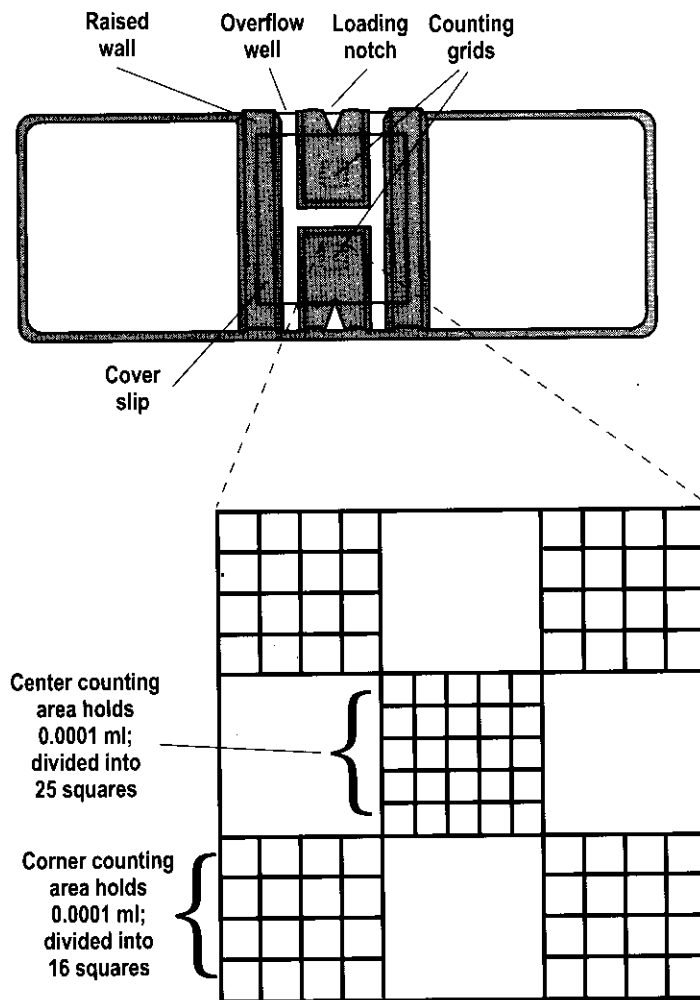
very fine film (0.1 mm deep) of the solution to be counted under a special coverslip. Two specially marked grids form counting areas and are surrounded by a well. The hemocytometer and coverslip must be very clean in order to work properly. Clean both with 10% isopropyl alcohol prior to use and wipe dry with a laboratory grade tissue or lens paper. Be particularly careful to remove any fingerprint grease. Lightly dampen a fingertip with saliva and touch the raised wall - not the counting surface. Immediately place the coverslip on the slide so that its edges rest on the dampened spots, which hold the coverslip in place.

A small (e.g. 100 ml) sample of the algal culture to be counted is procured in a beaker. If the algae is a motile species (e.g. *Isochrysis*), it must be killed prior to counting. An effective way to do this is with a drop of Lugol's solution. Lugol's solution is made by mixing 6 g of potassium iodide and 4 g of iodine in 10 ml of seawater. Lugol's solution is also available ready-mixed from most scientific houses. Non-motile species such as most diatoms do not need to be killed prior to counting.

A long-tipped disposable Pasteur pipet is used to load the hemocytometer. Before drawing the sample, mix the algal cells by submerging the pipet and blowing gently through it. Then allow the pipet to draw a small sample by capillary action. Do not suck algae into the main body of the pipet. Hold the pipet at a 45° angle and gently touch the tip to the "V" slot at one end of the coverslip. A small amount of sample

should immediately be sucked under the coverslip by capillary action, completely covering the silvered counting grid. Repeat this process to load the other counting grid. If the sample spills over into the well area, empty the slide and start over. If the sample does not fully cover the silvered grid, reapply. The time from drawing the algal sample to filling the chamber should be short to minimize settling of cells in the pipet.

Place the loaded hemocytometer on a compound microscope and set the magnification to 100X (10X ocular and 10X objective). Move the slide around until you locate one of the counting grids (**Fig. 16**). Typically, the slide is divided into 5 counting areas: four corner squares and a central square, each of which contain exactly 0.0001 ml of the sample. These counting areas are subdivided: 16 small squares in the corners and 25 small squares in the central region. (If your hemocytometer has a different type of counting grid, refer to the instructions with the slide). If all algal cells in any one of these five regions are counted, that count multiplied by 10,000 is the number of cells per milliliter in the original sample. Since algal cultures are usually very dense, a subsample of the smaller squares may be counted. For instance, 5 of the 25 small squares in the central region may be counted. This number is then multiplied by 5 (to get the total in the entire central region) and by 10,000 to yield the number per milliliter. When algal concentration is <500,000 cells/ml, the cells in all five counting regions should be counted and that total multiplied by 2,000 to yield the number per milliliter. When densities are



**Figure 16.**

*Hemocytometer slide for counting phytoplankton*

very low (<100,000), all cells in all five regions of both counting grids may be counted and that total multiplied by 1,000.

## Troubleshooting

Most algal culture failures are due to contamination. Contaminated cultures are characterized by foaming of the surface, clumping within the culture, unusual coloration, or failure of the algae to reach appropriate densities in a reasonable period of time. If any of these symptoms are observed, the culture should be examined under the microscope. A healthy culture will be very clean—all the algal cells similar in appearance, no extraneous organisms (e.g. ciliates) or foreign objects (e.g. hair, dust), and no clumping of algal cells. If the species is a flagellate (e.g. *Isochrysis*), the cells should be moving. If there are any other organisms (usually ciliates) present, or if the algal cells are clumped (a sign of bacterial contamination), the culture should be discarded. Some possible sources of algal culture problems and corrective measures are listed in **Table 11**.

## Choice of phytoplankton species to culture

There are many marine phytoplankton which could be cultured for use in shellfish hatcheries, but relatively few are in common use. The choice of algal species to culture depends on a number of factors, including availability, food value, and ease of culture. Some sources of starter cultures are listed in Appendix D. There are many

reports of feeding studies in the literature (e.g. Davis and Guillard 1958, Epifanio 1979, Enright et al. 1986, and Tan Tiu et al. 1989) which can be consulted to determine whether a species is a suitable food source. Probably the most commonly cultured species is *Isochrysis galbana*, which is often used as the control diet in feeding studies. It is desirable to culture two or more types of algae in order to provide a nutritionally complete diet for the clams. Usually one of the species cultured is a diatom. Some of the commonly cultured species are described in Appendix D. An algal species which grows well in one hatchery may not in another, so it may be advisable to start with several species and determine which grow best in your facility. Tahitian *Isochrysis galbana*, *Chaetoceros muelleri*, and *Chaetoceros gracilis* have proven reliable in the culture system at MRRI.

## GENETIC CONSIDERATIONS FOR HATCHERY MANAGERS

Some hatcheries have elaborate breeding programs to improve stock performance. If your hatchery needs are small (e.g. you only need one or two spawnings per year to produce the required seed) it may not be necessary to have an elaborate breeding program. Even small hatcheries, however, usually choose broodstock from their own seed once it reaches maturity. For this reason it is very important to follow certain protocols to insure the genetic “health” of your stocks. The most important of these is to include enough genetic variation in each spawning. If genetic

**Table 11.**  
**Sources of algal culture problems and corrective measures.**

Source	Corrective measures
Water not properly sterilized	Review procedures Check operation of autoclave Verify chlorination with check strips or kits
Aeration supply contaminated	Add/replace in-line air filters Sterilize airstones Sterilize distribution pipes
Subcultures contaminated	Examine flasks and tubes for contamination Review inoculation procedures Verify media sterilization Procure new starter cultures if needed
Nutrients contaminated	Examine microscopically Cold sterilize by 0.2 $\mu\text{m}$ filtration Prepare new nutrients
Temperature too high or fluctuating	Decrease thermostat setting Remove heat sources (e.g. chillers) Install more powerful air conditioners Substitute natural illumination for some of the artificial lighting
pH too high or low	Check CO <sub>2</sub> supply Check regulator Increase/decrease injection frequency or injection pressure
Nutrients inadequate	Verify prescribed formula is being used Modify formula if needed
Airborne contamination	Cover culture containers Remove contamination sources (e.g. unwashed culture vessels; uncovered wastebasket; used airline)

variation is low in the original spawning, there is a high probability that the clams selected to be the next generation of broodstock will be closely related. In only a few generations of selection the genetic variation can be dramatically reduced, potentially resulting in lowered productivity. This is prevented by using as many parents as possible to “found” the broodstock and produce each subsequent generation. Alternatively, new broodstock may be introduced from a different population at each generation.

### Effective parental number

If only one male and one female were spawned, there would be a limited number of genotypes in the resulting offspring population. Each additional spawner used increases the amount of genetic variation in the offspring. It is important not only to spawn enough clams, but to maximize the effective parental number ( $N_e$ ), defined by the equation:

$$N_e = \frac{(4 N_m N_f)}{(N_m + N_f)}$$

where  $N_m$  and  $N_f$  are the number of male and female spawners, respectively.  $N_e$  is equal to the number of spawners only if the sex ratio is 50:50 and the contributions of all spawners are equal, which never happens. The effect of varying sex ratio on  $N_e$  is illustrated in **Table 12**. Our experience suggests that an effective number of 20 is a minimum for a successful spawn, and many geneticists would argue that 20 is not enough. When  $N_e$  is less than 20, the

performance of the offspring may be unreliable. An  $N_e$  of 20 may be obtained by spawning 10 males and 10 females. If fewer of one sex are available, more of the other is required, as illustrated in **Table 12**. If the number of spawners of one sex is 5 or less, it is not possible to achieve  $N_e$  of 20.

$N_e$  can be reduced inadvertently by the method of handling the spawners and gametes. To maximize  $N_e$ , each male must have an equal chance of fertilizing eggs from each female, and each female must contribute an equal number of offspring. To accomplish this you would have to:

- (a) spawn in individual containers so that each spawner can be identified and fertilization controlled;
- (b) equalize the number of eggs contributed by each female;

**Table 12.**  
Effect of unequal sex ratio on effective parental number ( $N_e$ )

Females	Males	Total spawners	$N_e$
10	10	20	20
9	11	20	19.8
8	12	20	19.2
7	13	20	18.2
6	14	20	16.8
5	15	20	15
7	18	25	20.2
6	30	36	20
5	195	200	19.5

(c) divide the eggs into as many separate lots as there are male spawners, each lot to be fertilized by only one male; and

(d) combined all resulting zygotes to create the offspring population.

Even following this laborious procedure, there is no guarantee that each spawner has been equally represented, since there are differences in viability of eggs, fertility of sperm, etc.

The degree of ripeness of the spawners also affects the  $N_c$ . Inadequately conditioned females may spawn small eggs with reduced lipid reserves. These are less likely to survive to metamorphosis. It is important that all potential spawners have been subjected to the same degree of conditioning, although this does not totally eliminate the problem.

Probably the only way to insure an adequate  $N_c$  is to use more spawners. For instance, if you want an  $N_c$  of 20, spawn 15 males and 15 females. Unfortunately, it is not always possible to convince that many clams to spawn at one time. In this case, it is possible to combine offspring from several smaller spawns made on subsequent days. For example, you might spawn 10 clams each day for three days and then combine all the larvae together.

As mentioned previously, gametes deteriorate quickly. Therefore, early spawners may be under-represented in the offspring generation because their gametes may have reduced viability by the time the eggs are

fertilized. For this reason it may be best to allow eggs of early spawners to be fertilized by whatever males are available. This is the same as combining several small spawns to create one larger one.

## Larval and seed handling protocols

Genetic variation will inevitably be reduced during the culture process. Many subtle selection pressures are applied on a regular basis. These include high feeding levels, monoalgal diets, absence of inter-specific competition, lack of predation, removal of silt, and temperature control. Obviously, hatchery production would be impossible if these were eliminated. However, it is wise to remember that all of these are reducing genetic variation, emphasizing the need to maximize the variation available at spawning.

Some culture practices which are not necessary, although they are certainly convenient, are probably counterproductive. One of these is the common hatchery and nursery practice of culling larvae and seed at regular intervals to remove smaller individuals. Some hatcheries even cull their eggs and most cull 24 hour veligers. Eggs of a particular female tend to be similar in size and quality, and the size of 24 hour veligers is closely tied to the size and quality of the eggs. Thus, culling eggs or veligers may selectively remove all offspring of particular individuals. Related individuals continue to be similar in size at least through the nursery stages. Culling small individuals is a form of anti-family selection.

Repetitive culling of small individuals is potentially disadvantageous for another reason. The hatchery is only one of several environments your clams will experience. Animals which perform poorly in the hatchery may exhibit superior performance in the nursery or growout phases. Conversely, the best hatchery performers may be only mediocre, or in fact dismal, during growout. It has been demonstrated that it is not economically advantageous to discard slow growers in the nursery (Malinowski 1988). In fact, there is evidence that the true growth potential of clams may not be reached until they have been in the field for at least 6 months.

It is impractical to attempt to retain every single larva or post-set, regardless of its performance. However, it may be advisable to retain at least a portion of the slow growers. If they can be nurtured through to the next culture phase, their performance may well improve. In fact, they may eventually "catch up" or surpass their cohorts.

## **PROBLEMS/TROUBLESHOOTING**

Failures in the hatchery can, for the most part, be avoided if seawater sources are of good quality and free from pollution, and gametes are suitable for culture. Even these qualities, however, will not save a hatchery that is poorly operated. Most hatchery problems can be traced to one of these sources: contaminated source water; contaminated algal cultures; cross-contamination between broodstock

holding area and larviculture area; or dirty equipment. Preventing these problems is much easier than correcting them once they have occurred. Hatchery operations must be methodical and hatchery workers must practice extreme cleanliness and be highly observant. This requires constant vigilance and attention to detail.

### **Some basic rules for hatchery success**

1. The most important factor in a successful hatchery operation is site selection. A hatchery is dependent on a supply of clean, high-salinity (>24 ppt) water. If this is not available, water can be brought from a clean source or can be treated to improve its quality. However, this greatly increases the cost of operating the hatchery. While some research facilities operate in this fashion, it is probably uneconomical for a commercial facility.
2. Hatchery materials should be carefully chosen to avoid toxicity problems. The following should be avoided entirely:
  - galvanized metal
  - brass, bronze, copper, zinc, other heavy metals
  - treated wood
  - lead-based paint

Plastic materials (PVC, polyethylene, etc.) must be leached to remove volatile compounds. Volatile chemicals, such as those in various glues, should not be applied within the hatchery (after initial hatchery start-up). Anything which has

been glued, painted, or sealed with chemicals should be leached prior to use. Leaching can be accomplished by washing with biodegradable detergent, rinsing with hot tapwater ( $>60^{\circ}\text{C}$ ), and then rinsing with seawater. Alternatively, a 24 hour soak in running water or shorter soaks with several water changes will usually remove volatile compounds. It is a good idea to test any new material for toxicity before using it in the hatchery. Expose a sample of newly fertilized eggs or young larvae to the material for 24–48 hours. Their survival and growth should be compared to that of an identical sample which was not exposed to the material being tested.

3. One of the most common sources of problems in a hatchery operation is the unwillingness of staff to throw out larvae, post-set and juveniles. This results in system overcrowding and stress. Never attempt to operate at higher densities than the system was designed to accommodate. Never try to maintain more cultures than can be adequately tended.
4. Pesticides or bug repellants should not be used in the hatchery. Personnel who have used bug repellant should wash thoroughly with soap and water prior to entering the hatchery.
5. Use of hatchery equipment for other purposes should be avoided. If such use is necessary, equipment should be cleaned and disinfected before using it

in the hatchery.

6. The hatchery should be a closed environment devoted entirely to clam culture. No other species (e.g. crabs, fish) should be cultured in the hatchery as this will probably lead to contamination of the clam cultures.
7. The hatchery should not be used as a storage area. A minimum of equipment and supplies needed for use in the hatchery itself should be stored within the hatchery. All equipment should be dry prior to storage. Wet equipment is an invitation to bacteria. Materials for use in the nursery or field should be kept out of the hatchery or disinfected prior to being brought into the hatchery.
8. Smoking should not be permitted in the hatchery. There is evidence that cigarette smoke is harmful to larvae.

### **Hatchery mortalities**

Mortality of hatchery-cultured larvae may be attributable to any number of problems, including poor quality gametes, poor culture techniques (e.g. insufficient food, overcrowding), and disease. Occasional failures in a single cohort of larvae may well be due to poor quality gametes. Repetitive failures are often a sign of poor culture techniques. Sudden mass mortalities or mortalities which effect many cohorts may be due to disease. Very little is known about diseases of clams. Once a disease problem occurs in larval or post-set cultures there is little that can be done



about it, except to discard the diseased cultures and sterilize the hatchery. Therefore, the key is prevention. Frequent careful observation can alert the operator to problems before they become serious.

### **Warning signs**

- **Reduction in feeding rate**
  - Cultures not cleared as rapidly.
  - Guts of larvae not dark.
  - Feces not evident in brood tank.
- **Reduced activity level**
  - Larvae are not “rafting” in the culture container.
  - Many larvae are observed on the bottom of the container.
  - Larvae do not swim when placed on a microscope slide.
  - Larvae gape with velum extended or rotate slowly on one valve.
  - Post-set do not cling to sides of container or form byssal masses.
- **Fouled shells**
  - Shells appear to have fuzzy edges, evidence of something growing on the shells.
- **Ciliates in culture**
  - Ciliates are bacterial feeders. A sudden increase or appearance of ciliates in one of the best indicators of a bacterial bloom.
- **Recent mortality**
  - Cultures have unpleasant odor.
  - Fresh tissues seen in culture water (post-set, broodstock).
  - Bacteria and ciliates visible inside shells of dead larvae.

### **Prevention**

The major preventive measure is to keep everything CLEAN. All hatchery equipment should be washed regularly with hot water and biodegradable detergent. Culture containers must be cleaned in the same manner and sterilized weekly or whenever contamination is noted. Hoses should be rinsed with hot freshwater after each use and hung to dry. Water cannot be allowed to stand in the hoses. If any hose shows signs of developing a film inside, or any unusual discoloration, it should be scrubbed with a brush attached to a length of PVC pipe and bleached or acid-washed. If a hose cannot be cleaned, it should be discarded. All glassware, beakers, buckets, etc. should be cleaned after use and stored face down on an open-grid rack to dry. After drying, the equipment should be stored. Before the next use, rinse to remove any dust. Never stack wet buckets or beakers inside each other.

Algal cultures may become infested with bacteria or ciliates. Examine cultures routinely and be suspicious of any culture which shows unusual color change, foaming on the surface, growth on the culture vessel walls or airlines, presence of clumps in the culture. Any suspect cultures should be discarded immediately. Between cultures, all algal culture vessels should be thoroughly cleaned. New cultures are always provided with new airline and clean airstones. Airstones can be cleaned with clorox or acid. The filter on the air pump will need to be changed occasionally, as will the in-line filters. Algal cultures should

always be covered to prevent airborne contamination.

The spread of disease is prevented by using different culture containers, screens, etc., for each group of larvae or post-set. Replication of each group in separate containers, while it does not prevent the occurrence of disease or mortality, protects against total loss.

### ***Identification and removal of problem***

When warning signs occur, the cause and extent of the problem need to be determined. Examine all cultures (being careful to avoid cross-contamination in the process) to determine which are affected. Within a culture, estimate how long the problem has existed and how many of the animals are affected. Are shells empty, indicating the animals have been dead more than 24 hours? How large are the shells (same size as live animals or smaller)? Are all culture stages (larvae, post-set, broodstock) affected? The extent of the problem will give some clues as to possible causes. For example, if only the post-set are affected, it is unlikely that the algal cultures, which are fed to all stages, are causing the problem.

### **Possible causes of hatchery mortalities and ways to eliminate them**

- **Poor water quality (this may vary seasonally, after storms, etc.)**
  - Increase or change methods of treatment of incoming water.
  - Get water from a different source temporarily.

- Make sure transmission lines are clean.

### • **Storage tank contaminated**

- Clean tank and sterilize with bleach.
- Change any airlines.
- Make sure all distribution lines, hoses and pumps are clean.

### • **Algal cultures contaminated**

- Examine cultures under microscope for signs of ciliates or clumping.
- Examine sub-cultures.
- Discard any suspect cultures and wash containers thoroughly.
- Change all air filters.
- Make sure Kalwalls® are protected from airborne contamination.
- Make sure Kalwalls® have clean airlines and airstones.
- Review procedures for sterilizing culture water.

### • **Contamination from broodstock**

- Scrub broodstock before spawning.
- Bleach broodstock before spawning.
- Depurate broodstock by holding in clean, UV-treated water with no food for 24 hours before spawning.
- Make sure all hoses, etc. used for broodstock water are washed thoroughly before being used for larvae or post-set; preferably have separate sets of equipment.
- Destroy diseased broodstock and sterilize tank.

### • **Inadequately cleaned or poorly stored equipment**

- Make sure all hoses are washed thoroughly and stored so that they dry.

- Make sure all pumps and other equipment are cleaned after use and allowed to dry.
- Wash equipment before use to remove any airborne contamination.
- Store only hatchery supplies/equipment in the hatchery; remove any other items to a different area.

• **Floor drains**

- Make sure all drains are flowing freely.
- Clean all floor drains and bleach.

• **Heavy metal contamination**

- Make sure there are no possible sources of contamination. Particularly examine any new pumps or equipment which might have metal parts.

• **Chemical contaminants**

- Make sure no insecticides are being used in the hatchery or nearby where they might drift in.
- Workers who have used bug repellent must wash thoroughly before entering hatchery.
- Make sure all chlorine is being removed after cleaning culture containers.
- Do not use volatile chemicals (e.g. some glues and cements) in the hatchery.

• **General cleanliness**

- Remove any sources of contamination (e.g. uncovered trash cans).
- Clean floors, countertops, etc..
- Remove any equipment which is used outdoors.
- Dip footwear in chlorine before entering hatchery.

- Make sure hatchery workers wash their hands before handling larval and post-set cultures.
- Do not allow smoking or eating in hatchery.

***Handling of diseased cultures***

Quite often the cultures are beyond salvage and should be discarded before further contamination occurs. However, if the problem has been noticed early, you may try the following remedies.

**Larvae**

1. Rinse larvae thoroughly and transfer to clean containers.
2. Clean and chlorinate containers .
3. Treat culture water with activated charcoal and UV radiation.
4. Rinse clams with freshwater at each water change (older larvae may tolerate this but it could kill young larvae).
5. Filter out any dead larvae if possible.

**Post-set**

1. Soak clams in cool freshwater for 5–10 minutes.
2. Isolate contaminated silos in separate reservoir.
3. Remove clams and scrub and chlorinate all silos as well as reservoir.
4. Replace all airlines and airstones.
5. Large post-set (approaching 1 mm) may be soaked in diluted chlorine bleach (1 ml/liter freshwater) for 5 minutes. Make sure to use freshwater, not seawater. Be sure to rinse thoroughly afterwards to remove the chlorine.

### **Broodstock**

1. Chlorinate tanks and trays.
2. Soak clams in dilute bleach (1 ml/liter freshwater) for up to 2 hours; scrub off any shell contaminants.
3. Replace all airlines and airstones.
4. Flush lines to chiller with freshwater and chlorinate.

### **Antibiotics**

Some hatcheries use antibiotics to control disease. While this may be warranted in some cases where the diseased cultures are of unusual importance, it is not recommended as a common practice. Many bacteria have the capability of developing resistant strains and the frequent or prolonged use of antibiotics therefore can be counterproductive. The use of antibiotics on organisms intended for human consumption is regulated by the Food and Drug Administration. For more information on antibiotic use, refer to Brown (1983).

### **RECORD KEEPING**

It is very important to keep accurate records of hatchery activities. These are needed for tracing the origin of problems, should they occur; modification of protocols; verification of feeding rates; documentation of experiments; and development of breeding programs. The following types of records are recommended: daily activity logbook; daily water quality/feeding sheets (one for each group being

cultured); daily water quality on source water; data forms for recording size, volume, counts at regular intervals on each group produced in the hatchery; data logbook for each group cultured (several groups may be in one book) in which all observations as well as data summaries are recorded; and broodstock logbooks.

### **HATCHERY MAINTENANCE**

The hatchery should be kept as clean as possible at all times. Floors should be mopped daily. Trash cans should be covered at all times and should be emptied daily. Seawater hoses should be rinsed with freshwater after use and allowed to dry. Freshwater hoses should be coiled neatly so people won't trip on them. Floor drains should be rinsed regularly to prevent buildup of silt, debris, algal film, or bacterial slime; drains may periodically require chlorination.

### **Equipment care and storage**

Only supplies and equipment in regular use should be stored in the hatchery proper. Storage closets should be used for long-term supplies, little-used equipment, etc. Hatchery supplies and equipment should not be shared with other operations (e.g. nursery). Everything should be thoroughly dry when stored. Store containers face down so they won't get dusty inside. Open shelves similar to refrigerator shelves can be used not only for storage but also for drying. Plastic trays such as those used by bread distributors also make

handy drying racks and are stackable. Larger items (buckets, Kalwalls®, small culture containers) should be stored upside down on slightly elevated racks to allow drainage. If containers are stacked one inside another, make sure they are thoroughly dry before nesting! If an item has been stored for a long time, wash with hot water before using it.

When not in use culture containers should be completely drained. Small containers should be stored upside down. Before reuse, the containers should be thoroughly washed. Water storage tanks must be emptied and cleaned regularly. After cleaning they should be allowed to air dry if possible. When they are cleaned, make sure to clean the drain pipes and various plumbing parts as well.

### **Water transmission system/plumbing**

All plumbing should be cleaned regularly. Flexible hoses should be rinsed with freshwater, drained and hung to dry. Fixed plumbing should be cleaned regularly by flushing with freshwater and bleach. This is easier to accomplish if dual lines are provided. Otherwise, it is necessary to shut down operations long enough to clean the lines.

Submersible pumps are sometimes used for transferring water within a hatchery. Many submersible pumps are available, but not all are suitable for hatchery use. Remember to consider the possible toxicity of the pump casing and any wettable parts. It is

safest to use pumps constructed entirely of plastic and/or fiberglass. Magnetic drive pumps do not require impeller shaft seals which could leak lubricants. Pumps should always be handled with care because of the possibility of electrical shock. Do not run pumps without water as they may overheat. Do not allow the electrical plug to contact seawater. Always make sure the plug is thoroughly dry before connecting to the wall socket. Ground fault receptacles provide additional protection from electrical hazards. Wash pumps after each use with hot water and allow to dry. Pumps should be chlorinated regularly (e.g. once a month) and after being used for moving any contaminated water. Don't chlorinate the plug. Oil-filled pumps must be examined regularly to ensure that no lubricant is leaking. The life expectancy of most submersible pumps is about one year under daily use.

### **Chillers**

Seawater chillers which exhibit reduced cooling capacity may need to be cleaned. Accumulation of dust on the radiator will reduce the cooling efficiency. The radiator may be washed with freshwater. If this does not correct the problem, the unit may be leaking freon. There are strict government regulations concerning freon release and a qualified serviceperson should examine the unit. If the refrigeration system requires recharging, it is important to simultaneously locate and repair the leak. Because of the corrosive atmosphere, seawater chillers have a life-expectancy of about 2 years and may fail without

warning. You should always have a backup chiller available. Chiller plumbing should be flushed with freshwater and chlorinated regularly. If you are not going to use a chiller over the winter, make sure to drain it to prevent freezing. Recirculating pumps used in conjunction with chillers usually are equipped with a strainer basket which should be cleaned regularly.

## SAFETY

Hatchery workers should be constantly cognizant of safety. Safety equipment (e.g., gloves, non-slip boots) should be supplied and each worker should be given safety instructions. Hatcheries are inherently dangerous and the greatest protection is caution and attention.

The floor in any wet lab is potentially very slippery. Non-slip products are available in various forms to mitigate this hazard but it cannot be totally eliminated. Do not wear open-toed or leather-soled shoes or go bare-footed in the hatchery. Rubber boots with non-slip soles are recommended. Protective aprons or waterproof coats may be worn to keep clothing dry. Do not leave hoses, etc. lying on the floor where somebody could trip on them.

There is inherently a great danger of electrical shock in a hatchery. Be careful! A ground fault system should be installed to reduce the risk of electrical shock, but the risk cannot be totally eliminated. Never touch light fixtures (e.g., those illuminating algal cultures) unless they are turned off.

Dry your hands before plugging in any electrical device. Do not handle electrical devices while standing in seawater. The only electrical devices which should be allowed near the water are submersible pumps. These must be kept in good working order to prevent short-circuits. When plugging in submersible pumps you should wear rubber boots and your hands should be dry. You could further improve your safety by wearing heavy rubber gloves. Never use electrical devices with spliced cords in the hatchery. Extension cords should be avoided if possible and should never be used where the plug could fall into a tank of water or on a wet floor. Extension cords may be installed on retractable reels overhead to give power in the center of the room. All electrical cords should be of the three-prong (grounded) type.

Be very careful whenever you are using hoses (either fresh or seawater). Watch where you are pointing them! Make sure any connections are tight so there is not danger of the hose blowing apart. If you drop a hose it may recoil unexpectedly and spray an electric plug. Watch out for other people who may be handling electricity. After use, turn off hoses which are fitted with nozzles to prevent bursting. Be particularly careful when you are filling Kalwalls<sup>®</sup> because of the proximity of the light fixtures.

Chlorine bleach and muriatic acid are used regularly in hatcheries, bleach for sterilizing water and culture vessels, muriatic acid for cleaning glassware and algal culture containers. These must never be mixed, as

---

toxic gas results. Be particularly careful when pouring either of these in a drain. If the other chemical is in the drain already, fumes will immediately result and may overwhelm you. Always flush the drain with plenty of freshwater after disposing of either of these. Chlorine bleach should be neutralized with sodium thiosulfate before putting it down the drain. If you always do this it will minimize the danger when disposing of acid. Introducing chlorine or acid into floor drains could effect your point source discharge permit.

Muriatic acid is strong enough to burn eyes and skin and eat holes in fabric. When using full-strength muriatic acid, gloves, safety glasses and respiratory protection are recommended. Avoid inhaling the fumes or allowing them to contact your eyes. If acid gets on your skin, wash it off with cold water immediately. Baking soda may be used to neutralize the acid. Be careful not to spill acid but if you do, rinse immediately with copious

amounts of freshwater. Chlorine bleach can also damage skin, eyes and clothing.

Another chemical in regular use in hatchery operations is formaldehyde, usually diluted to 10% formalin. Formalin use should be restricted to the support lab area and never used in the culture room. Microscope slides, pipettes and other supplies used with formalin should be labelled and reserved exclusively for that use. Formalin may burn if you get it on your skin. You should be in a well-ventilated area when using it and should not breathe the fumes. Never leave a jar of formalin open. Used formalin must be disposed of according to health department regulations.

Material safety data sheets should be obtained for each chemical which is used in the hatchery and laboratory. These should be posted where they can be easily read and should be reviewed by each worker.





# PART IV. DESIGN AND OPERATION OF AN UPWELLING NURSERY SYSTEM

## SYSTEM DESIGN

Upwelling nursery systems are flow-through systems with three basic components: 1) the pumping system which includes intake(s), pump(s), and distribution lines, 2) the upwelling system, and 3) the drainage system. An inexpensive system for seasonal use is described in Kemp (1991). Here we describe a nursery designed for year-round operation.

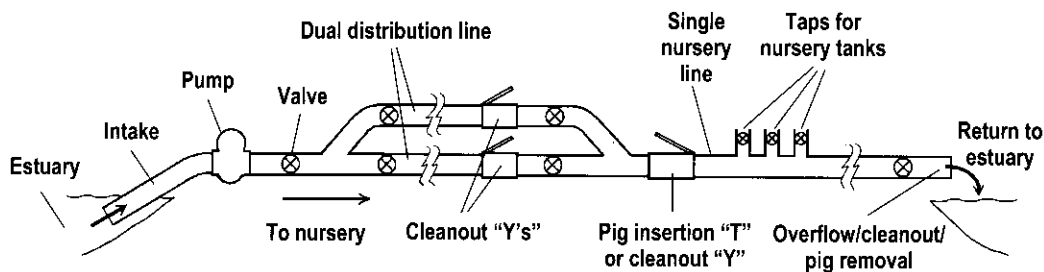
### Pumping system

The nursery must be located near a source of clean, high salinity water (i.e., averaging >24 ppt). This water will be pumped, without any treatment, directly to the juvenile clams, so choosing a site with

good water quality is very important.

Avoid obvious sources of pollutants such as marinas, industrial sites, and sewage treatment plants. Less obvious, but no less detrimental, are non-point source pollutants such as run-off from agricultural land or golf courses.

Choose a pump which is suitable for use in seawater. Avoid brass and bronze which will be subject to galvanic corrosion. The size of the pump will be determined by the desired production capacity of the nursery. For a small system (capacity  $\approx 100,000$  planting size seed), a swimming-pool pump is probably adequate. For larger capacity, a 5 hp or larger industrial pump will be needed.



**Figure 17.**

*Redundant seawater distribution system for a land-based nursery*

If at all possible, a redundant pump and distribution system should be installed (**Fig. 17**). By alternating pump and distribution line useage, the other system can be allowed to go anoxic, thereby killing fouling organisms. That will cut down on and possibly eliminate the need for cleaning the lines, which otherwise will need to be cleaned at least twice a year. A redundant system also provides a back-up pump. The size of the pumping system will depend on your water needs.

Intake line(s) extend from the pump(s) into the source water. If your water source has considerable freshwater input, it may stratify at times. It is important that your intake be deep enough to be in the saltwater layer if stratification is a possibility. A permit will be required from the Office of Coastal Resources Management at SCDHEC (see Appendix A) to install the intake lines. One requirement for intakes is that they must have screens on the end to prevent entrainment of fish. Current regulations require that the entrainment velocity not exceed 0.5 ft/sec. **Table 13** illustrates how to determine the size of the intake screens in order to meet this velocity restriction. Intake screens can be simply constructed using plastic mesh wrapped around a rigid frame. We have used plastic milk crates as a frame for an intake screen. The intake screens will need periodic (at least quarterly) cleaning to remove fouling organisms, and will probably have to be replaced at least once a year.

The intake lines may represent a hazard to navigation requiring appropriate signs. The

Coast Guard regulates marking of navigational hazards. Intakes may be damaged by boat anchors. Warning signs may prevent this. The DNR's Office of Boating Safety may be able to post buoy markers around your intake.

Distribution lines run from the pump to the nursery area. The system should be designed to allow access to the lines for cleaning, although cleaning may be unnecessary if redundant lines are installed. For most situations, PVC pipe will be the best choice for the distribution lines. Provision should be made for flushing the lines through a discharge which bypasses the upwelling system. If possible, include provision for running a "pig" through the distribution line (see description under system maintenance).

It would probably be too expensive to have a redundant distribution system in the nursery because of the many valves required to control flow to each individual tank. The dual distribution lines are therefore merged into one line at the nursery (**Fig. 17**). The distribution line is tapped and fitted with valves to control water flow to individual tanks.

## Upwelling system

The upwelling system is composed of reservoirs (tanks) and upwelling units (silos) (**Fig. 18**). Tanks are usually rectangular in shape and may be constructed of fiberglass, wood or concrete. The tank must have a cleanout fitting at one end, as close to the bottom of the tank as practi-

cable, to allow draining and cleaning. Flow to each tank is individually controlled by valves. PVC ball valves are relatively inexpensive and reliable.

Each tank accommodates several silos (usually 6–12). These silos are usually plastic cylinders 30 to 60 cm (12–24") in diameter and 30 to 45 cm (12–18") inches in depth. The bottom of the cylinder is covered with a plastic mesh screen to support the clams. Silos may be made out of other materials (e.g. wood) and may be other shapes (e.g. rectangular or square). Silos may be made from new or used 50 gallon ( $\approx 200$  L) plastic barrels. These may be cut in half and both ends removed, to yield 2 silos from each barrel. Smaller silos

may be made from 20 liter (5 gal) buckets. Exercise caution in selecting used containers. It is important that the previous contents were non-toxic. To be on the safe side, it would be best to only procure containers which had previously been used for food.

Each silo is connected to the tank wall by an outlet pipe. These drain pipes are usually short lengths of PVC pipe. They should have an elbow attached for adjusting flow rates. Water entering the reservoir must travel up through the mesh screens to reach the outlets which are located inside the silos. The outlet passes through the tank wall and empties into an external trough which carries the water to the drain system.

**Table 13.**  
**Calculation of intake screen surface area needed to meet entrainment restrictions**

**Variables**

A. Pump delivery volume (maximum) in gallons per minute = 500 gpm (example—use actual figure for your pump)

**Constants**

B. Gallons per cubic foot = 7.5

C. Entrainment velocity (maximum) = 0.5 ft/sec (constant) [SCDHEC regulation—check to verify applicability]

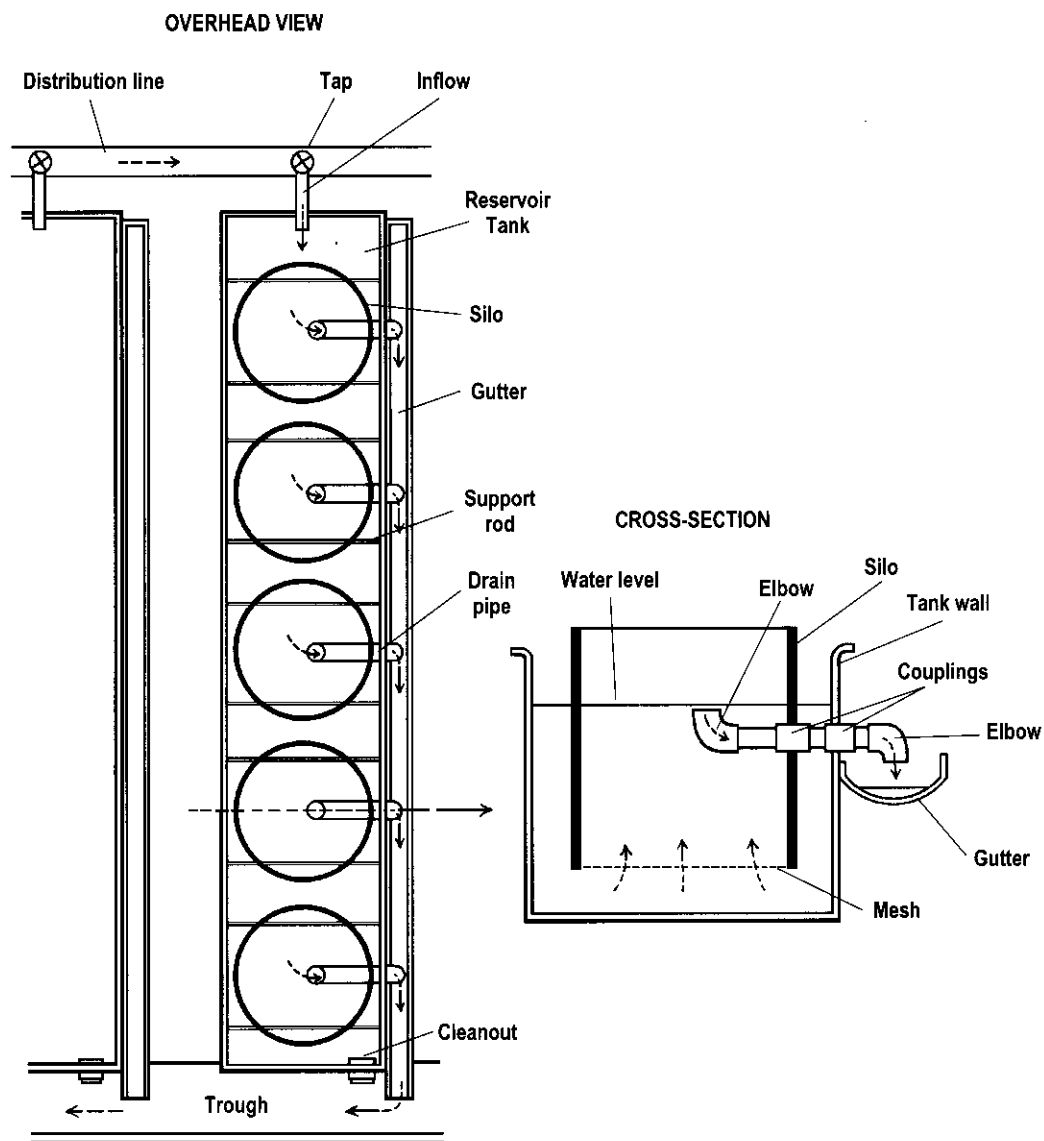
**Convert pump delivery volume to cubic feet per second**

D. Pump delivery in cubic feet per minute =  $A \div B = 500 \div 7.5 \approx 67$  cu ft/min

E. Pump delivery in cubic feet per second =  $D \div 60 \approx 1.1$  cu ft/sec

**Calculate needed intake screen surface area**

F. Surface area needed =  $E \div C = 1.1 \div 0.5 = 2.2$  sq ft



**Figure 18.**  
*Flow-through upwelling nursery system*

An upwelling system allows a large quantity of clams to be grown in a relatively small space. The water flow passing up through the bed of clams evenly distributes food and carries away silt and waste products. This system has the additional advantage that each silo is, in effect, isolated from all others because of the direction of water flow. This greatly reduces the risk of disease.

The number of tanks and their size will determine the capacity of the nursery. A system with six tanks, each holding 8 (24" diam) silos can accommodate  $\approx 1$  million planting size seed (8–10 mm). The flow rate to each tank should be 280–320 L/min (70–80 gpm). At least one extra tank should be included in the design to allow for cleaning needs and overflow capacity.

During certain times of the year, a filter bag must be placed over the water inflow into the tanks to screen out undesirable setting organisms (oysters, barnacles, tunicates, etc.). A mesh size of 190–210  $\mu\text{m}$  excludes most of these fouling organisms. Filter bags may be fabricated of polyester mesh sewed into a pillow-case shape. Polyester felt bags, such as those used for filtration in the hatchery, will not work for the high flow rate in the nursery. Although there may be some setting organisms year round, the problem is most severe from March to November in South Carolina. In addition to the filter bags, the tanks may need to be covered with a tarp or dense shade cloth to prevent the growth of macroalgae.

## Drainage system

All the outflow pipes from one tank drain into a common gutter running the length of the tank. This gutter in turn drains into a larger trough shared by all the tanks. The gutters and trough need to be open-topped to allow inspection of flow rates and access for cleaning. PVC pipe cut in half lengthwise can be used for gutters. The trough can be made of larger pipe, wood, or concrete. In some nursery systems, the common trough drains into a catch basin which can be used for the temporary storage of incoming or outgoing clams. The catch basin can also serve as a reservoir from which to pump seawater into a hatchery. The catch basin (or the common trough if there is no catch basin) drains back into the natural waterway. A discharge permit (NPDES) from SCDHEC will probably be required for this outfall.

## SYSTEM OPERATION

This section sets some basic guidelines for the nursery culture of clams. Although these requirements were developed specifically for clams, they can, in general, be utilized for the culture of other bivalve species.

Each group of clams in the nursery should be labelled for identification. This will enable you to track the progress of each group, essential for determining which stocks grow best and for recognizing problems. It is also a good idea to maintain a map showing the contents of each silo.

## Flow rates and stocking densities

In an upflow system, flow rates and stocking densities are interrelated. Flow rate is often expressed in flow per unit biomass of seed. If less water is available, fewer seed can be stocked in the system. Therefore it is difficult to recommend absolute flow rates in the absence of discussing stocking density.

Stocking densities are usually referred to in terms of biomass (volume) as opposed to total number of seed. This is because the total biomass in each silo is more relevant than the actual number of clams, in addition to being easier to determine. Smaller clams are stocked at lower volumes, but these actually represent a larger number of seed. All volumes referred to here are “packed volumes”. To determine the packed volume of a quantity of clams, partially fill a graduated vessel (cylinder or beaker) with freshwater. Slowly add clams, being careful not to let them overflow. Tap the vessel gently on a flat surface, or tap the side of the cylinder, to settle (“pack”) the clams and record the level of the top of the seed mass, the packed volume.

The recommended flow rate is 10–12 liters of water per minute (L/min) for each liter of large seed clams (>8 mm), 40–80 L/min per liter for 3–4 mm seed, and 100–200 L/min for 1–2 mm seed (**Table 14**). In practice, flow rate (L/min) is usually constant. For example, if your pump can supply 40 L/min ( $\approx$ 10 gallons per minute) to each silo, your silo capacity is about 4 liters for large seed, 1 liter for medium

(4 mm) seed, and 200–400 ml for small seed. Velocity is also important although the interaction between velocity, flow rate and carrying capacity has not been fully explored. Small seed appear to require a higher velocity than larger seed. In **Table 14**, examples of velocity/flow rate interaction are given for 2 and 3 mm seed. Less water is required at higher velocities. In practice, this means that your water is more effective and can support a higher biomass if your silo diameter is smaller (which increases the velocity of the water passing through it).

The above recommended flow rates are only guidelines. These recommendations were developed in South Carolina, but other nurseries in other areas have determined similar flow requirements. In most cases, the higher the flow rate the better. However, care must be taken when dealing with small clams. Very small clams form byssal threads which cause the clams to stick together in very tight mats. This can restrict the water flow and result in “fountaining”, with most of the flow coming up through small areas of the screen. The clams in this area are being tossed around, which disrupts their feeding. Seed in the remainder of the silo are receiving little flow at all. Also, high flow rates may actually force the small clams out the outflow pipe. Flow rates should be visually checked several times daily, as they may fluctuate with tidal height.

**Table 14.**  
Flow requirements and stocking densities for different  
size seed clams in an upwelling nursery

Seed Size (mm)	Velocity (cm/sec)	Flow ratio (L/min per liter of seed)	Seed Density (ml/cm <sup>2</sup> )
1	0.6	240	0.15
1.5	0.6	120	0.3
2	0.6	90	0.4
	0.5	100	0.3
	0.4	110	0.2
3	0.4	70	0.3
	0.2	80	0.15
4	0.2	40	0.3
5	0.2	30	0.4
6	0.2	20	0.6
7	0.2	15	0.8
8	0.2	12	1.0
9	0.2	10	1.2
10	0.2	9	1.4

### Expected growth

Clam growth is dependent on many factors, but the two most important are temperature and food availability. Clam growth is best when water temperatures are between 20 and 28°C. During times of the year when temperatures are within this range, seed should increase in size 2 to 3 mm per month and new growth (white lips on the shell) should always be apparent. If new growth is not apparent during these times, it may be a sign that biofouling within the system piping is removing a large portion of the food supply or is

restricting flow rates. Growth rates decrease below 20°C and clams will stop growing altogether below 10°C. Growth is also reduced above 28°C and may be negligible above 30°C. Food availability varies seasonally and is usually lowest in winter. In summer, food availability is high, but so are the metabolic requirements of seed, so that growth rate may be low despite a good food supply.

The nursery operator has no control over temperature and ambient food supply. However, it is possible to increase food availability by increasing flow rates or

decreasing stocking densities, thus providing more food for each individual. Higher flow rates improve growth and survival during periods of extreme heat (30°C) but have little effect when water temperatures are below 12°C.

## **Maintenance**

### ***Nursery system***

Regular maintenance of an upwelling nursery can be divided into two main categories based on frequency: Daily and Monthly.

#### **Daily maintenance**

1. Freshwater rinse for all clams and culture containers. This serves several purposes:

- remove accumulated silt
- flush planktonic fouling organisms (e.g. oyster and barnacle larvae) from the tank
- kill newly set fouling organisms
- kill surface bacteria

As much silt as possible should be rinsed from the bottom of the tank. If the silt is allowed to build up, it may go anaerobic and remove oxygen from the water. If weather is mild (40–70°F) it is advantageous to allow the clams and tanks to sit dry for 30 minutes before refilling. This will kill more fouling organisms and bacteria. Some nurseries fill the tank with freshwater after cleaning. The water is allowed to sit for 10–15 minutes before seawater flow is restored. It is important to monitor water temperature when using

this method and to restore flow if the temperature approaches 30°C.

After cleaning, the tanks are refilled. Very small clams (< 3 mm) will tend to float as the silos are being refilled; therefore, if the nursery contains clams of this size, be sure to check them as the silos are filling. Gently stirring or splashing the surface of the water will break the surface tension and allow the clams to sink to the bottom.

This routine cleaning may be performed less often (e.g. only three times a week) if observation indicates that there are no fouling problems. However, the nursery must be observed daily to insure proper flow rates, regardless of whether the tanks are drained and rinsed.

2. Check all flow rates.

As the silos are being cleaned, the outflow pipes can be knocked out of adjustment or become disconnected. After the tanks have been refilled and are flowing, check to see that all outlets are flowing at the proper rate and are securely connected to the tank wall. Flow rates should be inspected twice a day whether the tanks are cleaned or not.

3. Clean filter bags.

When filter bags are being used, they must be cleaned daily. They should be inspected at least twice daily because they sometimes become clogged and begin to overflow. As the bags are being cleaned, check them closely for holes or tears and replace or repair them as necessary. If possible, maintain two complete sets of bags so that they may be dried between use.



---

### **Monthly maintenance**

At least once per month, the tanks and silos need a thorough cleaning. It is convenient to schedule cleaning of 1–2 tanks per week, thus rotating through all tanks in a monthly period. Monthly cleaning involves draining the tank, transferring the clams to clean silos, and placing these into a previously cleaned tank. The dirty tank and the silos are then scraped to remove any fouling organisms. Paint scrapers or spackling knives work well. Particular care should be taken to remove even very small barnacles and oysters. Also clean all outflow pipes. After scraping, scrub the tank and silos with a plastic scrub brush and rinse with freshwater. Allow the tank and silos to air dry prior to reuse. If space permits, keep one tank at a time clean and empty, available for transferring clams. If there are not enough tanks available, clams must be left out of the tank while it is cleaned. If this is the case, make sure the clams are kept cool and slightly damp and keep them out of water as short a time as possible.

### ***Seawater distribution system***

The pump should be inspected daily to assure proper operation. Refer to the owner's manual for your pump for details on what to check. Excessive vibration or unusual noise in the impeller housing may indicate cavitation which should be corrected as soon as possible. Cavitation may result from an air leak on the intake side of the pump or from an intake restriction. It may indicate that the intake

screens are badly fouled. The intake screen should be checked monthly to determine the extent of fouling and ensure screen integrity. The screen will require cleaning or replacement at least twice a year.

If a dual distribution system has been installed, one line should be allowed to go anoxic for 7–10 days at a time. Thus, once a week, you will need to alternate which line is in use. Always flush the anoxic line through a bypass. In a dual system, further cleaning of the pipe may never be necessary. However, you should check occasionally by removing a cleanout and visually inspecting the interior of the pipe. A reduction in flow rate or food availability might indicate a need for cleaning.

If a dual system was not installed, the distribution line will need to be cleaned two or three times each year to remove barnacles, oysters, etc. which have set inside the line. Usually, this is done during the setting season (May/June) and immediately after the setting season (November/December). Depending on the size of the system, this cleaning may be done by hand with long-handled brushes, or you may have to hire a pipe-cleaning service.

Within the nursery it is usually impractical to have a dual service because of the great expense of duplicating all the valving for the individual tanks. Therefore the nursery line will need regular cleaning. If possible, the system should be designed to accommodate cleaning with a "pig", a device which fits snugly inside the pipe and is rough enough to scrape off small fouling

organisms. Our pig is constructed from a foam float wrapped with hardware cloth. The piping system must have a capped "T" or "Y" at the inflow end to allow the pig to be inserted. There must be a fitting at the outflow end to allow the pig to escape. With water flow diverted or shut off, the pig is inserted into the line. Water flow is then restored, pushing the pig through the line to the removal point. If a pig is run through the lines weekly, most fouling organisms should be removed. If not done that frequently, oysters and barnacles could become too large and prevent the pig's passage. If the system is not designed to accommodate a pig, the lines should be cleaned with wire brushes 2–3 times a year. It is imperative that the nursery design provide either ingress/egress for a pig or cleanouts for using a brush.

## MONITORING SEED STOCKS

Seed should be observed daily during cleaning to check for presence of empty shells, gapers, or decaying tissue (indicating recent mortality), fouling organisms (oysters, mussels, tunicates, barnacles), and growth rate. Recent growth is indicated by white bands on the shell margin. Lack of growth may indicate overcrowding, insufficient flow, or some other problem which should be corrected. Regular observation will indicate how frequently the seed need to be thinned, which will be site and season-specific. In South Carolina, seed may need thinning as frequently as once a week in spring, summer and fall. In the winter, once a month is probably adequate.

At least once a month, and more often during the rapid growing season, each silo should be assessed to determine growth and survival and to adjust stocking density. The silo is removed from the reservoir and all the clams are rinsed into a plastic pan, such as a dish pan, using freshwater. The clams are sieved on appropriate-size screens to separate them into various size categories. Some of the seed may be ready for transfer to field grow-out. These are moved to the field staging area, possibly in the catch basin or in a separate raceway. The remaining seed may be further subdivided into size categories by sieving. Growth is improved if seed in a silo are all similar in size. Water flow will be more uniform and large seed will not deprive smaller ones of food.

Working either with the entire group or with the separate size classes, determine total volume, total number and average size. The total volume of clams is determined by packed volume, described previously under stocking density. Subsamples of known volume (determined in the same way but using a smaller graduated vessel) are counted to determine the total number of seed according to the formula:

$$\frac{\text{average count}}{\text{volume of seed counted}} \times \text{total volume} = \text{total number}$$

A sample of 25–50 individuals should be measured with hand-held calipers. All this information is recorded and used to calculate growth (in terms of both volume and length) and survival since the last

sampling. Keeping track of growth and survival will allow you to anticipate problems (e.g. overcrowding), to identify groups with superior performance, and to improve predictions of nursery output.

If fouling organisms are present, these may need to be removed by hand-picking. The clams are then returned to one or more clean silos to achieve an appropriate stocking density in the new silos, remembering to allow for future growth.

## **PROBLEM SOLVING**

### **Biological problems**

Biological problems fall into 3 basic categories: mortality of seed; poor growth of seed; and presence of fouling organisms.

#### ***Mortality***

It is often difficult to determine the cause(s) of mortality in seed clams. The presence of gaping shells and decaying tissue in the silo indicates that clams are dying. These are usually accompanied by a distinctive unpleasant odor. Although some shell may be present when clams are initially stocked into the silos, any increase in the amount of clam shell should be taken as an indication of recent mortality. Clam tissue separates from the shell after the clam has died and is usually noticed floating in the water within the silo especially when the clams in the silo have been stirred. Some possible causes of mortality are discussed below.

#### **Inadequate food supply**

Occasionally, the source water will be naturally low in available food. If this is the case, there is not much that can be done. It is important, however, to insure that the piping system has not become fouled with organisms that are removing the available food from the source water before it reaches the clams in the tanks. One way to do this is to perform a visual inspection of the interior of the pipes and look for organisms such as oysters and barnacles. If these organisms are present in sufficient numbers, it is time to clean the pipes. Another way to insure that source water and tank water have the same amount of available food is to determine chlorophyll content of both the source and tank water. This can be done with a fluorometer. If you do not have a fluorometer, you may be able to have the samples analyzed by DNR, the Clemson Extension Service, a college or university or another clam culture facility. Instructions on performing a fluorescence check are in the section on Record Keeping. When available food is low it may be necessary to stock the clams less densely to prevent starvation. Alternatively, flow rates could be increased, if possible.

#### **Gas bubble disease**

Gas bubble disease is caused by culture water that is supersaturated with air. Mortality due to gas bubble disease should not occur if the nursery is being monitored properly. If the water is supersaturated, minute air bubbles are apparent where the

water enters the tank. Another sign of air in the water is accumulation of air under the screens of the silos. The seawater pump may be entraining air on the suction side. Some pumps will lose their prime if this is the case. Swimming-pool pumps usually have an observation port which allows you to see air in the incoming water. Air in the pumping chamber usually causes cavitation, which may be audible. Cavitation may quickly destroy the impeller and even the pump housing and should be corrected immediately.

Air entrainment can also occur in the distribution system. Because the system is under relatively low pressure, water moving past a loose fitting may suck air into the pipe, rather than pushing water out. This is often accompanied by a hissing or sucking sound. If the problem affects only one tank, check all fittings from the distribution line to that tank. If more than one tank exhibits the problem, there may be a faulty fitting in the distribution line or a bad pump seal.

#### **Temperature and/or salinity shock**

Clams do not do well when subjected to a rapid change in temperature or salinity. In order to prevent any adverse effects, allow the clams to acclimate. For example, if clams have been shipped in a refrigerated container, allow them to warm to ambient temperature before placing them into warm nursery water. If the clams have been held in 30 ppt water in the hatchery, wait for high tide before placing the clams into the nursery. Normal fluctuations in

salinity due to tidal flow are not sudden enough to harm the clams. However, prolonged periods of low salinity, such as may occur after heavy rainfall, may cause some mortality. This can only be prevented by site selection—i.e. choosing a location where the problem is not likely to occur.

#### **Lack of oxygen**

This is seldom a problem as long as the clams are in flowing seawater. However when in standing seawater, no matter the quantity, clams will continue to respire and can quickly use up the available oxygen. Clams can be placed in cool freshwater for an hour or two. Under no circumstances should clams be allowed to remain in standing seawater for any length of time!!! If water flow is lost in the nursery, mortality due to lack of oxygen may begin to occur within two hours, depending on the amount of clams in a tank, the temperature of the water, and the time of day. If flow cannot be restored within an hour, the tanks should be drained so that seed are not sitting in standing seawater. It may be necessary to spray them with water to keep them cool.

#### **Bacterial, fungal or fouling organism problems**

Sometimes mortalities are accompanied by obvious signs of bacterial contamination, such as bacterial plaques (usually pink) on the tanks or silos. Another symptom which may accompany mortality is the presence of a slimy or fuzzy coating on the shells. This may be bacteria, fungi, bryozoans,

hydroids, or some other unwanted pest. It may or may not be causing the mortality, but should nonetheless be treated. There are two possible treatments for bacterial and shell-fouling problems. One is to soak the seed in freshwater. A more effective method is to soak the seed in dilute chlorine (1 ml bleach in 1 liter freshwater) (see Kraeuter and Castagna, 1984). If the entire tank is filled with the bleach solution, the silos and tank will be sterilized at the same time. Make sure to use freshwater, not seawater. This soaking should last at least 15 minutes and no more than 2 hours. If soaking for a long period of time, monitor the temperature. The temperature should not be allowed to increase more than a degree or two, and never above 30°C. Soakings may be repeated daily for several weeks if needed. Recurring problems may be helped by daily soaking in freshwater, as described under nursery maintenance.

### **Poor growth**

Poor growth can result from poor quality seed stock, overcrowding, lack of food, or unfavorable environmental conditions. When the problem is caused by overcrowding, thinning the seed into additional silos to reduce density should immediately result in improved growth. If no more space is available it may be necessary to discard some of the seed stock. Chronic overcrowding will sooner or later result in increased mortality. If thinning the seed does not result in improved growth, there may be insufficient food. This could be caused by fouling within the distribution lines.

### **Fouling organisms**

Several fouling organisms can be a problem in the nursery, including oysters, barnacles, mussels, bryozoans, hydrozoans and tunicates. These can foul the piping system, occlude the silo screens, and foul the seed themselves. They make it difficult to clean the tanks and silos and can effect clam growth. It is difficult to remove these organisms from the seed clams, so it is best to prevent them from entering the reservoir tanks if possible. Fouling organisms probably enter the system as larvae because larger organisms would be damaged in passage through the pump. Since water is constantly flowing through the system and the tanks are drained regularly, the organisms which are the major problem are those which are ready to set when they enter the system. Filter bags on the tank intakes will trap most of these setting-size pests without interfering with water flow. If fouling organisms are evident in tanks equipped with bags, the bags may have holes in them, may be overflowing, or may be constructed of too large a mesh. The mesh size should be no greater than 210  $\mu\text{m}$ . Macroalgal growth can be a problem in the nursery at certain times of year. Covering the tanks with wooden covers, plastic tarps, or greenhouse shading material usually eliminates macroalgal growth.

---

## **System problems**

### ***Air bubbles in inlet water***

Abundant air bubbles in the water can be a serious problem to bivalves (e.g., gas bubble disease) and must be eliminated as soon as possible. Air bubbles indicate an air leak somewhere in the system. As water flows rapidly past the leak, air is sucked into the pipe. It may be accompanied by a hissing sound. The problem may often be localized by identifying which tanks are affected. If the leak is on the suction side of the pump, cavitation of the pump may be audible. Check for leaks especially around fittings and valves.

### ***Insufficient flow to one or more tanks***

If the problem is limited to only a few tanks, it is possible that the piping between the ball valve and the tank needs cleaning. If all tanks are involved, the intake may be fouled, the interior of the pipe may be fouled, or the pump may be malfunctioning. Scheduling regular cleaning of all pipes and the intake screens will prevent those problems and is often more convenient than having to perform emergency cleaning. It is advisable to have a back-up pump available in case of failure of the main pump.

### ***Silo screens clog***

Sometimes the screens on the bottom of the silos clog, causing reduced flow through that silo. Indications of screen clogging are reduced water level in a silo,

reduced flow through a silo, floating of the silo, or failure of the silo to drain when the tank is drained. Screen clogging can be caused by overcrowding in the silo, use of too fine a mesh, fouling organisms (especially sea squirts and bryozoans) growing on the bottom of the screen, or insufficient cleaning.

## **RECORD KEEPING**

Daily record keeping for any nursery system should include hydrographic data (e.g., temperature and salinity). After a period of operation, it will be possible to relate problems in the nursery (or hatchery) to variations in these parameters. For instance, reduced salinities often follow a period of heavy rainfall. Other problems may be associated with rainfall (e.g. runoff of pesticides or fertilizers) which are less easily measured. Water quality, particularly in a hatchery, may need to be monitored and/or modified more frequently during these episodes. The temperature record, accompanied by your growth measurements, will allow you to predict growth in future years.

Daily activities should be recorded in a log book. A chart showing the location of each stock in the nursery is useful if more than one stock is being grown. A record of seed movements into and out of the nursery should be kept. This is vital for determining nursery production, identifying any problems or bottlenecks, scheduling not only nursery but also hatchery and field activities, and predicting future perfor-

---

mance. All seed monitoring activities and data collected should be recorded.

If a fluorometer is available, occasional water samples should be collected for fluorescent analysis. Two samples are required: one from the water source and another from the inflow of the last tank in the nursery system. The difference between these is an indicator of the condition of the distribution piping. A higher fluorescence

for source water than for tank water indicates that the food (algae) is being stripped (eaten) by fouling organisms that have set on the interior of the piping system and a system cleaning is warranted (see section on Maintenance). Fluorescence records over time will also give you an idea of seasonal variations in food availability at your site. This will allow you to determine whether periods of slow growth are related to lack of food.





---

# LITERATURE CITED AND FURTHER READING

## GENERAL

- Castagna, M. 1983. Culture methods for growing the clam *Mercenaria mercenaria*. Virginia Institute of Marine Science, Wachapreague, VA.
- Castagna, M. and J. Kraeuter. 1981. A manual for growing the hard clam, *Mercenaria*. Special report in Applied Marine Science and Ocean Engineering No. 249, Virginia Institute of Marine Science, Gloucester Point, VA 23062. 107pp (\$3.00).
- DeVoe, M.R., and J. M. Whetstone. 1987. An interim guide to aquaculture permitting in South Carolina. South Carolina Sea Grant Consortium, SC-SG-TR-84-2. 27pp.
- Elston, R.A. 1990. Mollusc Diseases: Guide for the Shellfish Farmer. Washington, Sea Grant Program, University of Washington Press, Seattle. 73 pp.
- Flimlin, G. 1990. How to buy clam seed without getting shucked. New Jersey Sea Grant Extension Service Bulletin No. 5. 5pp.
- Gibbons, M.C. and W.J. Blogoslawski. 1989. Predators, pests, parasites, and diseases. pp 167-200 in Manzi, J.J. and Castagna, M., eds., Clam Mariculture in North America. Elsevier, NY.
- Hadley, N.H. 1993. Effects of hard clam hatchery management practices on productivity and on broodstock quality. World Aquaculture 24(3):30-31.
- Harbor Branch Oceanographic Institution. 1989. A Manual for Farming the Hardshell Clam in Florida. Harbor Branch Oceanographic Institute, Fort Pierce, FL. 27pp.
- Kemp, P.S. 1991. Clam Gardening: a manual for small-scale clam operations in North Carolina. NOAA, NMFS, Office of Sea Grant, UNC-SG-91-02. 35pp. \$5.00 from NC Sea Grant.
- Kraeuter, J. and M. Castagna. 1984. Disease treatment in hard clams *Mercenaria mercenaria*. Journal of the World Mariculture Society 15:310-317.
- Malinowski, S. 1986. Small-scale farming of the hard clam on Long Island, New York. (copies may be available from Aquaculture Innovation Program, NY State Urban Development Corporation, 1515 Broadway, NY, NY 10036; or from Steve Malinowski, The Clam Farm, Inc., Box 402, Fishers Island, NY 06390).
- Manzi, J. 1985. Clam aquaculture. pp. 275-310 in Huner and Brown, eds., Crustacean & Mollusk Aquaculture in the United States. AVI Publ., Westport, CT.
- Manzi, J. and Castagna, M., eds. 1989. Clam Mariculture in North America, Elsevier, NY.
- Manzi, J., V.G. Burrell, Jr. and W. Carson. 1980. A mariculture demonstration project for an alternative hard clam

- (*Mercenaria mercenaria*) fishery in South Carolina. Proceedings of the World Mariculture Society 11: 79-89 .
- NMFS. 1992. Fisheries of the United States, 1991. Current Fisheries Statistics 9100. U.S. Department of Commerce, National Marine Fisheries Service, Washington DC. 113 pp.
- Quayle, D.B. and G.F. Newkirk. 1989. Farming bivalve molluscs: methods for study and development. Advances in World Aquaculture, Vol. I. World Aquaculture Society, Baton Rouge, LA.
- Vaughan, D. 1988. Record keeping for aquaculture: clam farming. Publication of NJ Sea Grant, NJSG-88-172. 20pp.
- Walker, R. 1983. Feasibility of mariculture of the hard clam *Mercenaria mercenaria* Linne in Georgia. Journal of Shellfish Research 3(2):169-174.

## HATCHERY CULTURE

- Bricelj, V.M. 1979. Fecundity and related aspects of hard clam (*Mercenaria mercenaria*) reproduction in Great South Bay, New York. Masters thesis, Marine Environmental Sciences Program, State University of New York at Stony Brook.
- Brown, C. 1983. Bacterial disease in bivalve larval cultures and their control. pp. 230-242 in C.J. Berg, ed. Culture of Marine Invertebrates. Selected Readings. Hutchinson Ross Pub. Co. Stroudsburg, PA.
- Castagna, M. and J. Manzi. 1989. Clam culture in North America: Hatchery production of nursery stock clams. pp

111-126 in Manzi, J.J. and M. Castagna, eds. Clam Mariculture in North America. Elsevier, NY.

- Chanley, P. 1972. Laboratory cultivation of assorted bivalve larvae. pages 297-318 in W.L. Smith and M.H. Chanley, eds., Culture of Marine Invertebrate Animals. Plenum Press, New York.
- Davis, H.C. and R. R. Guillard. 1958. Relative value of ten genera of microorganisms as food for oyster and clam larvae. USFWS, Fishery Bulletin 136 (58): 293-304.
- Hartman, M. 1989. Manual for the design and operation of a low budget hatchery for the hard clam *Mercenaria mercenaria* in Florida. Aquaculture Report Series, Florida Dept of Agriculture and Consumer Services, Division of Marketing. (to get a copy write Aquaculture Program, Room 425, Mayo Building, Tallahassee FL 32399-0800 or call (904) 488-4033 -free or nominal cost)

- Loosanoff, V.L. and H. C. Davis. 1963. Rearing of bivalve mollusks. Advances in Marine Biology. Vol. 1. Academic Press, New York.

## NURSERY CULTURE

- Baldwin, R. B., W. Mook, N.H. Hadley, R.J. Rhodes, and M. R. DeVoe. 1995. Construction and Operations Manual for a Tidal-Powered Upwelling Nursery System. South Carolina Sea Grant Consortium, Charleston. 44pp.

- Bayes, J. C. 1981. Forced upwelling nurseries for oysters and clams using impounded water systems. pp. 73-82 in Claus et al., eds. Nursery culturing of Bivalve Molluscs. European Mariculture Society Special Publication #7. Belgium.
- Castagna, M. 1984. Methods of growing *Mercenaria mercenaria* from postlarval to preferred-size seed for field planting. *Aquaculture* 39:355-359.
- Hadley, N. and J. Manzi. 1984. Growth of seed clams (*Mercenaria mercenaria*) at various densities in a commercial scale nursery system. *Aquaculture* 36:369-379.
- Malinowski, S. 1988. Variable growth rates of seed clams in an upflow nursery system and the economics of culling slow growing animals. *Journal of Shellfish Research* 7(3):359-366.
- Malinowski, S. and S. Siddall. 1989. Passive water reuse in a commercial-scale hard clam upflow nursery system. *Journal of Shellfish Research* 8(1):241-248.
- Manzi, J.J. and M. Castagna. 1989. Nursery culture of clams in North America. pp 127-148 in Manzi, J.J. and Castagna, M. eds, *Clam Mariculture in North America*. Elsevier, NY.
- Manzi, J., N. Hadley, C. Battey, R. Haggerty, R. Hamilton and M. Carter. 1984. Culture of the northern hard clam in a commercial-scale, upflow, nursery system. *Journal of Shellfish Research* 4(2):119-124.
- Manzi, J. and N. Hadley. 1988. Recent advances in nursery culture of bivalve mollusks in North America. NOAA, Tech. Rep. NMFS 70, NMFS.
- Mook, W. 1988. Guide to construction of a tidal upweller. Mook Sea Farm, Inc. Damariscotta, ME. 23pp.
- Mook, W. and A.C. Johnson. 1988. Utilization of low-cost, tidal-powered floating nurseries to rear bivalve seed. Mook Sea Farm, Inc. Damariscotta, ME. 28pp.
- Vaughan, D. and L. Creswell. 1990. Field grow-out techniques and technology transfer for the hard clam *Mercenaria mercenaria*. Florida Department of Agriculture and Consumer Services, Aquaculture Report Series, Tallahassee, Fl. 22pp.
- Walsh, D.T., C. A. Withstandley, R.A. Krause, and E.J. Petrovitz. 1987. Mass culture of selected marine microalgae for the nursery production of bivalve seed. *Journal of Shellfish Research* 6:71-77.

## FIELD CULTURE

- Eldridge, P. J., W. Waltz, R.C. Gracy, and H. Hunt. 1976. Growth and mortality of hatchery seed clams *Mercenaria mercenaria* in protected trays in waters of South Carolina. *Proceedings of the National Shellfisheries Association* 66:13-20.
- Eldridge, P.J. A.G. Eversole, and J.M. Whetstone. 1979. Comparative survival and growth rates of hard clams, *Mercenaria mercenaria*, planted in trays subtidally and intertidally at varying densities in a South Carolina estuary. *Proceedings of the National Shellfisheries Association* 69:30-39.

- Gibbons, M.C. and M. Castagna. 1985. Biological control of predation by crabs in bottom cultures of hard clams using a combination of crushed stone aggregate, toadfish and cages. *Aquaculture* 47:101-104.
- Kraeuter, J. and M. Castagna. 1985. The effects of seed size, shell bags, crab traps and netting on the survival of the northern hard clam *Mercenaria mercenaria*. *Journal of Shellfish Research* 5:69-72.
- Kraeuter, J. and M. Castagna. 1989. Factors affecting the growth and survival of clam seed planted in the natural environment. pp. 149-166 in Manzi, J.J. and M. Castagna, eds. *Clam Mariculture in North America*. Elsevier, NY.
- Walker, R.L. 1984. Effects of density and sampling time on the growth of the hard clam, *Mercenaria mercenaria*, planted in predator-free cages in coastal Georgia. *Nautilus* 98(3):114-119.
- Walker, R. and P. Heffernan. 1990. Intertidal growth and survival of northern quahogs *Mercenaria mercenaria* and Atlantic surf clams *Spisula solidissima* in Georgia. *Journal of the World Mariculture Society* 21:307-313.
- Falconer, D.S. 1989. *Introduction to Quantitative Genetics*, 3rd Edition. Wiley, New York, 438 pp.
- Gall, G. and C. Busack, eds. 1985. *Genetics in Aquaculture II*. Elsevier Press, Amsterdam. 386pp (reprinted from *Aquaculture*, vol. 57)
- Gjedrem, T., editor. 1988. *Genetics in Aquaculture III*. Elsevier, Amsterdam. 340pp. (reprinted from *Aquaculture*, vol 85)
- Hadley, N., R. Dillon and J. Manzi. 1991. Realized heritability of growth rate in the hard clam *Mercenaria mercenaria*. *Aquaculture* 93:109-119.
- Heffernan, P., R. Walker, and J. Crenshaw. 1991. Negative larval response to selection for increased growth rate in northern quahogs *Mercenaria mercenaria* (Linnaeus 1758). *Journal of Shellfish Research* 10:199-202.
- Humphrey, C. and J. Crenshaw. 1989. Clam genetics. pp 323-356 in Manzi, J.J. and Castagna, M. eds, *Clam Mariculture in North America*. Elsevier, NY.
- Ryman, N. and F. Utter, eds. 1987. *Population Genetics and Fishery Management*. University of Washington Press, Seattle. 420 pp.
- Tave, D. 1986. *Genetics for Fish Hatchery Managers*. AVI Publishing, Westport, Ct. 299 pp.

## GENETICS/ BREEDING

- Dillon, R. T. and J. Manzi. 1988. Enzyme heterozygosity and growth rate in nursery populations of *Mercenaria mercenaria*. *Journal of Experimental Marine Biology and Ecology* 116:79-86.

## ALGAL CULTURE AND DIETS

- Enright, C. T., G.F. Newkirk, J.S. Craigie and J.D. Castell. 1986. Evaluation of phytoplankton as diets for juvenile

- Ostrea edulis*. Journal of Experimental Marine Biology and Ecology 96:1-13.
- Epifanio, C. E. 1979. Growth in bivalve mollusks: nutritional effects of two or more species of algae in diets fed to the American oyster (*Crassostrea virginica*) and the hard clam (*Mercenaria mercenaria*). Aquaculture 18:1-12.
- Fogg, G.E. 1966. Algae cultures and phytoplankton ecology. Univ. Wisconsin Press, Madison, 126pp.
- Guillard, R.L. 1983. Culture of phytoplankton for feeding marine invertebrates. Pages 108-132 in C.J. Berg, Jr. ed. Culture of Marine Invertebrates. Hutchinson Ross Publishing, Stroudsburg, PA.
- Guillard, R.L. 1972. Culture of phytoplankton for feeding marine invertebrates. pages 29-60 in W.L. Smith and M.H. Chanley, eds., Culture of Marine Invertebrate Animals. Plenum Press, New York.
- Hoff, F. H. and T.W. Snell. 1987. Plankton Culture Manual. Florida Aquafarms, Dade City. 126pp.
- Miyachi, S., O. Nakayama, Y. Yokohama, M. Ohmari, K. Komagata, H. Sugawara and Y. Ugawa, editors. 1989. World Catalogue of Algae. Japan Scientific Societies Press, Tokyo. 329 pp.
- Ogle, J. T. 1982. Operation of an oyster hatchery utilizing brown water culture technique. Journal of Shellfish Research 2: 153-156.
- Stein, J.R., ed. 1973. Handbook of Phyco-logical Methods. Culture methods and growth assessments. Cambridge University Press, New York.
- Tan Tiu, A., D. Vaughan, T. Chiles and K. Bird. 1989. Food value of eurotypic microalgae to bivalve larvae of *Cyrtopleura costata*, *Crassostrea virginica* and *Mercenaria mercenaria*. Journal of Shellfish Research 8:399-400.

## ECONOMICS

- Adams et al. 1991. Investing in commercial hard clam culture: a comprehensive guide to the South Atlantic states. Florida Sea Grant College Program, Report no. 104. 128pp. Available from Sea Grant.
- USDA. 1992. Aquaculture: situation and outlook report. US Department of Agriculture, Economic Research Service, AQUA-9. 45pp.



---

# GLOSSARY

***Airlift:***

A device for aerating and circulating water. Usually consists of a vertical cylinder submerged in water into which air is pumped.

***Aliquot:***

A measured quantity; a subsample of known quantity.

***Autoclave:***

A machine which uses steam and high pressure to sterilize.

***Axenic:***

Bacteria-free.

***Batch culture:***

A culture method in which water is not continuously exchanged. In batch culture of larvae, the larvae are grown in standing (not circulating) water which is completely changed on a regular schedule. In batch culture of algae, algae is allowed to bloom and then is completely harvested.

***Broodstock:***

Adult animals retained for reproduction.

***Calibrated:***

Marked in known units of measurement.

***Calipers:***

Precision hand-held measuring tool, useful for measurements of items ranging from 1 mm to 100 mm.

***Ciliates:***

Microscopic, unicellular animals which swim by means of cilia. These may contaminate algal cultures or larval cultures.

***Compound microscope:***

Same as light microscope. A microscope which provides relatively high magnification (usually 40 to 1000 times the actual size). The magnification provided is the product of the magnification provided by the ocular lens and the magnification provided by the objective lens. Used to examine microalgae, gametes and early larvae.

***Coulter® counter:***

A precision instrument which counts particles suspended in liquid. A Coulter® counter can be used for quantifying gametes, microalgae, and larvae.

***Dissecting microscope:***

A microscope used for examining relatively large objects. It can be used for surface examination of adult clams, for examination of late larvae, post-set and seed clams. Magnification is usually in the range of 4 to 40 times actual size.

***Downweller:***

A culture container in which a flow of water is directed down through a layer of clams supported on a mesh screen. Used for pediveligers and early post-set.

**Downwelling unit:**

See downweller.

**Fecundity:**

Reproductive capacity; ability to reproduce.

**Fluorometer:**

An instrument which measures fluorescence. Since chlorophyll and other photosynthetic pigments fluoresce (release energy as light) when stimulated by light, a fluorometer can be used to quantify microalgae.

**Gamete:**

Reproductive cell; egg or sperm.

**Gonad:**

Portion of body containing reproductive cells.

**Hemocytometer:**

A special microscope slide, designed to count blood cells, which is used for counting phytoplankton.

**Heritable:**

Determined by genetic factors, as opposed to environmental ones. Able to be passed on to offspring.

**Intertidal:**

Above the average low tide level. Exposed at low tide.

**Kalwall®:**

A cylindrical container made of clear fiberglass used for algal culture.

**Lugol's solution:**

An iodine solution which is used for preserving algae. Lugol's solution is made by mixing 6 g of potassium iodide and 4 g of iodine in 10 ml of seawater.

**Metamorphosis:**

Change from one morphological state to another. Hard clams metamorphose from trochophore to veliger and again from veliger to adult form. Metamorphosis is stressful and high mortalities may occur at these times.

**Micrometer:**

A calibrated device for measuring objects under magnification. An ocular micrometer can be inserted in the eyepiece of a microscope. It is calibrated with a stage micrometer (a special microscope slide).

**Milford method:**

A system for mono-specific algal culture. See description in chapter on algal culture.

**Muriatic acid:**

Dilute hydrochloric acid (HCl) used for cleaning glassware and other non-corroding materials.

**Notata:**

Having distinctive shell markings, often reddish. These may be in the form of bands or chevron-shaped marks.

**Objective lens:**

The lens nearest the sample on a compound microscope. Most compound scopes are equipped with several inter-



changeable objectives, allowing a range of magnification. The magnification is the product of the ocular lens magnification and the objective lens magnification.

**Ocular lens:**

The lens nearest the eye on a compound microscope. Total magnification on a compound microscope is the product of the ocular magnification (usually 10X or 15X) and the objective magnification.

**Pathogenic:**

Disease causing.

**Pediveliger:**

A late larval stage, intermediate between a swimming veliger and a non-swimming post-set. A pediveliger has both a foot and a velum.

**pH:**

A measure of the number of free hydrogen ions in a liquid. pH is measured on a scale of 0 to 14. 7 is neutral, <7 is acidic, >7 is basic or alkaline. Seawater usually has a pH of 7–8. Algal cultures become alkaline (pH increases) as the algae multiplies. Carbon dioxide is added to decrease the pH. pH can be measured with special paper strips which change color in response to pH, or with a variety of meters.

**Phytoplankton:**

Microscopic aquatic plants which are the primary food source for hard clams.

**Pig:**

A special device for cleaning the inside of pipes, such as in a flow-through nursery system.

**Polyspermy:**

A situation which results when more than one sperm penetrates a single egg, resulting in abnormal development.

**Post-set:**

Clams which have metamorphosed to the adult form but are still retained in hatchery culture, usually 1 mm or less in length.

**Pseudo-feces:**

Material which a clam has filtered from the water but not ingested. Copious pseudo-feces production indicates that the concentration of microalgae or other suspended particles is very high. The clams are filtering more particles than they can eat.

**PVC:**

Polyvinyl chloride. A common plastic used to fabricate piping. Commonly used in aquaculture for plumbing because it is non-corrosive and non-toxic.

**Ripe:**

Having mature gametes; ready to spawn.

**Salinity:**

Concentration of dissolved salts in seawater. Usually measured in parts per thousand (ppt), the grams of salt contained in a kilogram of water. Full-strength seawater is 35–36 ppt. Salinity can be measured by refractive index (refractometer), specific gravity (hydrometer) or conductivity.

**Secchi disk:**

A device for measuring turbidity. A white disk is submerged in liquid until the disk disappears from view. The depth at which

the disk disappears can be related to the quantity of suspended particles in the liquid (turbidity). A small Secchi disk can be calibrated for use in determining microalgae densities.

***Sedgewick-Rafter cell:***

A special microscope slide used for counting larvae.

***Seed:***

Any sub-market size (<45 mm) clams.

***Silo:***

Cylindrical culture container used as a downweller or upweller for culture of post-set and juvenile bivalves.

***Sodium thiosulfate:***

$\text{Na}_2\text{S}_2\text{O}_3$ . A compound used to neutralize chlorine.

***Spawning:***

Release of mature gametes (eggs/sperm). Clams release gametes into the water column where fertilization occurs.

***Spectrophotometer:***

An instrument which measures how much light of a given wavelength passes through a liquid.

***Subtidal:***

Below the average low-tide level.

***Trochophore:***

Early non-feeding bivalve larval stage with no shell, resembles a child's top.

***Upweller:***

A culture container in which continuous water flow is directed up through a layer of clams supported on a mesh screen. Used for late post-set and juveniles.

***Upwelling unit:***

See upweller.

***Veliger:***

Free-swimming larva which uses a "velum" for locomotion and feeding. Veligers have a shell, initially "D" shaped, later rounding out to a shape similar to adult clams.

***Wells-Glancy method:***

A system of algal culture depending on natural phytoplankton populations. Also known as "brown-water" culture.

***Wildstock:***

Animals procured from the wild, as opposed to those which have been produced in a hatchery.

***Zooplankton:***

Very small aquatic animals. Clam larvae are zooplankton. Other zooplankton may contaminate algal and larval cultures.

***Zygote:***

Fertilized egg.

---

# APPENDIX A. AGENCY/TECHNICAL ASSISTANCE CONTACTS

## REGULATORY AGENCIES

### **S.C. Department of Health and Environmental Control**

#### ***Critical zone activity permits***

Office of Ocean and  
Coastal Resources Management  
Ashley Corporate Center  
4280 Executive Place, Suite 300  
Charleston, SC 29405  
(803) 744-5838

#### ***Discharge permits***

Div. of Industrial and  
Agricultural Wastewater  
2600 Bull St.  
Columbia, SC 29201  
(803) 734-5300

#### ***Water quality and shellfish safety***

Bureau of Environmental Sanitation  
(Columbia)  
(803) 734-5071  
  
Div. of Water Quality and Shellfish  
Sanitation (Columbia)  
(803) 734-5232  
Environmental Quality Control  
(Charleston)  
(803) 740-1590

#### ***Water use***

Water Resources Division  
1500 Hwy. 17 N, Suite 212  
Surfside Beach, SC 29577  
(803) 238-4406  
or  
144 Ribault Square  
Beaufort, SC 29902  
(803) 524-1995

### **S.C. Department of Natural Resources**

Marine Resources Division  
PO Box 12559  
Charleston, SC 29422-2559  
(803) 762-5000

#### ***Warning signs***

Boating Safety Division:  
(803) 762-5041; 795-6800

#### ***Harvest, land and sell, wholesale dealers license***

Commercial License Office:  
(803) 762-5078; 762-5004; 762-5053

#### ***Law enforcement***

Charleston: (803) 795-7951  
Port Royal: (803) 524-9190  
Georgetown: (803) 546-8523.  
No answer: 1 (800) 922-5431

---

***Aquaculture regulations; importation regulations***

Office of Fisheries Management:  
(803) 762-5010

***Aquaculture permit***

Shellfish Management Program:  
(803) 762-5049; 762-5089

**U.S. Army Corps of Engineers**  
***Federal permits (Section 10 and Section 404)***

District Engineer  
PO Box 919  
Federal Building  
Charleston, SC 29402  
(803) 724-4330

**U.S. Coast Guard**  
***Navigation hazards; Marking of structures in navigable waters***

Marine and Safety Office  
196 Tradd St.  
Charleston, SC 29401  
(803) 724-4393

**PERMITTING ASSISTANCE/  
INFORMATION**

**S.C. Department of  
Natural Resources**

Office of Fisheries Management  
Shellfish Management Program  
PO Box 12559  
Charleston, SC 29422-2559  
(803) 762-5000; 762-5010; 762-5029;  
762-5049; 762-5089

**S.C. Department of Agriculture**

Permit Assistance Office  
PO Box 11280  
Columbia, SC 29211  
(803) 734-2210

**S.C. Sea Grant Consortium**

287 Meeting St.  
Charleston, SC 29401  
(803) 727-2078

---

## **TECHNICAL ASSISTANCE**

### **S.C. Department of Agriculture Marketing Section**

PO Box 11280  
Columbia, SC 29211  
(803) 734-2151  
Marketing assistance

### **S.C. Department of Natural Resources**

#### ***Marine Resources Division***

##### **Marine Resources Center**

PO Box 12559  
Charleston, SC 29412  
(803) 762-5000 - Switchboard  
Business/economic information:  
(803) 762-5040  
Culture assistance: (803) 762-5022;  
(803) 762-5071  
Marketing assistance: (803) 762-5017  
Site selection, regulations:  
(803) 762-5049; 762-5089; 762-5010

##### **Waddell Mariculture Research and Development Center**

PO Box 809  
Sawmill Creek Road  
Bluffton, SC 29910  
(803) 837-3795  
Culture assistance

### **S.C. Sea Grant Extension Program**

Aquaculture Specialist  
PO Drawer 1100  
Georgetown, SC 29440  
(803) 546-4481  
Culture/site selection assistance



---

## APPENDIX B. SEED SUPPLIERS

### ***Aquaculture Research Corporation***

PO Box 2028  
Dennis, MA 02638  
(800) 334-1380

### ***Aquarius Inc.***

2050 South Dixie Highway  
Palm Bay, FL 32905  
(407) 725-1829

### ***Atlantic LittleNeck ClamFarms, Inc.***

PO Box 12139  
James Island, SC 29422-2139  
(803) 762-0022

### ***Bagwell Enterprises***

PO Box 508  
Eastville, VA 23347  
(804) 678-5806

### ***Bayfarm***

586 Dock Rd.  
West Creek, NJ 08092  
(609) 294-0235

### ***Biosphere, Inc.***

1199 South Green St.  
Tuckerton, NJ 08087  
(609) 296-0945

### ***Bluepoints Co., Inc.***

PO Box 8  
West Sayville  
Long Island, NY 11796  
(516) 589-0123

### ***Chincoteague Island Oyster Farms***

101 Maddox Blvd.  
Chincoteague, VA 23336

### ***The Clam Farm, Inc.***

Box 402  
Fishers Island, NY 06390  
(516) 788-7889

### ***Cultured Aquatics***

Northport Envir. Res. Ctr.  
Eatons Neck Rd.  
Northport, NY 11768-1298  
(516) 757-8182

### ***Flomax***

H.C. Route, Box 207  
Westover, MD 21871  
(301) 651-9336

### ***Half Shell Farms, Inc.***

1260 Plum Avenue  
Merritt Island, FL 32952  
(407) 453-8906

### ***Harbor Branch Institute***

5600 Old Dixie Hwy.  
Ft. Pierce, FL 34946  
(407) 465-2400 Ext. 400

### ***Joe Huber***

Hwy 70 East  
PO Box 96  
Atlantic, NC 28511  
(919) 225-2071

---

***Intertidal Marine***

20 Whitehouse Drive  
Poquosin, VA 23662  
(804) 868-6058

***Bob Baldwin***

Lowcountry Seafood  
PO Box 262  
McClellanville, SC 29548  
(803) 887-3389

***Mercenaria Manufacturing***

R.D. 1, Box 293 B  
Millsboro, DE 19966  
(302) 945-8755

***Mook Sea Farms***

HC 64, Box 041  
Damariscotta, ME 04543  
(207) 563-1456

***Nelson Bay Aquafarms***

Star Rt. Box 22  
Sea Level, NC 28577  
(919) 225-0151

***The Oyster Bed***

12535 North A1A  
Vero Beach, FL 32963  
(407) 589-6138

***Oysterrific!!!***

PO Box 156  
Cape May Court House, NJ 08210  
(609) 465-4878

***Pleasant Bay Shellfish***

61 Eldredge Parkway  
Orleans, MA 02653  
(617) 255-5750

***Sea-Ag, Inc.***

2030 SE Old Dixie Hwy. #2  
Vero Beach, FL 32962  
(407) 468-3216

***Sea Breeding Farm, Inc.***

88 Knox St., A-2  
West Haven, CT 06516

***Sembler & Sembler***

Sebastian, FL  
(407) 724-0272

***Sloop Point Seafood Co.***

207 Pelican Walk  
Hampstead, NC 28443  
(919) 270-2438



---

# APPENDIX C. VENDORS AND PRODUCT DIRECTORY

## VENDOR DIRECTORY

Many of the supplies you will need are available at local hardware or building supply stores. The following vendors may be useful for obtaining more specialized supplies and equipment. This is undoubtedly not an all-inclusive list of sources. Inclusion does not imply endorsement; omissions are unintentional.

### ***Acry-tec, Inc.***

7868 Silvertown Ave., Suite J, San Diego,  
CA 92126. (619) 271-0045  
Fiberglass tanks; chillers; heat exchangers.

### ***Adams Mfg. Co.***

PO Box 339, Hardeeville, SC 29927.  
(803) 784-6756  
Custom fiberglass tanks.

### ***ADPI Enterprises, Inc.***

3621 B Street, Philadelphia, PA 19134.  
(800) 621-0275  
Plastic mesh, plastic netting, grow-out  
bags.

### ***AMETEK-Haveg Div.***

523 Fieldstream Way, Lawrenceville, GA  
30244. (404) 822-9707  
Heat exchangers, chillers.

### ***Aquacenter, Inc.***

PO Box 4877, Greenville, MS 38704.  
(800) 748-8921  
Wide variety of aquaculture supplies and  
equipment.

### ***Aquaculture Supply***

5532 Old Saint Joe Road, Dade City, FL  
33525. (904) 567-8540  
Wide assortment of aquaculture supplies  
and equipment; algal cultures.

### ***Aquafine Corp.***

25230 West Avenue Stanford, Valencia,  
CA 91355. (800) 423-3015  
Ultraviolet sterilizers.

### ***Aquanetics Systems, Inc.***

5252 Lovelock, San Diego, CA 92110.  
(619) 291-8335  
Variety of aquaculture equipment; seawater  
chillers, heat exchangers; sterilizers; pumps;  
air blowers; PVC valves and fittings.

### ***Aquatic Ecosystems, Inc.***

2056 Apopka Blvd., Apopka, FL 32703.  
(407) 866-3939  
General aquaculture supplies, air pumps,  
air blowers.

### ***Aquionics Inc.***

Erlanger KY. (606) 341-0710  
Ultraviolet Sterilizers

---

**AREA Inc.**

PO Box 1303, Homestead, FL 33090.  
(305) 248-4205  
General aquaculture supplies, submersible heaters, aeration equipment.

**Argent Chemical Laboratories**

8702 152nd Ave. NE, Redmond, WA 98052. (800) 426-6258  
General aquaculture supplies, chemicals.

**Baxter Scientific Products**

8350 Arrowridge Clvd., Charlotte, NC 28273. (800) 395-8900  
General scientific and laboratory supplies; submersible pumps.

**Bigelow Lab for Ocean Science**

McKown Point, West Boothbay Harbor, ME 04575. (207) 633-2173  
Algal cultures.

**Biomarine Aquafauna**

PO Box 5, Hawthorne, CA 90250.  
(213) 973-5275  
General aquaculture supplies.

**Boatcycle Mfg. and Chemical Co.**

Box 494, Henderson, TX 75654.  
(800) 333-9154  
General aquaculture & fishing supplies.

**Carolina Biological Supply Co.**

2700 York Rd., Burlington, NC 27215-3398. (800) 334-5551  
Algal cultures.

**Champlin Net Co., Inc.**

PO Box 788, Jonesville, LA 71343.  
(318) 339-9623  
General fishing/boating supplies.

**Cole Parmer Instrument Co.**

7425 N. Oak Park Ave., Chicago, IL 60648. (800) 323-4340  
General scientific and laboratory supplies; peristaltic pumps; metering pumps; air filters.

**Coulter Scientific Instruments.**

PO Box 2145, Hialeah, FL, 33012-0145.  
(800) 526-6932  
Coulter counters.

**C Squared Corporation**

7900 N. University Dr., Tamarac, FL 33321. (800) 448-3929  
Coulter counters.

**Dolphin Boats & Fiberglass**

24601 Packing House Rd., Princeton, FL 33032. (305) 257-2628  
Fiberglass tanks.

**Engineering Sales Associates, Inc.**

PO Box 35514, Charlotte, NC 28235.  
(704) 523-8535  
filter bags and filter cartridges

**Fisher Scientific**

2775 Pacific Dr., PO 4829, Norcross, GA 30091. (800) 282-0578  
General scientific and laboratory supplies.

**Florida Aquafarms**

See Aquaculture Supply.

---

***Gardeners Supply***

128 Intervale Rd., Burlington, VT 05401.  
(802) 863-1700  
Fluorescent plant-growing lights.

***Gilson Co.***

PO Box 677, Worthington, OH  
43085-0677. (800) 431-5935  
Sieves, screening materials.

***W.W. Grainger, Inc.***

7401 Pepperdam Ave., North Charleston,  
SC 29418. (803) 552-0633  
General industrial supplies; pumps; metal  
halide lamps; fluorescent bulbs.

***Hach Co.***

PO Box 389, Loveland, CO 80539.  
(800) 227-4224  
Water quality test kits.

***ICN Biomedicals, Inc.***

PO Box 19536, Irvine, CA 92713-9921.  
(800) 854-0530.  
Chemicals.

***Internet, Inc.***

2730 Nevada Ave. North, Minneapolis,  
MN 55427. (800) 328-8456  
Plastic netting and mesh.

***Jelliff Corp.***

354 Pequot Ave., Southport, CT 06490.  
(203) 259-1615  
Nylon and polyester mesh.

***La Motte Co.***

PO Box 329, Chestertown, MD 21620.  
(800) 344-3100  
Water quality test kits.

***Memphis Net and Twine Co.***

PO Box 8331, Memphis, TN 38108.  
(800) 238-6380  
General fishing/boating supplies.

***Nalle Plastics, Inc.***

203 Colorado, Austin, TX 78701.  
(800) 531-5112  
Plastic mesh and netting.

***National Netting Inc.***

PO Box 2562, Norcross, GA 30091.  
(800) 233-7896  
Plastic netting, cages.

***Nylon Net Co.***

615 E. Bodley Ave., PO Box 592,  
Memphis, TN 38101. (901) 774-1500  
General fishing/boating supplies.

***Package Supply and Equipment Co.***

PO Box 19021, Greenville, SC 29602.  
(803) 277-0900  
Buckets, bottles.

***Packaging Products Corp.***

198 Melville Blvd., Box 6002, New  
Bedford, MA 02742. (800) 225-0484  
Shipping containers.

***Plastic Piping Systems***

(PPS) 3603-07 Tryclan Dr., Charlotte, NC  
28217. (800) 438-3535  
PVC pipe and fittings.

***PPS***

See Plastic Piping Systems

---

***PVC Supply House***

120 Fortenberry Rd., Merritt Island, FL  
32952. (407) 452-1540  
PVC pipe and fittings.

***Rainbow Plastics, Filter Div.***

PO Box 4127, El Monte, CA 91734.  
(818) 443-6114  
Filters, UV sterilizers.

***Red Ewald, Inc.***

PO Box 519, Karnes City, TX 78118.  
(800) 531-3606  
Fiberglass tanks.

***Riverdale Mills Corp.***

130 Riverdale St., PO Box 200,  
Northbridge, MA 01534. (800) 762-6374  
Vinyl coated wire for field cages; hog-nose  
rings.

***Rowland Fiberglass Inc.***

PO Box 971, Ingleside, TX 78362.  
(512) 776-7753  
Fiberglass tanks.

***C.E. Shepherd Co., Inc.***

7206 Dallas, PO Box 9445, Houston, TX  
77011. (713) 928-3763  
Vinyl coated wire; hog nose rings.

***Sigma Chemical Co.***

PO Box 14508, St. Louis, MO 63178-9916.  
(800) 325-3010.  
Chemicals.

***SIP***

See Southeastern Industrial Plastics

***Solar Components Corp.***

88 Pine St., Manchester, NH 03103.  
(603) 668-8186  
Kalwalls®.

***Southeastern Industrial Plastics (SIP)***

2740 S. Cobb Industrial Blvd., Smyrna,  
GA 30082. (800) 325-0121  
PVC pipe and fittings.

***Southern Industrial Sales,  
Pureflow Division***

4958 Hammermill Rd., Tucker, Ga 30084.  
(409) 939-7715  
Ultraviolet sterilizers.

***Techpak, Inc.***

2 Fifth St., Peabody, MA 01960.  
(800) 225-5019  
Styrofoam shipping containers.

***Tenax***

8291 Patuxent Range Road, Jessup, MD  
20794. (800) 356-8495  
Plastic netting; plastic mesh.

***Tetko Inc.***

420 Saw Mill River Road, Elmsford, NY  
10523. (914) 941-7767  
Nylon and polyester mesh.

***Universal Marine Industries (UMI).***

1815 Williams St., San Leandro, CA  
94577-2389. (415) 352-9856  
Seawater heat exchangers and chillers.

**US Plastic Corp.**

1390 Neubrecht Rd., Lima, OH 45801.  
(800) 537-9724

Wide variety of plastic supplies; pans;  
tubing; PVC; pumps.

**V-J Growers Supply**

Charlotte, NC (800) 222-4504;  
Apopka, FL (800) 327-5422  
Plastic netting, nylon and polyester mesh.

**Water Treatment Technology**

5332 Selton Ave., Jacksonville, FL 32211.  
(904) 744-9393  
Water purification systems.

**West Coast Aquatics**

906 Calle Collado, Thousand Oaks, CA  
91360. (805) 499-7866  
Seawater chillers.

**W.P. Law, Inc.**

PO Box 448, Johns Island, SC 29457.  
(803) 559-3945  
PVC pipe and fittings; pumps.

## PRODUCT DIRECTORY

**Algal cultures:**

See Appendix D.

**Algal nutrients (premixed):**

Aquacenter; Aquaculture Supply; Argent.

**Algal nutrients**

**(chemicals to mix your own):**

Argent; Baxter; Fisher; ICN; Sigma.

**Airline:**

Aquaculture Supply; Aquatic Ecosystems.

**Airstones:**

Aquaculture Supply; Aquatic Ecosystems;  
Area; Argent; Boatcycle; Biomarine  
Aquafaun.

**Air pumps/blowers:**

Aquacenter; Aquanetics; Aquatic Ecosystems;  
AREA; Boatcycle.

**Air filters:**

Baxter; Cole Parmer; Fisher.

**Boots:**

Argent; Boatcycle; Champlin; Grainger;  
Memphis Net; Nylon Net.

**Buckets:**

Package Supply & Equipment.

**Calipers:**

Aquaculture Supply; Aquatic Ecosystems;  
Baxter; Fisher.

**Carboys:**

Aquaculture Supply; Baxter; Cole Parmer;  
Fisher; Package Supply.

**Chemicals:**

Argent; Baxter; Fisher; Hach; ICN;  
LaMotte; Sigma.

**Chillers (seawater):**

Acry-tec; AMETEK-Haveg Div.;  
Aquanetics; UMI; West Coast Aquatics.

**Chlorine test kits, test papers:**

Aquaculture Supply; Argent; Hach;  
LaMotte.

***Clothing, foul weather:***

Boatcycle; Champlin Net; Memphis Net; Nylon Net.

***Coulter counter:***

Coulter Scientific; C Squared Corp.

***Crimps (=hog-nose rings):***

Riverdale Mills; C.E. Shepherd.

***Filter bags:***

Aquaculture Supply; Aquatic Ecosystems; Engineering Sales Associates; Grainger; Tetko.

***Filter cartridges:***

Aquaculture Supply; Aquatic Ecosystems; AREA; Engineering Sales; Rainbow Plastics.

***Fittings (plastic):***

Aquacenter; Aquaculture Supply; Aquanetics; Aquatic Ecosystems; AREA; Cole Parmer; US Plastics.

***Fittings (PVC):***

Aquacenter; Aquaculture Supply; Aquanetics; Aquatic Ecosystems; AREA; PPS; PVC Supply; SIP; US Plastics; WP Law.

***Fittings (tubing/specialty):***

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; AREA; Cole Parmer; US Plastics.

***Flasks:***

Aquacenter; Aquaculture Supply; Baxter; Cole Parmer; Fisher.

***Fluorometer:***

Baxter; Fisher.

***Foul weather clothing:***

Argent; Champlin; Boatcycle; Memphis Net; Nylon Net.

***General aquaculture supplies:***

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; AREA; Argent; Biomarine Aquafauna; Boatcycle.

***Glassware:***

Baxter; Fisher; Package Supply & Equipment.

***Gloves (heavy duty):***

Champlin; Boatcycle; Memphis Net; Nylon net.

***Heaters (submersible):***

Aquacenter; Aquanetics; AREA.

***Hemocytometers:***

Aquaculture Supply; Baxter; Fisher.

***Hog-nose rings (for fastening wire and mesh on field cages):***

Riverdale Mills; C.E. Shepherd.

***Hose, hose fittings:***

Aquacenter; Aquaculture Supply; Aquanetics; Aquatic Ecosystems; Cole Parmer; US Plastics.

***Kalwalls®:***

Solar Components.

***Lab supplies:***

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Baxter; Cole Parmer; Fisher.

---

**Lights for algal culture:**

Aquacenter; Aquaculture Supply; Gardeners Supply; Graingers.

**Mesh, nylon and polyester  
(for sieves, silos):**

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Argent; Jelliff; Tetko.

**Mesh, plastic (for field cages):**

ADPI; Aquatic Ecosystems; Argent; Internet; Nalle Plastics; Tenax; V-J Growers Supply.

**Mesh, wire (for field cages):**

Aquatic Ecosystems; Boatcycle; Riverdale Mills; C.E. Shepherd.

**Microscopes:**

Aquacenter; Aquaculture Supply; Argent; Baxter; Cole Parmer; Fisher.

**Netting, plastic:**

ADPI; Aquatic Ecosystems; Argent; Champlin; Memphis Net; Nalle Plastics; Nylon net; Tenax; V-J Growers Supply.

**Nylon mesh:**

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Argent; Jelliff; Tetko.

**pH meters, testers:**

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Argent; Baxter; Biomarina Aquafauna; Cole Parmer; Fisher.

**pH strips:**

Aquaculture Supply; Aquatic Ecosystems; Argent; Baxter; Biomarine Aquafauna; Cole Parmer; Fisher.

**Pipe:**

See PVC.

**Pipets:**

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Baxter; Cole Parmer; Fisher.

**Polyester mesh:**

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Argent; Jelliff; Tetko.

**Pumps, air:**

Aquacenter; Aquaculture Supply; Aquatic Ecosystems; Aquanetics; AREA; Boatcycle.

**Pumps, gasoline:**

Aquanetics; Grainger.

**Pumps, metering:**

Cole Parmer; Grainger; US Plastics.

**Pumps, peristaltic:**

Cole Parmer; US Plastics.

**Pumps, submersible:**

Aquacenter; Aquaculture Supply; Aquanetics; Aquatic Ecosystems; Baxter; Boatcycle; Cole Parmer; Fisher; US Plastics.

**PVC pipe:**

PPS; PVC Supply; SIP; US Plastics; WP Law.

**Refractometers:**

Argent; Aquacenter; Aquaculture Supply; Aquatic Ecosystems; AREA; Baxter; Fisher.

**Rope:**

Aquacenter; Champlin; Memphis Net;  
Nylon net.

**Sand filters:**

Aquanetics.

**Secchi disk (for algal culture):**

Aquaculture Supply.

**Sedgewick-Rafter cells:**

Aquaculture Supply; Baxter; Fisher.

**Shipping containers (styrofoam):**

Packaging Products; TechPak.

**Sieves:**

Aquaculture Supply; Gilco.

**Sodium thiosulfate:**

Aquacenter; Aquatic Ecosystems; Argent.

**Spectrophotometer:**

Baxter; Fisher.

**Tanks, fiberglass:**

Acry-tec; Adams Mfg.; Aquacenter;  
Aquanetics; Dolphin; Red Ewald;  
Rowlands.

**Tanks, plastic:**

Aquacenter; Aquaculture Supply;  
Grainger; Solar Components; US Plastics.

**Test kits:**

Aquacenter; Aquaculture Supply; Argent;  
Hach; LaMotte.

**Test tubes:**

Aquacenter; Aquaculture Supply; Baxter;  
Cole Parmer; Fisher.

**Tubing:**

Aquacenter; Aquaculture Supply;  
Aquanetics; Aquatic Ecosystems; Cole  
Parmer; US Plastics.

**Tubing fittings:**

Aquacenter; Aquaculture Supply; Aquatic  
Ecosystems; Cole Parmer; US Plastics.

**Ultraviolet sterilizer:**

Aquacenter; Aquaculture Supply;  
Aquafine; Aquanetics; Aquionics; Rain-  
bow Plastics, Filter Div.; Southern Indus-  
trial Sales, Pureflow Div..

**Valves (air):**

Aquacenter; Aquaculture Supply; Aquatic  
Ecosystems.

**Valves (water):**

Aquacenter; Aquaculture Supply; Aquatic  
Ecosystems; AREA; PPS; PVC Supply;  
SIP; US Plastics; W.P. Law.

**Wire mesh:**

See Mesh, wire.



---

# APPENDIX D. COMMONLY CULTURED PHYTOPLANKTON SPECIES AND SOURCES FOR STARTER CULTURES

## COMMONLY CULTURED PHYTOPLANKTON SPECIES

### Class Prymnesiophyceae

(formerly Haptophyceae)

Golden brown flagellates

*Isochrysis galbana* Size: 3-7  $\mu\text{m}$

The most commonly cultured strain is Tahitian *Isochrysis* (T-Iso). Recently a strain isolated in the Caribbean has become popular (C-Iso). T-Iso is generally considered to be the optimal feed for larvae. It is also utilized for post-set and broodstock. It thrives at relatively high temperatures.

*Pavlova* (*Monochrysis*) *lutheri* Size: 4-6  $\mu\text{m}$

A formerly popular flagellate whose use has been largely superseded by T-Iso.

### Class Bacillariophyceae

Diatoms

*Chaetoceros* spp.

Several species of these diatoms are commonly cultured. Slightly larger than *Isochrysis* and non-motile, these are fed to older larvae, post-set and broodstock.

Usually less tolerant to warm temperatures than T-Iso and C-Iso.

Size: *C. gracilis*: 7-12  $\mu\text{m}$

*C. muelleri*: 4-6  $\mu\text{m}$

*C. calcitrans*: 3  $\mu\text{m}$

*Skeletonema costatum*

Size: 3-22  $\mu\text{m}$  X 3-12  $\mu\text{m}$

This diatom has been much used in shellfish hatcheries but recently has been largely replaced by *Chaetoceros* spp., which are often easier to culture. Often the most abundant diatom in natural waters.

*Thalassiosira* spp.

*T. pseudonana* (clone 3H) has been used in shellfish hatcheries for years and can be used for all life stages. *T. weissflogii* (formerly *T. fluviatilis*) is a large diatom which is an excellent feed for broodstock.

Size: *T. pseudonana*: 4  $\mu\text{m}$

*T. weissflogii*: 6-20  $\mu\text{m}$

*Cyclotella* spp. Size: 10-90  $\mu\text{m}$

These diatoms are very hardy and multiply rapidly. They are a good feed for broodstock.

---

### **Class Chlorophyceae**

Green algae

*Nannochloris* spp. Size: 4 µm

A small green alga which multiplies rapidly.

### **Class Prasinophyceae**

Greenish algae

*Tetraselmis suecica* Size: 7-13 µm

Widely used in bivalve culture.

#### ***Sources for starter cultures***

Many state universities may maintain algal collections and be able to provide starter cultures. Often your best source for starter cultures may be established hatcheries in

your area. Your extension agency may be able to help locate a source for starter cultures. There is also an extensive list of algal culture collections contained in Miyachi et al. (1989). The following vendors sell monospecific phytoplankton cultures:

#### ***Aquaculture Supply***

5532 Old Saint Joe Road, Dade City, FL 33525. (904) 567-8540

#### ***Bigelow Lab for Ocean Science***

Provafoli-Guillard Center for Culture of Marine Phytoplankton. McKown Point, West Boothbay Harbor, ME 04575. (207) 633-2173

#### ***Carolina Biological Supply Co.***

2700 York Rd., Burlington, NC 27215-3398. (800) 334-5551

---

# APPENDIX E. UNITS OF MEASUREMENTS

## SCIENTIFIC NOTATION

Scientific notation uses exponents to simplify very large or very small numbers. It is particularly useful when precision is not important; in other words, if you do not care whether the number is 1,000,000 or 1,000,100—1 million is close enough—scientific notation can be used to simplify calculations.

$10^0 = 1$
$10^1 = 10$
$10^2 = 100$
$10^3 = 1000$
$10^4 = 10,000$
$10^5 = 100,000$
$10^6 = 1,000,000$ (million)
$10^9 = 1,000,000,000$ (billion)

Negative exponents indicate fractions:

$10^{-1} = 0.1 = \text{one tenth}$
$10^{-2} = 0.01 = \text{one hundredth}$
$10^{-3} = 0.001 = \text{one thousandth}$
$10^{-6} = 0.000001 = \text{one millionth}$

Other numbers are represented as multiples of these base units:

3.5 million = $3.5 \times 10^6$
1.5 billion = $1.5 \times 10^9$

## THE METRIC SYSTEM

Scientific measurements are usually made in metric units. The metric system is a decimal system. Basic units are multiplied or divided by ten. The following prefixes are the ones you will most commonly encounter. These prefixes are combined with the basic units (gram, liter, meter) to produce larger or smaller units. For example, “kilo-” + “meter” yields kilometer, meaning 1000 meters.

<u>Prefix</u>	<u>Abbreviation</u>	<u>Multiple</u>
kilo-	k	$10^3$
centi-	c	$10^{-2}$
milli-	m	$10^{-3}$
micro-	$\mu$	$10^{-6}$

1000 micrograms = 1 milligram
1000 milligrams = 1 gram
1000 grams = 1 kilogram
1000 microliters = 1 milliliter
1000 milliliters = 1 liter
1000 micrometers = 1 millimeter
1000 millimeters = 1 meter
10 millimeters = 1 centimeter
100 centimeters = 1 meter
1000 meters = 1 kilometer

The following approximations can be used to convert between US measurements and the metric system.

LENGTH	<u><i>If you know</i></u>	<u><i>Multiply by</i></u>	<u><i>To find</i></u>	<u><i>If you know</i></u>	<u><i>Multiply by</i></u>	<u><i>To find</i></u>
	millimeters	0.04	inches	inches	25.40	millimeters
	centimeters	0.39	inches	inches	2.54	centimeters
	meters	3.28	feet	feet	30.48	centimeters
	meters	1.09	yards	feet	0.30	meters
	kilometers	0.62	miles	yards	0.91	meters
				miles	1.61	kilometers

AREA	<u><i>If you know</i></u>	<u><i>Multiply by</i></u>	<u><i>To find</i></u>	<u><i>If you know</i></u>	<u><i>Multiply by</i></u>	<u><i>To find</i></u>
	sq. centimeters	0.16	sq. inches	sq. inches	6.54	sq. centimeters
	sq. meters	1.20	sq. yards	sq. feet	0.09	sq. meters
	sq. kilometers	0.39	sq. miles	sq. yards	0.84	sq. meters
	hectares	2.47	acres	sq. miles	2.60	sq. kilometers
				acres	0.40	hectares

VOLUME	<u><i>If you know</i></u>	<u><i>Multiply by</i></u>	<u><i>To find</i></u>	<u><i>If you know</i></u>	<u><i>Multiply by</i></u>	<u><i>To find</i></u>
	milliliters	0.20	teaspoons	teaspoons	4.93	milliliters
	milliliters	0.06	tablespoons	tablespoons	14.78	milliliters
	milliliters	0.03	fluid ounces	fluid ounces	29.57	milliliters
	liters	4.23	cups	cups	0.24	liters
	liters	2.12	pints	pints	0.47	liters
	liters	1.06	quarts	quarts	0.95	liters
	liters	0.26	gallons	gallons	3.79	liters
	cubic meters	35.32	cubic feet	cubic feet	28.32	liters
	cubic meters	264.21	gallons	cubic feet	0.03	cubic meters
	cubic meters	1.35	cubic yards	cubic yards	0.76	cubic meters

WEIGHT	<u>If you know</u>	<u>Multiply by</u>	<u>To find</u>	<u>If you know</u>	<u>Multiply by</u>	<u>To find</u>
	grams	0.035	ounces	ounces	28.35	grams
	kilograms	2.21	pounds	pounds	0.45	kilograms
	metric ton (1000 kg)	1.10	tons (2000 lbs.)	tons	0.91	metric tons

TEMPERATURE

If you know Celsius (= centigrade), multiply by 9/5 and add 32 to get Fahrenheit.  If you know Fahrenheit, subtract 32 and multiply by 5/9 to get Celsius.	Commonly encountered temperatures:	
	<u>Fahrenheit</u>	<u>Celsius</u>
	32	0
	50	10
	59	15
	68	20
	77	25
	86	30
	98.6	37
	104	40
	122	50
	140	60
	212	100

