Manual for Hatchery Culture

of the

Bay Scallop

Sea Grant
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MANUAL FOR HATCHERY CULTURE OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS IRRADIANS*

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FOREWORD

This manual is dedicated to those Milford Laboratory scientists who earlier worked in the disciplines described in this manual and who were instrumental in the development of shellfish culture techniques now being used worldwide and from which the bay scallop culture techniques described here are derived. Dr. Victor Loosanoff, along with his associate Mr. Harry Davis, originally described culture techniques for many species of bivalves. Dr. Ravenna Ukeles first established protocols for the culture of microalgae which she then used in shellfish nutrition studies. Mr. Haskell Tubiash was one of the first individuals to study diseases of shellfish larvae in culture. Dr. Arlene Longwell was one of the first geneticists to study selective breeding in marine bivalves, particularly the oyster. These individuals made significant contributions to the field of shellfish culture as we know it today and we thank them for their contributions.

NOTE

This manual describes methods used at the National Marine Fisheries Service's Milford Laboratory to culture bay scallops in the northeastern U.S. from eggs to the point at which they can be placed in nets or cages in the environment. As you go through the manual, certain questions may arise regarding particular techniques. The laboratory can be contacted at (203) 579-7000 for further clarification. Individual training in techniques discussed here can be provided at the Milford Laboratory at no cost.

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I. INTRODUCTION

The native New England bay scallop, *Argopecten irradians irradians*, is a high-value seafood species with an existing market but an unstable yield from the wild-caught fishery. Wide inter-annual fluctuations in bay scallop populations can be attributed largely to two factors: 1) the short life history of the bay scallop, i.e., most live less than two years and spawn only once (Orensanz *et al.*, 1991), and 2) their sensitivity to environmental stresses (Tettlebach *et al.*, 1985), particularly winter mortality (Mercaldo & Rhodes, 1982; Bricelj *et al.*, 1987). The bay scallop is, however, an extremely fast-growing bivalve, reaching a minimum market size of 40+ mm (millimeters) (one mm equals 0.03937 inches) in less than one year (Oesterling & Rose, 1996). For comparison, oysters require 3-5 years to reach market size (Burrell, 1985), and quahogs grow even slower, requiring 4 years or more to reach littleneck size in New England waters (Menzel, 1989). When available, New England bay scallop adductor muscles (the only portion of this animal currently marketed widely) sell for $9-16 per pound or more. The high market value, coupled with limited wild-fishery yield and rapid growth, make the bay scallop an attractive candidate for aquaculture (Gates *et al.*, 1974; Webber & Riordan, 1976).

An aquaculture strategy for the bay scallop must address the basic biological constraints that limit the wild fishery; in fishery biology terms, these constraints are recruitment and survival to market size. Recruitment is the addition of new individuals to the population. In shellfish aquaculture, "recruits" are referred to as "seed."

There are two ways to obtain bivalve seed: one is collection of wild "spat," or seed, in natural waters, usually employing an artificial substrate deployed in the water. The other source of seed is through hatchery production. Spat collection is dependent upon a sufficient population of "spawning stock," i.e., reproductive adults, and conditions suitable for development and retention of planktonic larvae in the estuary. Efforts to collect bay scallop spat in southern New England estuaries have met with limited and inconsistent success (Tammi *et al.*, 1997). We, therefore, have based our strategy for bay scallop aquaculture development in New England upon hatchery production of seed scallops.

Practical methods for artificial spawning of bivalve mollusks and rearing of larvae and young spat were developed at the Milford Laboratory over four decades ago (Loosanoff & Davis, 1963). Preparation of adult bivalves for induced reproduction (referred to as "conditioning" or "ripening") can be accomplished at almost any time of year by increasing or decreasing water temperature incrementally over several weeks to the desired conditioning temperature and providing sufficient microalgal food to fuel the energy-intensive process of gamete (eggs and sperm) formation. "Ripeness" is easier to determine in bay scallops than in other bivalves because the gonads (reproductive organs) can be inspected visually without sacrificing the animal. Conditioned scallops held at 17-20°C (see Appendix C for conversion of °C to °F) can be stimulated to spawn by increasing the temperature of the water to 25°C over a period of one hour. Gametes (both eggs and sperm) may be released from the same

individuals (hermaphroditism). Fertilization occurs immediately and embryos may be collected, counted, and dispersed into suitable containers. Embryos require no supplemental feeding for 24-48 hours, deriving their nutritional needs from storage reserves of the egg. For this reason, it is important that broodstock be nutritionally robust.

When larvae reach the veliger stage (characterized by the presence of a locomotory and feeding organ called the velum), after about 24 hours at 25°C, they are about 70 μm (one μm or *micrometer* equals one thousandth of a mm) in size; at this time they are fed a diet of cultured microalgae. Effective microalgal diets for larval bay scallops must be: 1) non-toxic, 2) sufficiently small to be ingested, 3) digestible, and 4) nutritionally complete. Whereas toxicity and size of potential microalgal diets are evaluated easily, digestibility and nutritional composition are more difficult to ascertain. For bay scallop larvae, we have shown that algal diets high in specific, "essential" fatty-acids and sterols support more rapid growth and result in a higher percentage of larvae successfully undergoing metamorphosis (a physiological change from one form to another) to the post-set stage (Alix *et al.*, 1996).

After 7-14 days of larval development and growth to about 200 μm, scallops undergo metamorphosis, also called "setting," when adult morphological characteristics are established. As this process includes loss of the velum and development of a new, gill-feeding apparatus, feeding may be interrupted or become less efficient. Thus, nutritional condition of larvae at the pre-setting stage may be important in determining setting success.

Metamorphosing scallops attach to surfaces by means of byssal threads similar to those of mussels. It is thought that byssal attachment to eelgrass and other living and non-living structures in nature serves to keep scallops off the bottom to avoid crustaceans and other predators and to ensure a current-mediated supply of phytoplankton food (Brand, 1991). In the hatchery, scallops are set on mesh "onion bags" filled with nylon monofilament line, plastic strips or burlap, fiberglass, or directly on the container sides. Setting success in the hatchery generally ranges from less than 1% to about 10%; this variation offers a potential area for improvement, but from a practical standpoint, does not constrain hatchery production seriously because of the large number of larvae (millions) that can be obtained from spawning several individuals. Post-set scallops then enter a stage of culture referred to as the "nursery." Traditionally, young, post-set scallops, as well as other bivalves, are reared in tanks through which coarse-filtered seawater is pumped (Rhodes & Widman, 1980) or in protected enclosures placed in the natural environment (Widman & Rhodes, 1991). In either case, young animals are exposed to ambient seawater temperatures and depend upon natural phytoplankton, not cultured microalgae, for food.

In nature, the bay scallop in northeastern U. S. waters usually spawns in July, although peak spawning may occur from June through mid-August (Belding, 1910), with a lighter secondary spawning possible in the fall (Tettlebach *et al.*, 1999). The life cycle of the bay scallop (Figure 1) begins when the fertilized egg develops into a D-shaped larva, known as a veliger or prodissoconch, within 24–48 hours. The veliger has two valves (or shells) and a ciliated velum which it uses for feeding and locomotion. After 7 to 14 days, the larva undergoes metamorphosis, a physiological change whereby it develops into a juvenile scallop, or dissoconch, which is similar in appearance to an adult. Just prior to metamorphosis, the larva develops a foot which it uses for crawling, while maintaining its ability to swim.
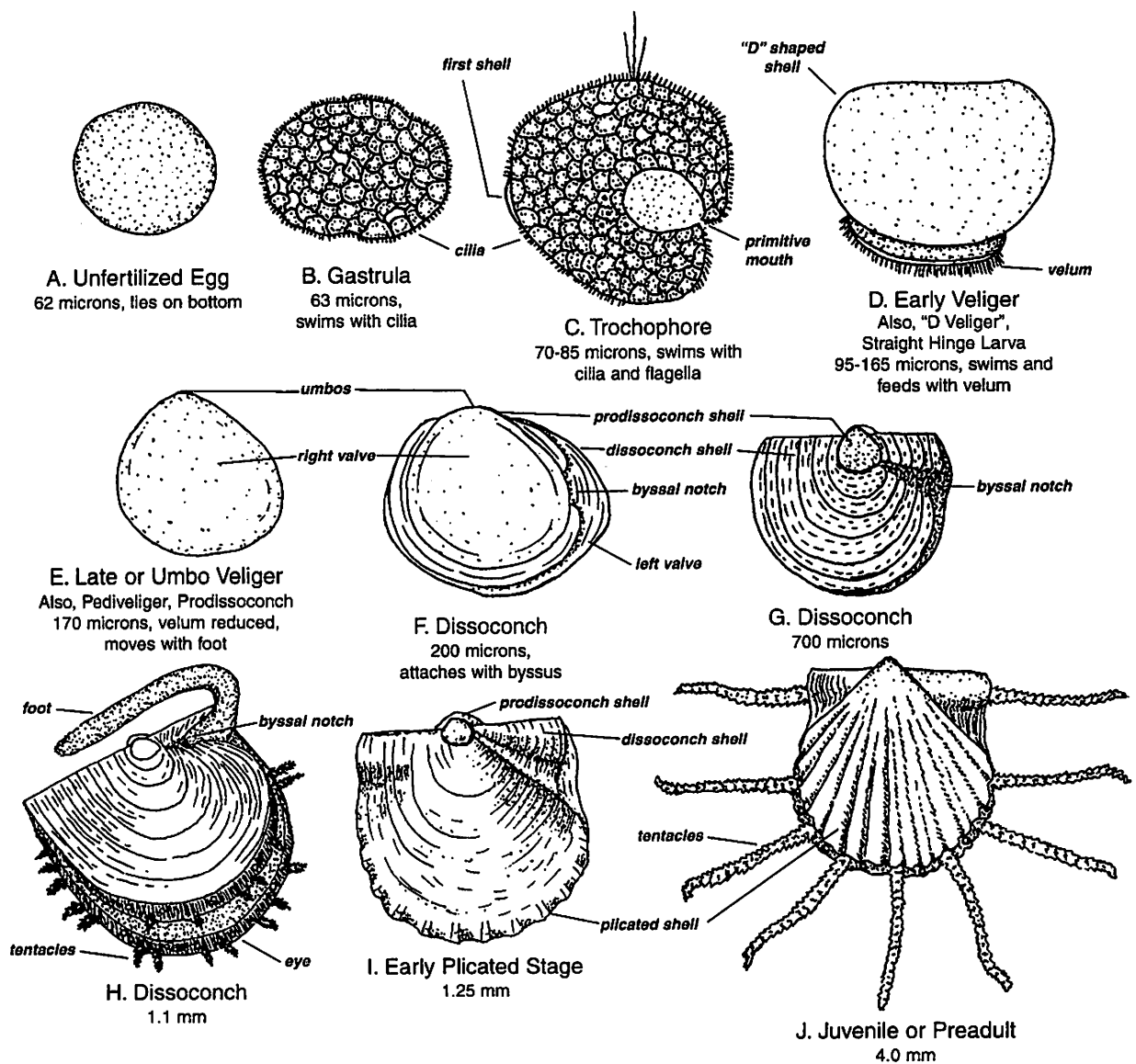
Figure 1. Early life cycle of the bay scallop. [Bottom (right) valve is shown uppermost in E-J, A-D after Sastry, 1965; E-J after Belding, 1910].

During this period the juvenile scallop tests various substrate materials to determine their suitability as places for attachment or settlement. A preferred substrate is eelgrass, *Zostera marinus*, although seaweed, pilings, rocks, etc., are used as well. The scallop grows rapidly at this stage, as long as the water temperature remains warm and sufficient food is available. Juvenile scallops typically attach and detach a number of times because of the growth and shedding of the eelgrass frond. As they get larger, they settle to the bottom. By late November to early December they grow to a shell height of 20 to 40 mm, although growth can range from 7 to 60 mm depending on environmental conditions. As water temperature decreases to below 15°C, growth slows considerably and eventually stops. There appears to be no growth at temperatures below 7 to 10°C. As temperature increases the following spring, growth resumes. Considerable energy is needed for maturation of the gonads during this period. Adults mature at approximately one year of age when spawning normally occurs, and resume growing until late fall when water temperatures cool. The majority of scallops appear to live for only 20 months, although this is not likely due to aging alone but to environmental stresses as well. Some scallops have been observed to be at least two years old, and some even three.

Citations for further reading on the life history of the bay scallop are presented in an Indexed Bibliography of the Bay Scallop (*A. irradians*) by Sabo & Rhodes (1987), which is available at the Milford Laboratory.

II. FACILITIES

Facilities for the culture of the bay scallop require access to a continuous supply of high-quality seawater. The salinity of the water should range from 25 to about 33 ‰ (parts per thousand). In the northeastern U.S., wide fluctuations of ambient seawater temperature necessitate the heating and chilling of the seawater supply. Conditioning broodstock, spawning, and culture of larvae all require temperature-regulated seawater. Heat exchangers can be used to raise or lower the temperature in flow-through systems. Electric aquarium heaters can be used in static systems by placing them directly into the holding systems. It is most likely that any facility that draws seawater from a natural source and discharges waste waters into a natural water body would require permits from the appropriate state and/or local government agencies.

The size of the facility would be dependent upon the seed production anticipated. The facility should be of such size to house the proper numbers of hatchery and nursery tanks needed, seawater pumps, heating and chilling units with associated energy sources, as well as storage areas and lab space. Associated facilities for production of microalgal feed cultures should be conveniently located adjacent to or within the hatchery installation.

The facilities at Milford described in this manual are just one design of a shellfish hatchery. Numerous hatcheries throughout the U.S., both public and private, provide alternative design ideas.

All new materials to be used in the culture of larvae should be cleaned with a biodegradable soap solution and household bleach; i.e., we routinely scrub with 5 milliliters (mL) of Liquinox® (or any similar biodegradable liquid detergent) plus 5 mL of bleach in 10 liters (L) of tap water. Then the material should be submersed in seawater for a minimum of 24 hours prior to use. After the submersion period the materials should again be washed in a soap and bleach mixture. After washing, materials should be rinsed three or more times with hot fresh water and stored dry. Prior to contact with larvae, the materials should be rinsed with 10-μm filtered seawater to remove any chemical residues.

References to use of seawater in this hatchery manual imply 10-μm cartridge-filtered seawater at 25°C unless otherwise noted. While cartridge filtration is recommended, it should be noted that there have been great strides made in bag filtration methods, and these may be acceptable alternatives.

No metallic items (except titanium) that would come in contact with seawater should be used. Copper and brass are extremely toxic to shellfish, and should never come in contact with the seawater system. Seawater is extremely corrosive; it has been our experience that stainless steel #316 still corrodes in these environments. For most needs, PVC, fiberglass, polycarbonate, plexiglass, most plastics, and glass are acceptable for use. If metal items are used, they should be coated prior to use; one acceptable coating is Teflon®.

Hatchery culture typically uses elevated seawater temperatures, thus requiring scallops to undergo an acclimation period before they are placed in warmer or cooler waters. Normally, in the hatchery, scallops can tolerate temperature changes, up or down, of 1°C/day very well. Changes above that rate may increase mortalities. During this phase, follow the normal standard operating procedure of periodic water changes and feedings. As temperatures decrease, scallops feed less, hence water changes can be made less frequently.

FACILITY REQUIREMENTS

- Seawater source
- Size dependent upon production goals
- Must accommodate tanks, pumps heaters/chillers, lab work counter space
- Room for associated algal culture
- No metal in contact with seawater

III. BROODSTOCK MANAGEMENT

A. BROODSTOCK SELECTION

The main objective of broodstock management is selection of good quality broodstock in adequate numbers to ensure genetic diversity, especially over the long term. Selection of good quality broodstock is critical to the success or failure of any cultured species, including scallops. The best broodstock often is found at a site where scallops occur naturally.

Knowledge of life history characteristics of bay scallops, such as growth and survival, is useful in predicting the expected performance of hatchery broodstock. For example, scallops are hermaphroditic, i.e., contain both male and female sex organs (Figure 2). Accordingly, under hatchery conditions, it is possible that some inbreeding through self-fertilization will occur when the scallops are spawned; therefore, maintain sufficient numbers of scallops in the hatchery to allow for genetic diversity. Inbreeding depression manifested as reduced growth and survival can occur.

Figure 2. Bay scallop with fully ripe female (pinkish-orange) and male (white) sex organs.

B. SIZE AND APPEARANCE

Generally, larger animals produce greater quantities of gametes. Typically, scallop populations in the wild consist of only two year classes, as the normal life span is only about 20 months. A mixed-size group of both year classes could be used for spawning stock. Maturing scallops generally display a black integument (tissue) (Figure 3) that covers the immature gonad which is quite distinct prior to gametogenesis (the development of eggs and sperm). As gametogenesis progresses, the integument disappears and the discrete white-colored male and pinkish-orange female gonads ripen and become prominent, indicating spawnability. When scallops are collected in the wild, shells should appear well formed without indentations or blunt or abnormal margins. Healthy scallops usually close their valves when touched. Scallops with valves that remain open or are gaping are probably dying. Hinges should close tightly, and should fit together evenly.

A check of tissues should be made to see if they are plump and healthy rather than flaccid. If assessment of tissue quality and gonad cannot be made adequately from external observations while holding the valves open, 2 to 3 scallops can be shucked for examination of tissue quality. If
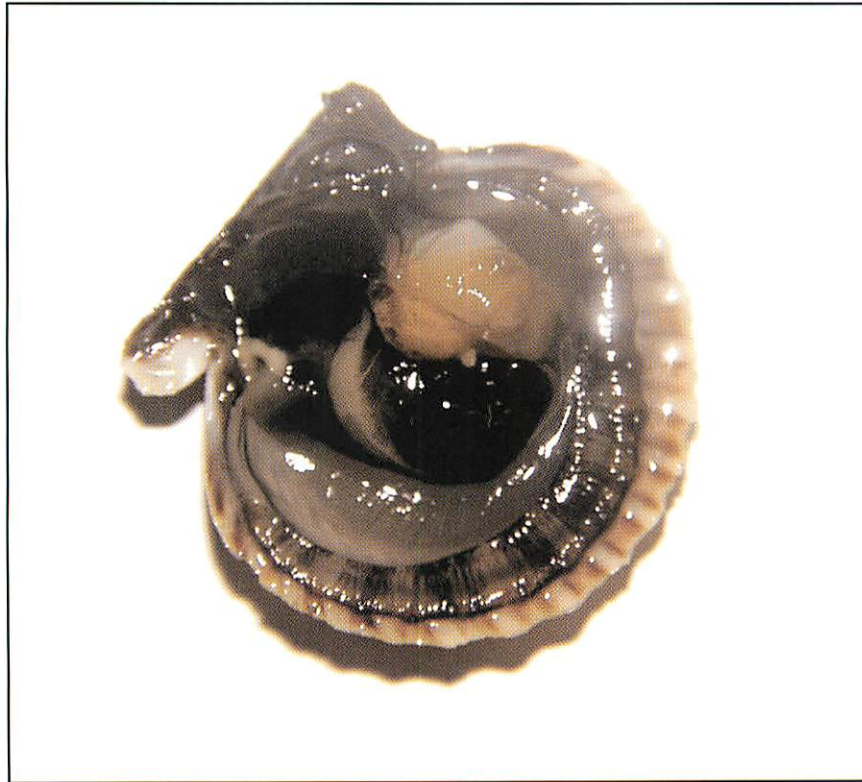
Figure 3. Bay scallop with black integument covering immature gonad.

most of the scallops in a broodstock tank recently have died, it is better to seek a new source of broodstock or use scallops from a separate tank where few mortalities are evident.

C. BROODSTOCK NUMBERS

An adequate supply of broodstock should be available to initiate spawning at appropriate times of the year. At a minimum, 300 adult scallops should be collected and maintained in the hatchery. When mass-spawning scallops, 40 to 50 animals are used since only about half will spawn. Of those that spawn, only a few contribute most of the eggs and sperm.

BROODSTOCK MANAGEMENT

- Local stock is best to use
- Select large & healthy individuals
- Use sufficient numbers to ensure genetic diversity; about 300

IV. HATCHERY PRODUCTION

A. Broodstock Conditioning

Conditioning of broodstock is the process of artificially ripening the gonad so that mature gametes will be released upon spawning. The conditioning process is required when attempting to spawn scallops outside of their normal spawning period (June - August). When spawning scallops just prior to their natural cycle, no conditioning usually is required.

Scallops typically are conditioned at 17-20°C in the northeastern United States, specifically Connecticut, during the winter/spring months. Conditioning temperatures will vary with location (latitude); it may be done statically in a container that provides sufficient bottom area for the adults to spread out (Figure 4), with daily or every-other-day water changes. Scallops also can be conditioned in flowing seawater (Figure 5), but heating or chilling of flowing seawater can be expensive. If the salinity of the incoming seawater is below 17‰ and water temperature is above 15°C, mortalities will occur (Mercaldo & Rhodes, 1982). Nutrition is crucial in the successful conditioning of scallops out-of-season. A drip system or peristaltic pump (metering pump to deliver liquid at precise doses) can be used to feed the scallops from an algal source. Numerous daily feedings will improve effectiveness of feeding. We recommend that at least two groups of broodstock be conditioned at the same time so that at least one group is still available in the event that one group spawns inadvertently during the ripening process. Spawning may occur if the water temperature varies too
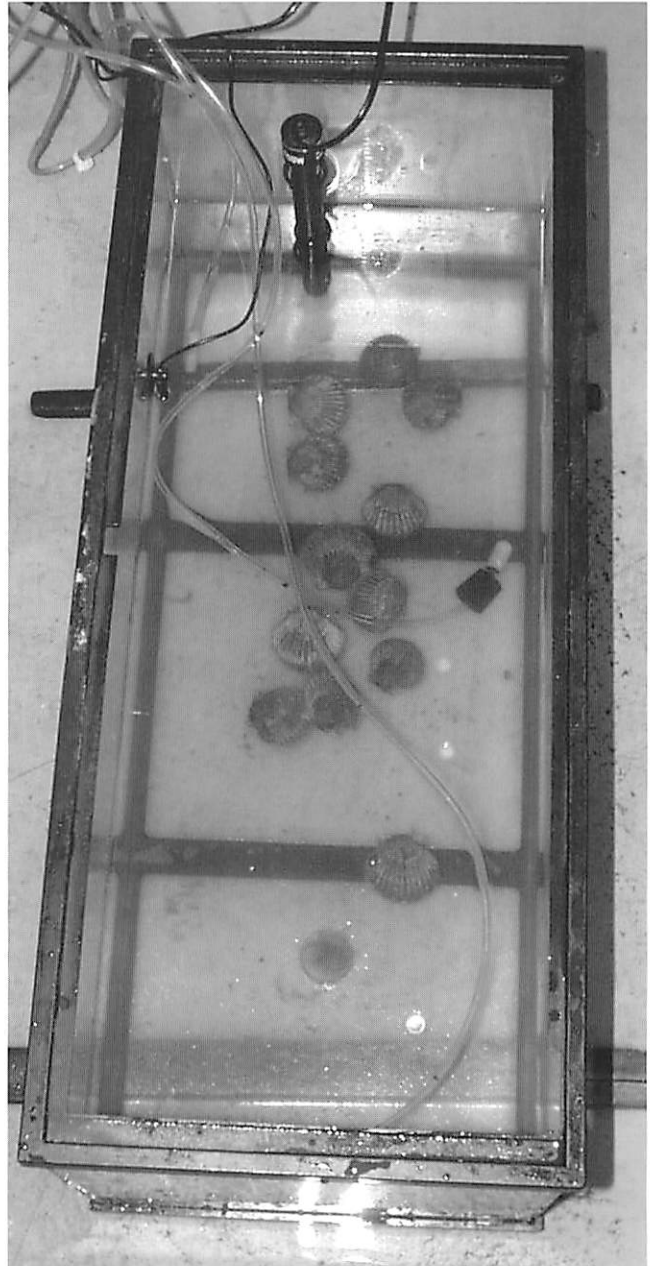
Figure 4. Conditioning scallops in static culture.

much during the conditioning process. In New England, the process of ripening the gonads takes approximately 6-8 weeks when starting in December. Closer to the natural spawning season, less time is required to condition the gonads. Spawning also can be delayed by holding the broodstock in cooler (10°C) water during the warmer months.

Figure 5. Conditioning scallops in trays of flowing seawater.

TEMPERATURE CONTROL

Providing a stable temperature environment (plus or minus 1° Celsius) is important for both conditioning broodstock and larval rearing. Lower temperature variations usually provide better and more consistent results. Heating can be as simple as using aquarium immersion heaters and water baths or as complex as a titanium heat exchanger. Many hatcheries, including this one, rely on multiple heating methods. There are numerous digital controllers on the market that provide excellent temperature control. Even some inexpensive (less than $50) immersion heaters come with fairly sophisticated controllers now. Do not use non-submersible aquarium heaters as these usually fail rather quickly in a seawater environment. Cooling can also be done by immersion titanium heat exchangers, water baths or special chillers made for seawater.

gonad are observed, normally the scallop can be spawned successfully. Scallops that have spawned, but are still ripe (capable of producing sperm and eggs), will appear to have "rivers" traversing the colored parts of the gonad (Figure 6). Gonads that are spent or depleted typically have a translucent beige coloration, and usually do not release eggs or sperm. More vivid coloration of the gonad usually indicates a riper gonad, with the potential for more gametes to be released as well as a greater 48-hour larval survival of the released gametes. Scallops having a white-colored gonad only usually indicates a nutritional deficiency traceable to the microalgae being fed to the broodstock.

B. CONDITION/RIPENESS

Visual observations can determine whether the scallop appears to be ripe enough to spawn. With the scallop held open, peer in between the valves and observe the coloration of the gonad. The scallop, as mentioned earlier, is a functional hermaphrodite, i.e., contains both male and female sex organs. The pinkish-orange area produces eggs, whereas the whitish area produces sperm. It also is common to find a black integument covering the gonad during the conditioning process. This integument usually disappears as the gonad ripens, but can be scraped away gently to reveal the coloration of the gonad. If both colors (pinkish-orange and white) of the
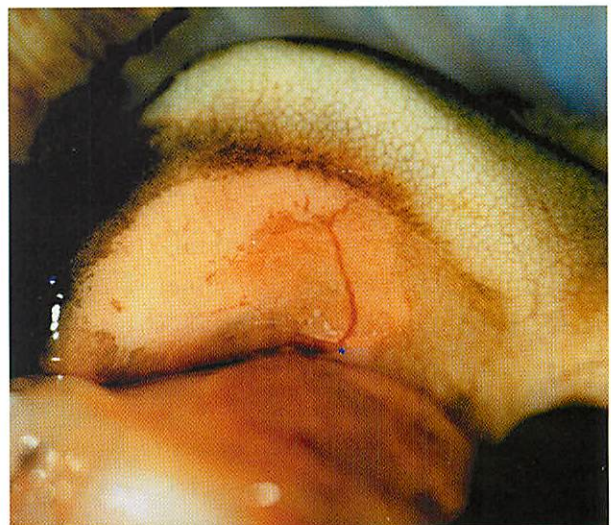
Figure 6. Scallops that have partially spawned with traversing "rivers" through the gonad.

BROODSTOCK CONDITIONING

- Necessary to spawn out of season
- Temperature varies; usually 17-20°C
- Salinity > 17 ‰
- Up to eight weeks required
- Ripeness evaluated by color of gonad

C. SPAWNING

We use two approaches to spawning, both of which rely on increased water temperature to stimulate the scallops: 1) mass spawning, i.e., spawning a number of individuals collectively to induce spawning of the group more easily, and 2) individual spawning, i.e., scallops are spawned in individual containers and the gametes are kept separate, which is particularly important when scallops with special traits are desired. As the bay scallop is hermaphroditic, spawning must be monitored closely during individual spawning to prevent self-fertilization. See Section VI.A.3 for cleaning of scallops prior to spawning.

For both spawning methods, scallops are placed into the spawning container(s) with water at the same temperature from which the scallops were removed (usually 17-20°C). They are allowed to acclimate to this new environment for one hour and then the temperature is increased gradually to approximately 21°C over a 20-minute period. Usually, no spawning occurs during this time, but if the scallops are very ripe, even a temperature change of 1°C can be enough to induce spawning.

Once the temperature reaches 21°C the temperature is held steady for 20 minutes; it is then increased to 25°C over another 20-minute period. After the temperature reaches 25°C, it should be maintained for at least one hour (**do not exceed 26°C**). Typically, spawning will begin during this time. If the scallops still have not started spawning, the temperature can be lowered to 17-18°C and later increased following a 20-minute temperature change and 20-minute holding cycle.

1. **Mass spawning** (Figure 7). Two methods of mass spawning are described here. One method is to place 40-50 scallops into a large, flat-bottomed, dark-colored tray or tank and allow the scallops to spawn by increasing the temperature as described above. (Black is preferred to determine visually when gametes are released.) When an individual scallop spawns, it is removed and placed in a separate container with seawater at the same temperature. (Removing the scallop will minimize any self-fertilization that may take place.) The spawner is allowed to continue spawning in this container, but must be watched closely
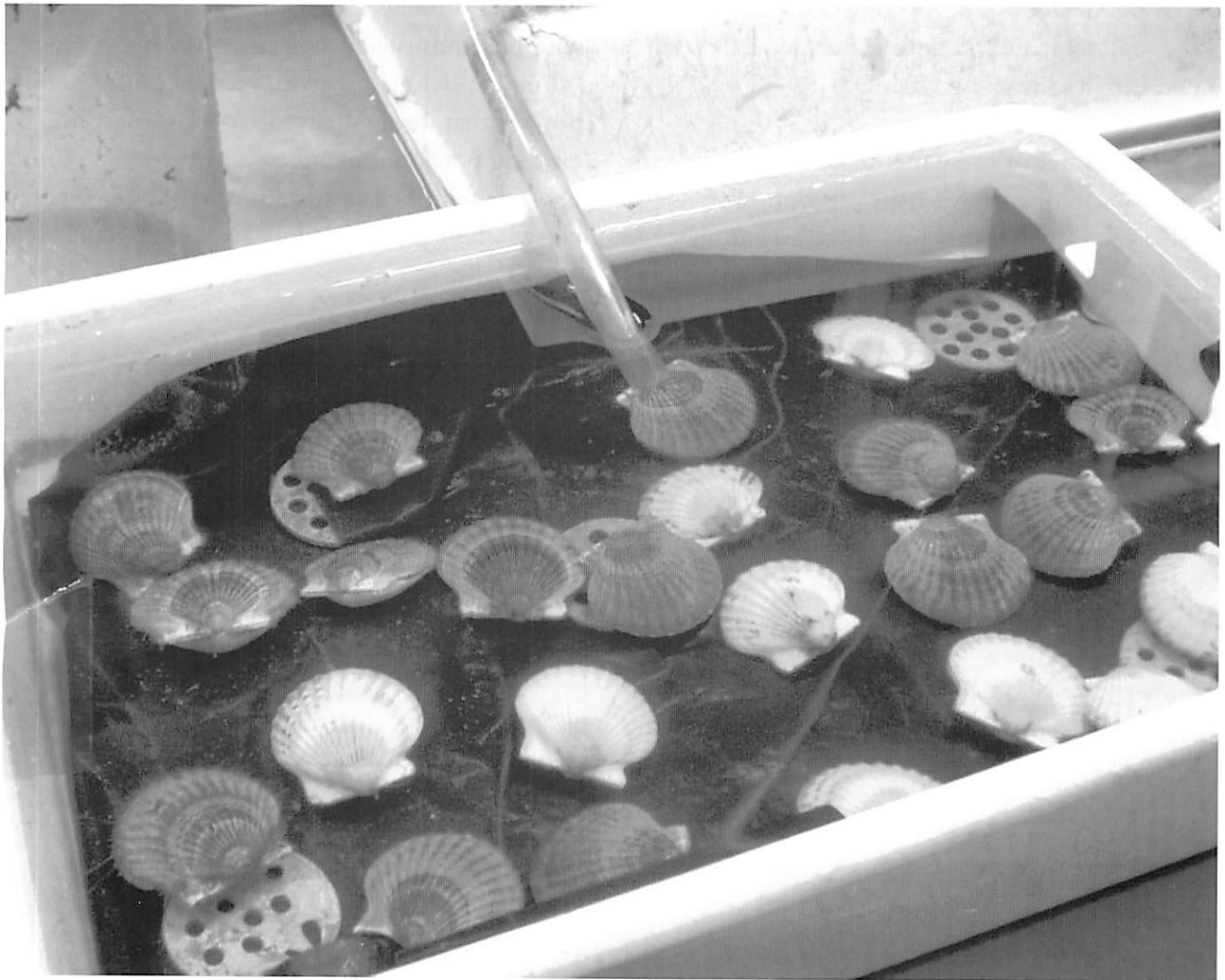
Figure 7. Mass spawning of scallops.

to prevent self-fertilization from occurring should the scallop start releasing gametes of the other sex. To help prevent self-fertilization, we recommend the spawner be placed in a new container of sea-water every 7-10 minutes, or when the scallop appears to have ceased spawning. As the culturist becomes more adept at controlling (or observing) the spawning process, he or she will be able to determine the type of gamete being released into the water column. A thin milky stream is an indication of sperm, whereas a grainy appearance indicates eggs have been released.

A second method of mass spawning is to place scallop broodstock (in nets) into a conical tank or other large container in which the larvae are to be reared. (This method may result in increased rates of self-fertilization.) The temperature of the water in these containers should be pre-heated to 25°C to allow spawning to take place. Periodic sampling of the spawning container is required to determine the number of eggs produced; this is done so that the spawning process can be stopped when the desired number of eggs is obtained. The embryos/larvae resulting from this spawn should

not be handled for 48 hours unless the numbers of larvae are too great and need to be reduced or separated into additional containers.

Using either of these methods, it is possible to stop the scallops from further spawning so as to be able to re-spawn this broodstock a number of times (typically four). To stop the spawning process, the broodstock are removed and typically placed in chilled seawater (17-18°C) - although sometimes cooler water is needed.

2. **Individual spawning** (Figure 8). Individuals are placed into separate containers (Pyrex® bread pans work well) placed over a dark background and the temperature is increased (as described above), usually by flowing warm tap water around the outside of these containers, until spawning takes place. This method of spawning offers considerable control, and it is possible to cross individual animals if desired. This process is more laborious and usually slower than mass spawning. During individual spawning, one must exercise care so that individuals do not self-fertilize by releasing both sperm and eggs in the same spawning container. Self-fertilization may be prevented by switching each scallop to a new container periodically.

SPAWNING

- Two methods: mass or individual

- Temperature increase to 21°C, then to 25°C in two stages
  - **do not exceed 26°C**

Figure 8. Spawning of scallops individually.

D. DETERMINING EGG AND LARVAL COUNTS

Eggs spawned in the containers are separated from debris by passing the egg suspension through a 100-µm or larger screen size (Figure 9). Seawater and eggs that pass through the screen are collected in an appropriately-sized container. If the egg suspension is too dilute during mass spawning, it may be necessary to concentrate the eggs on a 20-µm screen; however, frequent screening of the eggs may be detrimental to normal development. Another approach to concentrate the eggs is to siphon out the excess liquid in the container by covering the inlet of the siphon with a 20-µm screen. To count the eggs, it is necessary to evenly distribute them in the collection container by mixing with a perforated plastic plunger (Figure 10) in an up-and-down motion.
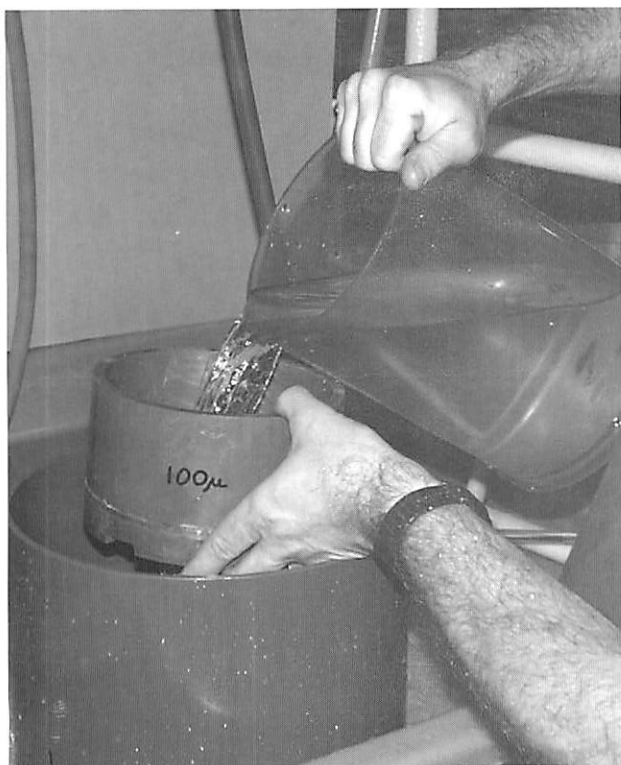
Figure 9. Separation of eggs from debris by passage through a 100-µm screen.
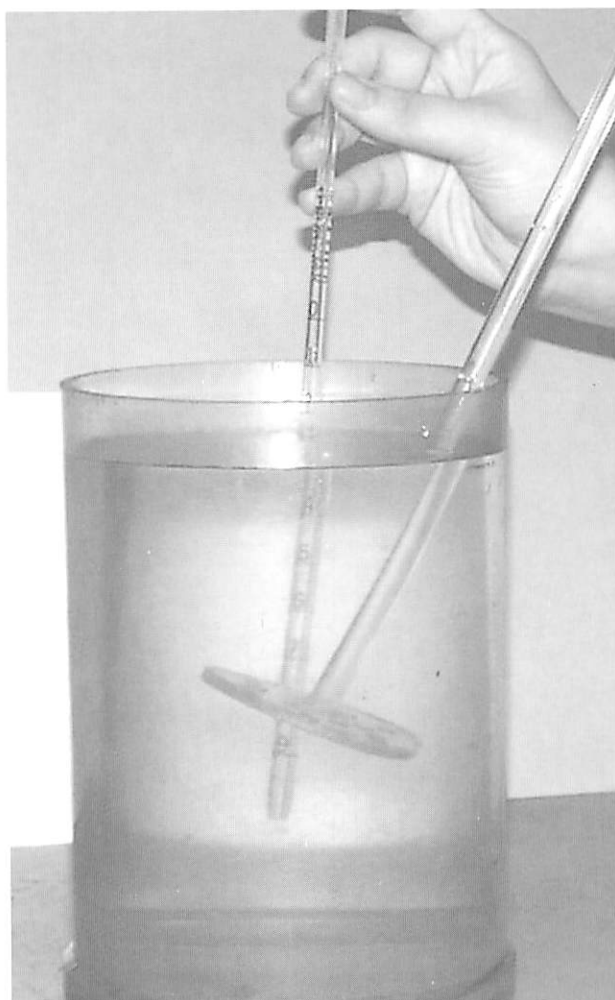
Figure 10. Mixing of egg suspension with a perforated plastic plunger.

This must be done in a predetermined volume. If the mixing motion is circular, the eggs can become concentrated at the bottom and not evenly distributed, resulting in an erroneous count. Once the eggs are mixed in a known volume such as a 250-mL or 1,000-mL graduated cylinder, a 1-mL pipette with a large bore opening is used to remove eggs from the container to count them (Figure 10). This sample is placed on a Sedgwick-Rafter counting cell (Figure 11). This must be done quickly; no more than 7-10 seconds from pipette to slide to count the eggs under a

Figure 11. Dispensing a 1-mL sample of egg suspension on a Sedgwick-Rafter counting cell.

compound microscope. By scanning the entire slide at 60 X, the number of eggs in 1 mL is determined. By multiplying the number of eggs in 1 mL by the total number of mL in the container, you obtain the total number of eggs in the container. This number then is used to determine the volume of egg suspension to add to each rearing container.

See Table 1 (page 17) for stocking densities and Table 2 for screen sizes to be used. Counting eggs is relatively simple because they are not motile.

EGG COUNTING

- Screen to concentrate and clean
- Mix thoroughly with plunger
- Subsample 1 mL with wide-tip pipette
- Count in Sedgwick-Rafter Cell at 60 X magnification
- Multiply number in 1 mL X total volume in milliliters to calculate total number of eggs (embryos)

Table 1.
Recommended stocking densities of eggs and larvae based on age and size.

Age/Size*	Density
embryos/fertilized eggs	30/mL
48 hours	17.5/mL
150 µm	10/mL
175 µm	1.25/mL - near setting size

*Sizes may vary with variations in the nutritional value of the microalgae being fed.

Table 2.
Recommended mesh sizes of screens for larvae based on age and size.

Mesh Size*

20 µm	-	retains eggs (usually only need a 6" diameter screen)
54 µm	-	retains 48-hour-old larvae
75 µm	-	retains 4-to 5-day-old larvae
100 µm	-	retains 6-to 7-day-old larvae
135 µm	-	retains 7-to 14-day-old metamorphosing & metamorphosed larvae

*Screens generally used are 10" diameter, although other sizes can be used. At our facility we use 10" screens when draining 400-L conical tanks.

Figure 12. "Standard" 400-liter fiberglass tanks for culture of embryos and larvae.

Figure 13. Small plastic containers for culture of embryos and larvae.

AERATION

Aeration is vital in a hatchery when rearing shellfish in recirculating or static systems. Small, fine bubbles diffuse more air into the water than larger bubbles because of the greater surface area. The longer the bubble is in contact with the water the better, hence an air diffuser should be placed as deep in the water column as possible. Adding air to the water does a number of things: it provides oxygen which is consumed by the shellfish, mixes the water, and helps in the removal of various waste compounds. Aeration can be provided by a small aquarium-style air pump, an oil-less compressor or air blower. The method chosen should be based on total usage. Since air is so vital to the shellfish, a backup system is recommended.

REARING CONTAINERS

- Size variable
- Smooth surface
- Light color
- Aeration

E. REARING CONTAINERS

Shellfish hatcheries use a variety of containers to rear shellfish larvae. Some hatcheries use containers as large as swimming pools, others use the "standard" 400-L fiberglass conical tanks (Figure 12), and smaller operations successfully use plastic garbage containers (Figure 13). When selecting a container, choose one with a smooth surface to help reduce bacterial growth and allow easier cleaning (we prefer light-colored containers) and rigid enough to support the volume of water held. Gently aerate the seawater in these containers to maintain uniform temperatures and provide oxygen to the larvae.

F. LARVAL REARING

Optimal larval rearing conditions (See Table 1 on page 16 for stocking densities and Table 2 for screen sizes to be used) are at a water temperature of 25±1°C and a salinity of 25‰ (Tettlebach & Rhodes, 1981). Temperatures should not exceed 26°C. All items that are used in spawning and larval rearing should be washed immediately prior to use with a biodegradable soap and chlorine bleach solution (see section VI.A.1.). Then wash with at least three hot water (as hot as tolerable) rinses, followed by a rinse with 10-μm filtered seawater. During the first 24 hours of life, the fertilized egg develops into the following stages: gastrula, trochophore, and, finally, a swimming veliger (Figure 1). Larvae are first fed 24 hours after spawning and daily thereafter. Water in the culture containers remains static for the first 48 hours to allow the fertilized eggs to develop into "D-shaped larvae" (veligers). At 48 hours, the larvae have already developed shells and are able to swim by moving their cilia. Movement of the cilia causes water to move within the larval shell cavity, maintaining an oxygen-rich environment and providing food to the larvae. Healthy larvae can be identified under a microscope at 60 X by their circular swimming pattern and their dark, rich gold/tan coloring (although the coloring can differ depending on the microalgae being fed). Occasionally, after the larvae are disturbed—possibly by changing the water—they become "shocked"
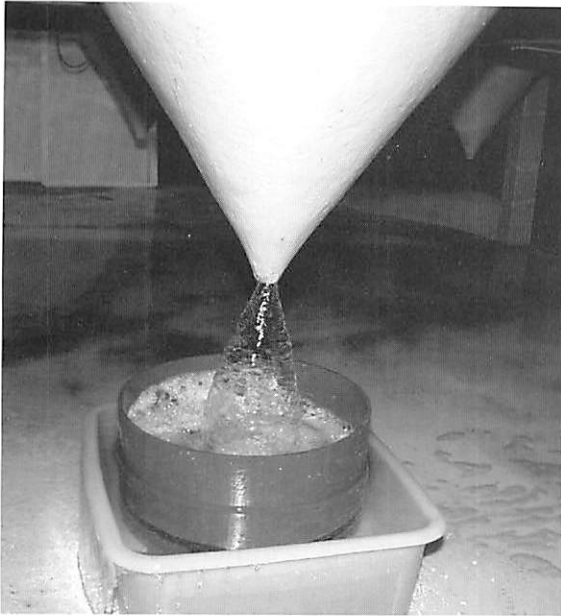
a. Screening culture water   b. Larvae on screen

Figure 14a,b. Passing larval culture water through an appropriate-size screen to collect larvae.

a.   b.

Figure 15a,b. Larval-collection screen placed in seawater to prevent damage.

and are not very mobile. It is then important to view the larvae under a microscope at 120 X power or higher to determine whether the cilia are moving within the shell. Another method to determine how well the larvae are doing is to keep the larvae on the slide for a longer period of time (minutes) in order to observe whether they begin to exhibit normal swimming behavior.

NOTE: Do not keep the microscope light on during this time as you may heat-stress the larvae.

Water changes are required to maintain a healthy growing environment and remove the waste products that accumulate in the larval culture. Water for larvae should be changed on a daily basis after the first 48 hours, although some shellfish hatcheries change every other day. To change the larvae, the container is drained through an appropriate-sized mesh-screen (mesh size varies with the size of the larvae-Table 2) (Figure 14a) so that the larvae are retained on the screen (Figure 14b). Depending upon the length of time it takes to drain the larval container, it may be necessary to place the screen in seawater to help prevent damage to the larvae (Figure 15a,b). Increasing the mesh size of the screen as the larvae grow aids in the removal of debris that would otherwise accumulate in the culture and provide additional substrate for bacteria. As an example, we typically use a 54-μm screen after 48 hours and a 75-μm screen after four to five days. If you are trying to maximize genetic diversity, we recommend using a 36-μm screen at 48 hours to retain all larvae.

Occasionally, you may want to separate the culture based on larval size. This can be accomplished by passing the culture water through a series of mesh screens, a process known as culling. It is called culling because some hatcheries discard the runts (slowest growers), especially if there is a shortage of phytoplankton or larval rearing containers. Always discard larvae that you are unable to feed or rear, rather than starve or crowd them, which usually results in higher mortalities.

Sampling of the larvae in the culture containers should be done on a daily, or at least every other day, schedule. When larvae are sampled, a preservative that immobilizes the larvae on the slide must be used to obtain an accurate count (3 to 5 drops of ethanol or a 10% household chlorine bleach will suffice). Generally, it is a good idea to concentrate/dilute larval suspensions to obtain counts of larvae in the 250-300 per mL range.

It is important to note that even if the larvae are not counted and measured on a daily basis, they at least **SHOULD be observed under the microscope on a daily basis.** (This cannot be emphasized enough!)

LARVAL REARING

- Temperature 25°C; salinity 25‰
- Start feeding after 24 hours
- Change water after 48 hours
- Sample and observe under a microscope daily
- Feed 400-liter conical 1liter of algae at packed-cell-volume of 0.010 daily
- Record survival and growth

A
85x70

B
95x81

C
105x91

D
115x99

E
126x112

F
135x120

G
145x130

H
155x142

I
167x150

J
177x159

K
187x167

L
197x184
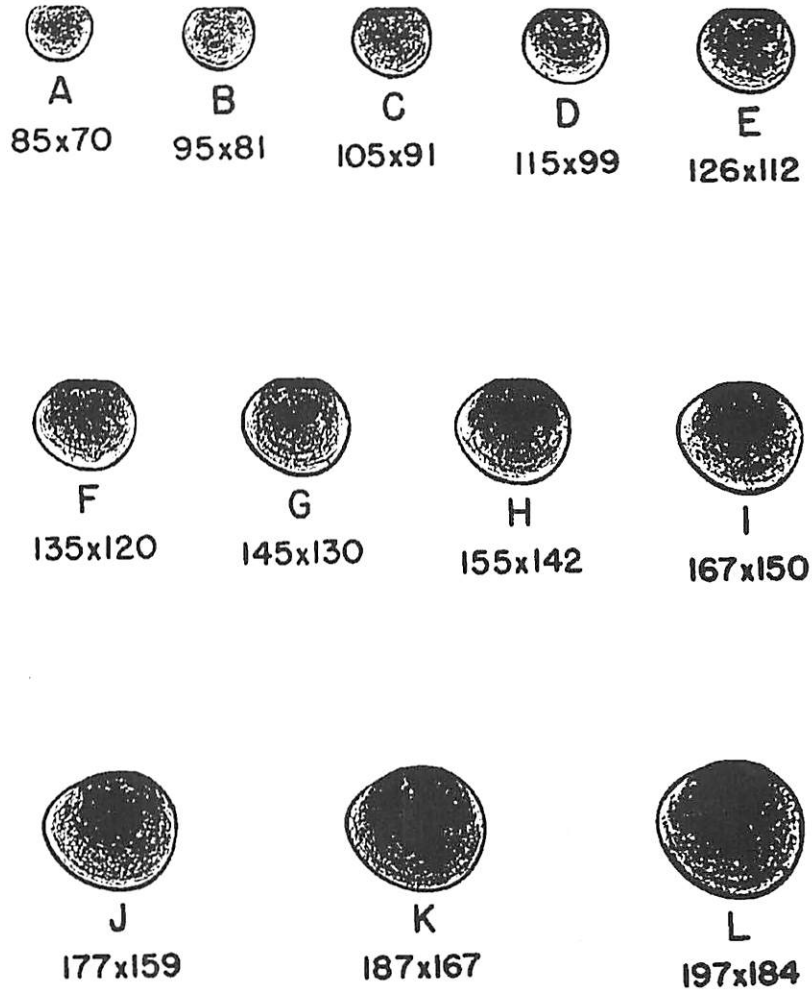
ARGOPECTEN IRRADIANS

x 112

Figure 16. Size of larvae (in μm) from about 48 hours to about 10 days of age. Largest dimension is in a line parallel to the hinge.

Larvae are fed microalgae at a rate equivalent to 1000 mL at a packed-cell volume (PCV) of 0.010 per 400 L of larval culture per day (See Table 3 and Appendix A). As an example, we normally feed a 400-L larval culture 15×10^9 cells of *Isochrysis* sp. strain T-ISO or 13.5×10^9 cells of *Pavlova* strain 459. To provide a good nutritional mix, at least two types of microalgae are recommended as a food source.

Records of survival and growth should be maintained for each spawning batch. By tracking growth of the larvae (Figure 16), one can get a feel for the robustness of a particular spawn and any possible problems. Growth of the larvae can be measured with an ocular-micrometer placed in one of the eyepieces of the binocular microscope. Normally, a decrease or cessation of growth indicates some type of problem. Two common problems are bacterial contamination and nutritional deficiencies.

G. GENETIC CONSIDERATIONS AND SELECTIVE BREEDING

In most hatcheries, faster-growing larvae are selected for further grow-out. It is common practice in a hatchery to screen-out and discard bottom material, or "dregs", and slow-growing or dying larvae. Doing this may prevent a buildup of harmful microorganisms in the culture. If possible, however, it is wise to save some of the moderately-growing larvae. It is important to realize that inadvertent selection and, therefore, narrowing of the gene pool, occurs with standard hatchery practices such as spawning small numbers of broodstock and screening or culling larvae and juveniles for size. Some unexplained "crashes" (near total mortality) of larval cultures may be attributable to a narrow gene pool. These crashes can be reduced by mass spawning more diverse broodstock.

If some inbreeding from self-fertilization does occur, one can minimize effects by taking certain steps. If possible, maintain sufficient numbers of adult broodstock in the hatchery so as to be able to spawn several times. Also, spawn as many scallops as possible at one time. Split a batch of larvae into several containers to minimize loss of the entire population from one mistake or malfunction. This is based on the practical advice not to "put all of your eggs in one basket".

In some hatcheries, certain characteristics may be desired to improve or increase production. Crossing of selected scallops could increase the frequency of certain traits like faster growth, disease resistance, or shell color or markings (Stiles *et al.* 1998 a, b). Some scallops have obvious genetic markings or color patterns on their shells, such as stripes (Figure 17), which could be used in identifying stocks from a particular hatchery. For example, dark red vertical bands on shells of the hard clam, called Notata clam, are used for this purpose in the clam industry (Stiles & Choromanski, 1995). More detailed

Figure 17. Scallops with distinct striped markings.

discussion of genetics and selective breeding is beyond the scope of this manual.

GENETICS AND SELECTIVE BREEDING

- Moderate culling removes dregs, but preserves genetic diversity
- Replicate spawnings minimize risk
- Crosses may be made to select for desired traits·

H. JUVENILE NURSERY

The term "juvenile scallop" typically refers to post-metamorphosed (180-μm) animals up to 25 mm in size. These scallops are hardier than the larvae, but still require special handling. Scallops cannot close their valves tightly and are subject to dehydration when they are exposed to air. Small scallops experience detrimental effects more quickly than larger ones. Freshwater exposure will cause mortalities as well. When transporting scallops, care should be taken to make sure they remain moist and cool.

Post-set scallops attach to substrates by means of byssal threads and the substrates take on a textured appearance. By taking advantage of this attachment phenomenon, scallops can be allowed to set on various materials. Typically, used gill net in "onion" bags, plastic sheets, or strips of burlap are placed in the larval containers for scallop attachment. Scallops, unlike oysters, can readily detach from these setting materials, so gentle

handling is important. Scallops metamorphose within 1-2 weeks inside the larval containers and juveniles can be found attached to the bottom and sides. Scallops that are not attached can be rinsed away with a gentle stream of seawater (soft rinse); the metamorphosed scallops remain attached to the sides. To remove the attached metamorphosed scallops, a hard stream of seawater (hard rinse), similar to that formed by restricting the flow of water from a hose, is used to dislodge the scallops from the container/conical tank. From here, the scallops normally are transferred to either static or flow-through tanks of seawater. Static cultures are drained and refilled every day or less often.

Once the scallops have set they can be moved to various systems, including placement in the natural environment. The circumstances chosen for this stage of culture will depend upon final product/harvest size and natural growth cycle/temperature regime. When transporting scallops, keep them moist with seawater, cool, and out of direct sunlight. One useful method is to keep them covered with moist burlap or newspaper in a cooler with a cooling block.

Scallops in static tanks are fed daily and water is changed every other day. To improve growth rates, microalgae can be added on a semi-continuous basis (manually, drip-feed, or use of a peristaltic pump) over a 20-22 hour period. This assures that very little algae is wasted when the water is changed. The available microalgal production will determine how long and how many scallops can be reared in this way.

Flow-through systems such as raceways (Figure 18) or trays (Figure 19) also can be used (Rhodes & Widman, 1980). Scallops can be placed in these systems (more vertical attachment areas) as long as the ambient water temperature is greater than 15°C. Stocking densities are based on

Figure 18. Raceway system for culture of juvenile scallops.

Figure 19. Tray system for culture of juvenile scallops.

the microalgal food supply at each hatchery site. See Section VI.C.1. and 2. for disease diagnosis and cleaning of trays or raceways. Downwellers (where water flows from the top down through the scallops and the mesh bottom) are used successfully to grow post-set scallops up to shell heights of 10 mm (Figure 20). Upwellers can be used as well, but must be modified to prevent scallops from swimming out the drain.

Another technique for culturing juvenile scallops involves suspension culture in the natural environment, typically employing either spat bags (Figure 21) and/or pearl nets (Figure 22). Spat bags are fine-mesh bags filled with a substrate, e.g., gill netting, on which scallops have set. Larger scallops (>4 mm) can be placed directly in pearl nets which utilize a larger mesh and are pyramid-shaped. Scallops can be placed out in natural waters successfully at temperatures as low as 5°C in the spring in the northeastern United States in these types of gear. Little growth occurs until the water temperature reaches about 15°C (Widman & Rhodes, 1991).
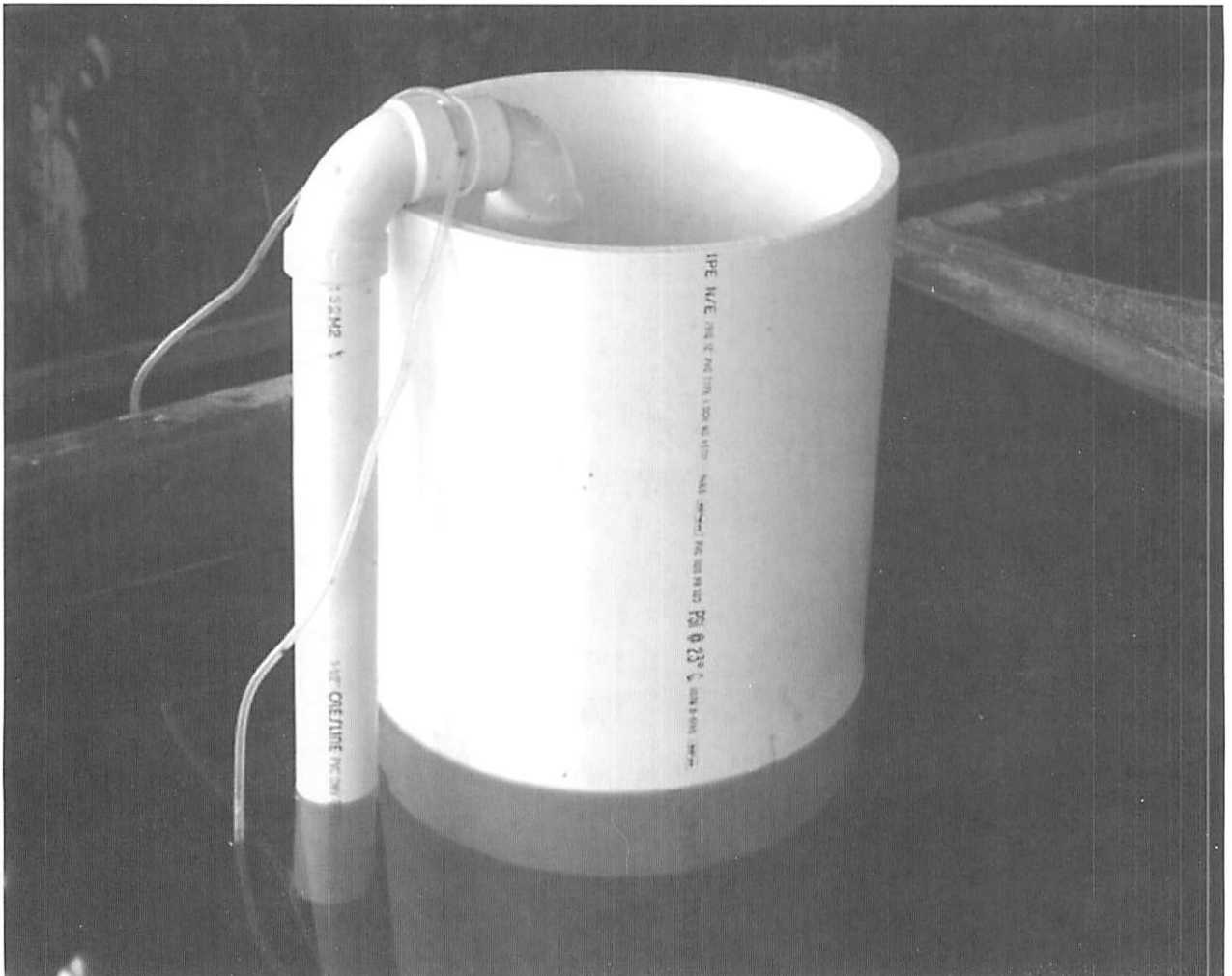
Figure 20. Downwellers for the culture of scallops up to 10 mm.

Figure 21. Spat bags for the culture of juvenile scallops in the natural environment.

> **JUVENILE NURSERY**
>
> - Do not allow post-set to dry out
> - Static tanks with supplemental algal food
> - Flow-through with raceways, downwellers, or upwellers
> - Nets in the sea

Figure 22. Pearl nets for the culture of juvenile scallops in the natural environment.

V. ALGAL FOODS

A. Diets for Pre-set Larvae

To feed any animal, one must know three things: what, how much, and how often to feed. Feeding rations and regimes–how much and how often–are covered elsewhere in this manual. To answer the first question–what to feed–dietary items must be found that are not toxic, are ingested and digested, and supply the energy and materials needs of the animal being fed. As with larvae of other molluscan shellfish reared in captivity, living cultures of selected microalgae have proven to be most successful in practical application. Not all algal strains, however, satisfy the criteria listed above for a suitable diet. Further, not all algal strains satisfy an additional criterion for success in the hatchery– ease of culture. Table 3 summarizes findings of controlled feeding studies conducted at Milford to compare growth and metamorphosis of larval bay scallops on unialgal diets of some commonly-cultured microalgal strains, ranked in order of nutritional value with the best diets at the top.

It is clear from Table 3 that many easily-cultured microalgal strains are not particularly good diets for larval scallops. Reasons for poor larval growth on diets ranked as "awful" appear to be related to either size (PLY429 is too big to be ingested by first-feeding larvae), indigestibility (UTEX2341, Sticho-GSB, and 580), or biochemical composition (3H and perhaps Chaet cal). Several *Pavlova* strains appear to be the best diets, but these can be difficult to culture in large scale; nevertheless, some hatcheries have used these strains successfully. T-ISO combines attributes of a good larval diet and dependable growth in industrial settings; therefore, this strain is recommended for those inexperienced in microalgal culture. Another strain of *Isochrysis* – strain

C-ISO – appears to have similar nutritional characteristics to T-ISO and may grow better than T-ISO under some conditions. *Pavlova* strains may offer substantial benefits if the facility's conditions are conducive to their growth; at present, we do not know what these conditions are. Significance of the final column, involving relationship between cell size and packed-cell volume is described in Appendix A..

B. Diets for Larvae During Metamorphosis

In addition to feeding through the larval stage, suitable diets must be provided as larvae are undergoing metamorphosis. As scallops set, capture of food particles shifts from the larval feeding organ, the velum, to the developing gills. Particles in the size range suitable for larval ingestion, including the useful larval diets listed in Table 3, are too small to be retained efficiently by the gill-feeding structures of post-set larvae. Hence, it is critical to include algal feeds larger than about 6 μm during the time that scallops are setting. We have achieved best setting success by beginning to add one or more strains of *Tetraselmis*, PLAT-P (*T. striata*, about 9 μm) and/or PLY429 (*T. chui*, about 12 μm) between days 5 and 7 of larval life, first as about a third of the ration and then shifting to 100% of the feed after setting is complete. These two *Tetraselmis* strains contain extremely high levels of essential fatty acids and dietary sterols that are also associated with rapid growth in post-set scallops and oysters. Broodstock conditioning also can be done using these *Tetraselmis* strains; however, inclusion of a diatom or a larger brown flagellate (e.g., *Pavlova* #93) may be necessary to provide fatty-acids not found in *Tetraselmis*.

Table 3. Microalgal strains tested for feeding larval bay scallops through to metamorphosis.

Algal Strain	Identification	Nutritional Value	Ease of Culture	Size (µm)	10^6 cells/mL @ pcv=0.010*
CCMP459	*Pavlova* sp.	Excellent	Very difficult	6x5	13.5
CCMP609	*Pavlova* sp.	Excellent	Difficult	6x5	13.5
MONO	*Pavlova lutheri*	Very good	Difficult	5x4	15
DICRAT	*Dicrateria inornata*	Very good	Difficult	5x4	15
T-ISO	*Isochrysis* sp.	Very good	Easy	6x5	13.5
GBF	*unident. prymnesiophyte*	Good	Easy	3x2	17
Chaet cal	*Chaetoceros calcitrans*	Poor	Easy	7x6	9.5
PLY429	*Tetraselmis chui*	Awful	Easy	12x10	2.5
3H	*Thalassiosira pseudonana*	Awful	Easy	3-5	12
UTEX2341	*Nannochloropsis* sp.	Awful	Easy	2-3	20
Sticho-GSB	*Nannochloropsis salina*	Awful	Easy	3-4	17
580	*Chlorella autotrophica*	Awful	Easy	3-4	18

*pcv (packed-cell volume units are mL of packed cells per 10 mL of culture.

How to use the last column of this Table to calculate daily rations for larvae from cell counts:

1. Divide the volume of your larval culture (in liters) by 400 to determine the liters of algal food at 0.010 PCV needed (ex. 400 L/400 = 1L).
2. Find the cells per mL @ 0.010 on this Table (ex. 13.5×10^6 for T-ISO)
3. Multiply the result of step 1 by the result of step 2 and divide by the cell count in your culture (ex. 1,000 mL x 13.5/6= 2,250 mL)

Note: This calculation assumes linearity between pcv and cell count, a reasonable assumption between 10^5 and 10^7 cells per mL.

C. Precautions in Producing Microalgae

Detailed instructions on how to culture microalgae are beyond the scope of this manual; the first volume of *The Handbook of Phycological Methods* (Stein, 1973) is a good place to obtain such basic information. Nevertheless, a few comments are in order about precautions that should be taken to ensure that microalgal cultures provide a wholesome and nutritional diet for young scallops. Although it is impractical to produce bacteria-free feed cultures in a farm setting, care must be taken to minimize the potential for infection of microalgal cultures with pathogenic microorganisms. Saline water from a well may be the best option for algal-culture water; generally, microbial numbers are very low, salinity is stable, and further treatment prior to inoculation with seed algal cultures usually is unnecessary. If coastal surface water is used, some combination of filtration, pasteurization, or other treatment methods may be necessary. Nutrients that must be added to seawater to support algal growth should be sufficiently refined to avoid introducing chemical contaminants that may harm both algae and shellfish. Reagent chemicals can be combined according to recipes listed in algal-culture manuals (e.g., Stein, 1973); however, pre-mixed nutrient products (usually described as 'f/2' mixes) available from aquaculture supply companies are suitable, convenient, and widely-used.

D. Seed Cultures

Starter, or "seed" cultures of microalgae are available from the Milford Laboratory, as well as from commercial aquaculture supply companies and a few research institutions that maintain collections of microalgal strains. Hatcheries need to maintain their own seed cultures, usually in test tubes or small flasks that are "transplanted" periodically using aseptic microbiological techniques,

for re-starting production cultures after the initial starter is obtained from an outside source. Beyond the expense or potential delays of repeatedly sending for new starter cultures from an outside source, there are advantages to maintaining one's own seed cultures. First and foremost, inocula for production cultures are available as needed. In addition, algal strains may become adapted to local salinity and/or other seawater chemistry and out-perform new starters. Guidelines for maintaining small cultures of microalgae under aseptic conditions can be found in *The Handbook of Phycological Methods* mentioned previously or adapted from bacteriological procedures. The hatchery operator should be prepared to accomplish all practical aspects of microalgal culture before attempting to rear animals.

ALGAL FOODS

- Best larval diets are nutritious and easy to culture — T-ISO and other flagellates
- Provide setting larvae and post-set with larger cells — PLAT-P or PLY429
- Broodstock conditioned on mix of *Tetraselmis* and diatoms or "brown" flagellate
- Microalgae culture precautions
- **Do not** attempt to grow larvae until you are able to feed them!

VI. DISEASE PROBLEMS

Bay scallops are relatively resistant to infectious diseases. Infections, however, may be introduced into aquaculture systems by several routes; when they become established, they can spread rapidly and may kill most of the scallop larvae or juveniles within the system. Likely routes for pathogen entry into the hatchery or nursery are through:

    (1) contaminated equipment or materials,
    (2) the seawater source,
    (3) the broodstock,
    (4) algal-food stocks, and
    (5) addition of scallop stocks from
         new sources.

Even when diseased scallops are removed, the pathogen may be maintained in the system when it grows within a biofilm on pipes, grading screens, or tank walls.

These biofilms have been found to consist of layers of microorganisms bound together with a polysaccharide "glue" that is resistant to cleaning and to penetration of chemical agents. Although they are protected within this film, pathogenic microorganisms can be shed back into the system. Therefore, maintenance of hygienic conditions is very important in controlling pathogenic agents in the hatchery.

Although the literature about pathogens of bay scallops is sparse, bacteria (primarily *Vibrio* species), rickettsiae, *Chlamydia*, and fungi have been described. Regardless of the type of infecting agent, the recognition of a disease condition and the procedures for limiting disease problems in the hatchery are similar. Information in the following sections is offered as "best practice", with the

recognition that individual hatchery operators will make decisions about available resources, and relative need for control of disease in the hatchery.

DISEASE PROBLEMS

- Potential pathogens are everywhere
- Hygiene is critical

A. PROCEDURES TO CONTROL INTRODUCTION OF PATHOGENS

1. Equipment and Materials. Routine washing and drying of all equipment and materials may be the best single means to control disease. It is, however, also wise to use separate handling equipment for each group of scallops so that a non-apparent disease (in its early stages) is not transferred between groups. Equipment and tanks should be washed with a biodegradable detergent (we routinely scrub with 5 mL of Liquinox® plus 5 mL of household bleach in 10L of tap water); multiple rinses (preferably with hot tap water and once with filtered seawater) are needed to remove all traces of detergent. Items should be allowed to dry completely–this includes screens and hoses which may allow growth of microbes if left damp. Water delivery and holding containers should be designed to avoid pockets where debris and moisture can collect. Also, pipes and valves should be large enough so that a brush can be used in cleaning if disease problems occur. Damp filters used for filtering the incoming seawater provide excellent substrates for microbial growth;

therefore, filters should be washed and dried. (Old filters should be bagged carefully prior to disposal, to contain any disease organisms.)

Even the most heat-resistant bacteria will be killed when exposed to water at 71°C (160°F) for 60 seconds. On the other hand, bacterial spores may survive boiling water for several hours; however, spore-forming bacteria are not known to be pathogenic for molluscs. Screens, sponges, brushes, and other equipment can be sterilized effectively by dipping them in hot water for one to two minutes, provided that they are clean. Presence of organic material may protect microbes from the penetration of heat; therefore, items either should be cleaned first or the heat treatment should be longer (i.e., 5-10 minutes).

2. **Seawater.** Filtration will remove most microorganisms from the seawater used during the rearing of scallop larvae to the 10- to 12-day stage. Water filtration becomes impractical for larger, post-set animals which require greater amounts of natural foods in flowing systems. A useful first step is to pass raw seawater through a 10-µm bag-filter (Figure 23) to remove large particles and most fouling organisms from the water as it enters a holding tank. Water pumped from the holding tank through two 10-µm nylon filters provides further clean-up of the water. Usually it is not necessary to sterilize the water with ultraviolet (UV) light (Figure 24) after the filtration step; in fact, some experience suggests that complete removal of competing microorganisms will allow unrestricted growth of microbial pathogens in the system. However, if incoming seawater is determined to be the source of high levels of disease organisms, the pre-filtered water should be UV-treated by passing it through a commercial UV unit before it is added to larval scallops. Filtration must occur before UV treatment because particulate matter in raw seawater can block the penetra-

tion of light, and silt can damage light-conducting surfaces of most UV units.

3. **Broodstock.** Bay scallop broodstock should be healthy. If many scallops in a broodstock tank recently have died, it is better to seek a new source of broodstock or use animals from a separate tank where no mortalities are evident. Although the surface of other molluscs such as clams or oysters (which close tightly when confronted with stressful agents) may be chemically disinfected, this is not possible with bay scallops. Therefore, removal of surface films and organisms must be done by scraping any hard deposits from the shell with a blade followed by scrubbing it with a clean brush. The scallop should be rinsed well with clean, filtered seawater before placing it in a holding-tank of clean seawater. Although not routinely practiced, it may be possible to reduce transfer of pathogens to gametes even further by allowing the cleaned broodstock to purge for 24 hours in 17-20°C filtered seawater, with several water changes, before the scallops are used in spawning.

4. **Algal food.** Food used in rearing larval scallops may be delivered in several forms. The most controllable feeding is done with a suspension of selected, cultured microalgae (a single species grown in seawater filtered or otherwise treated to eliminate microorganisms) that is known to have good nutritional qualities. In some high-volume aquaculture operations, it may be necessary to feed larvae a mixture of natural algae in bag-filtered (25 µm) seawater supplemented with cultured algae. This mixture will include many natural bacteria, which may be benign or even helpful in maintaining the health of scallops. However, *Vibrio* spp. often grow in association with natural marine algae; some of these *Vibrio* spp. are pathogenic to bay scallop larvae. Surprisingly, even carefully-controlled algal cultures may become contaminated with bacteria despite careful efforts of

the culturist; this often includes *Vibrio* spp. which seem to grow better in association with algae.

If the resources are available, food and tank-water containing larvae can be checked for *Vibrio* spp. by counting bacterial colonies which grow on TCBS agar (Difco Laboratories) (Figure 25). The step-wise procedure for counting *Vibrio* is given in Appendix B.

5. Scallop stocks from new sources. If you plan to bring in scallop stocks from new sources (larvae, juveniles or adults), the first consideration should be whether the source (natural or hatchery) is known to be disease-free. There should be an initial period of quarantine (i.e., complete separation from existing stocks for a period long enough to determine whether scallops are growing normally and show no evidence of disease). Imported

Figure 23. Bag filter systems for filtratiion of seawater.

larvae can be tested for disease by grinding 500–1,000 larvae in a tissue grinder or between two pieces of clean glass and then placing the ground mash in an isolated culture vessel containing about 1,000 one-day-old, healthy, native larvae. Compare larvae in the test vessel with healthy native larvae in a second vessel containing no ground mash; look for disease symptoms as in Section VI.5.B.1., below, or evidence that the shells do not develop in the normal D-shape. If no evidence of disease is seen in 48 hours, then serious pathogens probably are not present. Whole larvae or ground mash also can be placed on TCBS agar to test for presence of *Vibrio* spp. as described in Appendix B. Juvenile and adult animals should be observed in isolation for several weeks before being introduced into water systems in common with those of native stock.

B. LARVAL DISEASE

1. Diagnosis. Once per day inspect larvae with a microscope (60 X to 100 X total magnification). Healthy scallop larvae actively swim, have good (not weak or transparent) color, and clean vela. Healthy larvae should grow about 10 μm per day when held above 20° C. Sick larvae exhibit slow growth, poor color, and little or no swimming activity (finally sinking to the bottom); also they may have debris attached to the velum, detached velar cells, and protozoans swimming around them. Bacterial infection may show up as swarms of bacteria and protozoans around a gaping shell.

If you have the resources, the water in tanks containing scallop larvae can be monitored daily for evidence of increased *Vibrio* counts as described in Appendix B. Counts increasing to 10,000 per mL (possibly as low as 1,000 per mL) indicate a problem with vibriosis. If the food source is free of *Vibrio* spp., then increased counts in water indicate either a larval infection or insufficient removal of the biofilm during the cleaning process.

Figure 24. Ultraviolet (UV) light systems for sterilizing seawater.

2. Treatment. Very mild infections may not be evident except that some larvae may exhibit poor growth (disease is only one of several factors that can cause slow growth). Therefore, it is good practice to size the larvae daily (or at least every other day) through several screens. Discard larvae on the smallest screen after first soaking the larvae and screen in hot >71°C (160°F) water for 10 minutes to kill any pathogens. To maintain clean conditions in hatchery conical tanks, scrub them daily with mild detergent and bleach and rinse them thoroughly as in Section VI. A.1. If possible, place the larvae in another, clean tank of seawater and allow the first tank to dry for at least 24 hours.

If it is obvious that larvae are dying of a serious infection (infections tend to kill all the larvae over a two- to four-day period rather than all together within one day; also see symptoms under Section VI. B. 2.), the entire system should be drained, cleaned and disinfected. Disinfection can be done by scrubbing all surfaces with a solution of tap-water containing both household bleach diluted 1:20 and Liquinox® diluted 1:1600 (i.e., 500 mL of bleach and 15 mL of Liquinox to 10 L of tap-water or 6 ounces of bleach and 1 teaspoon of Liquinox per gallon of tap water). Since this concentration of bleach is very strong, rubber gloves, an apron, and eye protection should be worn. Inlet and outlet pipes also should be cleaned with appropriate brushes if possible. Then, surfaces should be rinsed at least eight times with hot water to remove chlorine and detergent. As a precaution, leach out any residual chlorine by re-filling the system with freshwater or seawater and allow it to stand overnight. Drain the system, rinse it again with seawater, and allow it to dry. This chemical disinfection procedure is recommended because, if done thoroughly, it provides the most certain elimination of infectious agents. However, it should be mentioned that some culturists
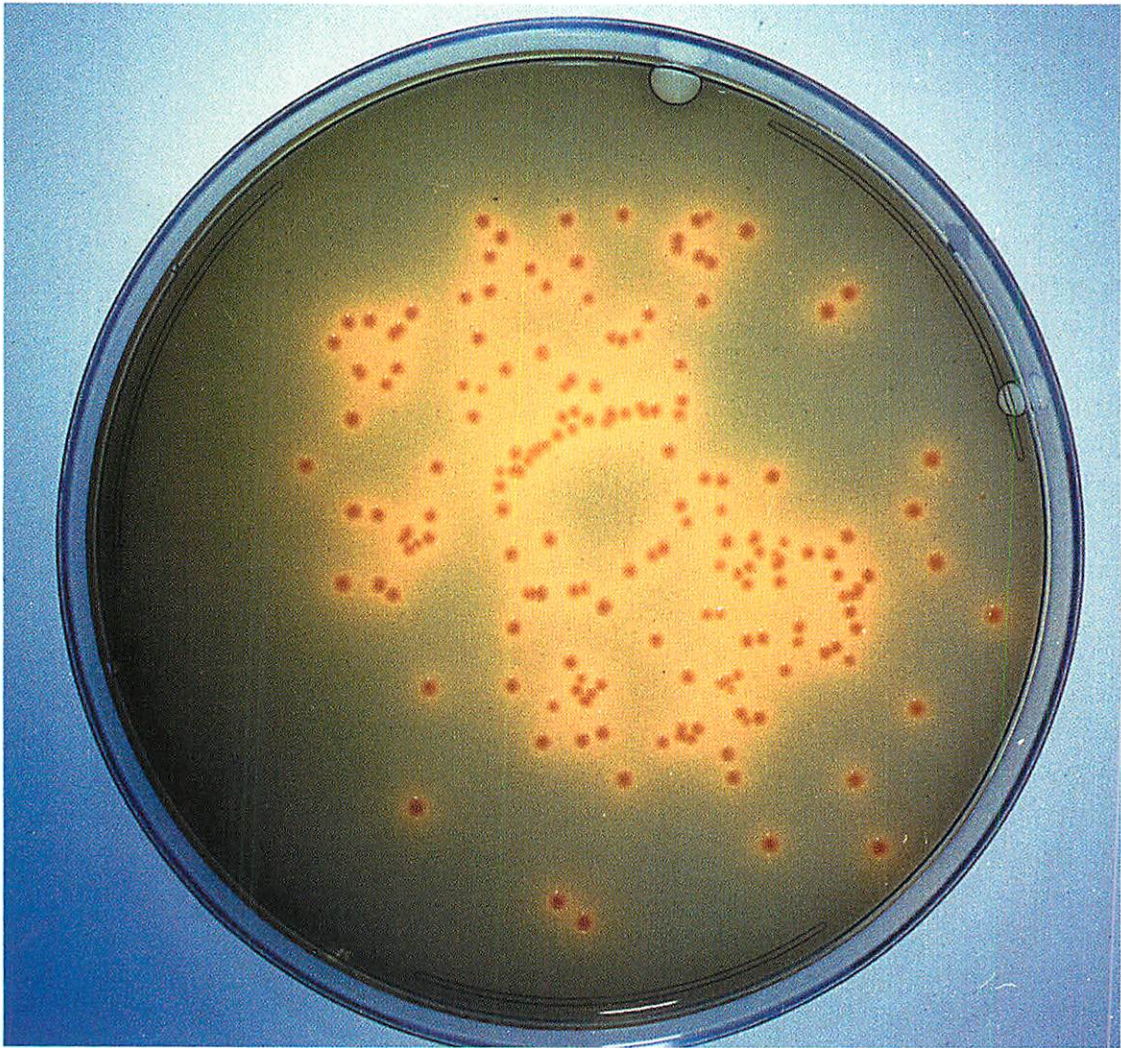

Figure 25. TCBS agar plate showing bacterial colonies.

working with other molluscan species rely instead on rigorous scrubbing of system surfaces with abrasive pads, thorough rinsing with freshwater, and then allowing the system to stand dry for several days.

The use of antibiotics to treat or prevent disease is not recommended for several reasons. First, it is illegal (FDA has not approved any antibiotics for use in seafood), and, second, routine use of antibiotics promotes the emergence of antibiotic-resistant strains of bacteria. Health studies surrounding the use of antibiotics in cattle feeds show that antibiotic-resistant strains which emerge in cattle can transfer their resistance to bacterial strains which are pathogenic in humans (for example, *Salmonella, Campylobacter, E. coli* O157, and some strains of *Enterococcus*).

C. GROW-OUT DISEASES

1. Diagnosis.

Post-set scallops may be affected by the same disease organisms as larvae; however, recognition of a problem is more difficult. A sample consisting of 15 - 30 scallops should be examined under a microscope several times each week to determine general health, percent survival, and growth. Pre-cleaned medicine droppers and other sampling equipment should be kept separate for each grow-out table and sterilized by heat or disinfectant after use. Notes should be maintained on each group of scallops on each grow-out table. Slow growth or the presence of red stain around the scallop are indicators of stress or death - possibly caused by disease (a red bacterium, a pseudomonad, may grow on the increased ammonia being released by a dying scallop, but the bacterium is not in itself pathogenic). If inspection reveals a high percentage of gaping scallops or empty shells, a severe disease problem may exist.

2. Treatment. Once scallops reach a size where they are placed in trays receiving flowing seawater as a food source, measures that can be taken to prevent disease become limited. Complete removal of biofilms from trays would require detachment and removal of scallops; this seems to retard scallop growth. Bi-weekly cleaning of the trays by washing away fecal material and other detritus with a mild stream of water is the preferred route. If fouling is a problem, the incoming seawater can be passed through a 50-μm bag-filter which will remove most of the fouling organisms, but allow algal food to pass through. If a severe disease problem occurs, the remaining scallops should be disinfected and discarded and the trays cleaned and disinfected with bleach as described in Section VI.5.B.2.

PATHOGEN CONTROL

- Routine washing and drying
- Separate equipment for each population
- Hot-water dip effective bactericide
- Filter and/or UV-treat seawater
- Spawn only healthy broodstock
- Check for *Vibrio* in algal food with TCBS agar if materials and expertise are available
- Quarantine any new scallops until health can be evaluated
- Discard diseased populations and disinfect all materials in contact

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X. SUPPLIERS GUIDE

Aquaculture Magazine. 2000. Buyer's Guide and Industry Directory 2000. 29th Annual Edition. Aquaculture Magazine, P. O. Box 2329, Asheville, NC 28802.

This guide provides the names of hundreds of vendors providing supplies, equipment, and services to the aquaculture industry.

APPENDIX A

QUANTIFYING MICROALGAL FEEDS

Unlike cells of metazoan plants and animals, microalgal cells separate from each other after dividing; therefore, as algal cultures "grow," you get more rather than bigger algae. For this reason, microalgal feeding rates for scallops must take into account the volume of algal culture fed to a population of scallops, how many cells are in that volume, and also how big cells of the algal species are. Three practical methods have been developed for quantifying how much algal food is in a given volume of culture: 1) microscope cell counts using a hemocytometer; 2) determination of packed-cell volume in a centrifuged, hematocrit-type tube; and 3) percent transmittance of light through a culture measured with a colorimeter/spectrophotometer. All three of these methods require collection of a sub-sample from the culture and determination of the number of cells in that sub-sample; therefore, it is critical that the culture be mixed thoroughly before sampling so that the subsample measured will be representative of the whole culture. Information provided in this manual facilitates use of cell counts or packed-cell volume to adjust feeding rates on a daily basis.
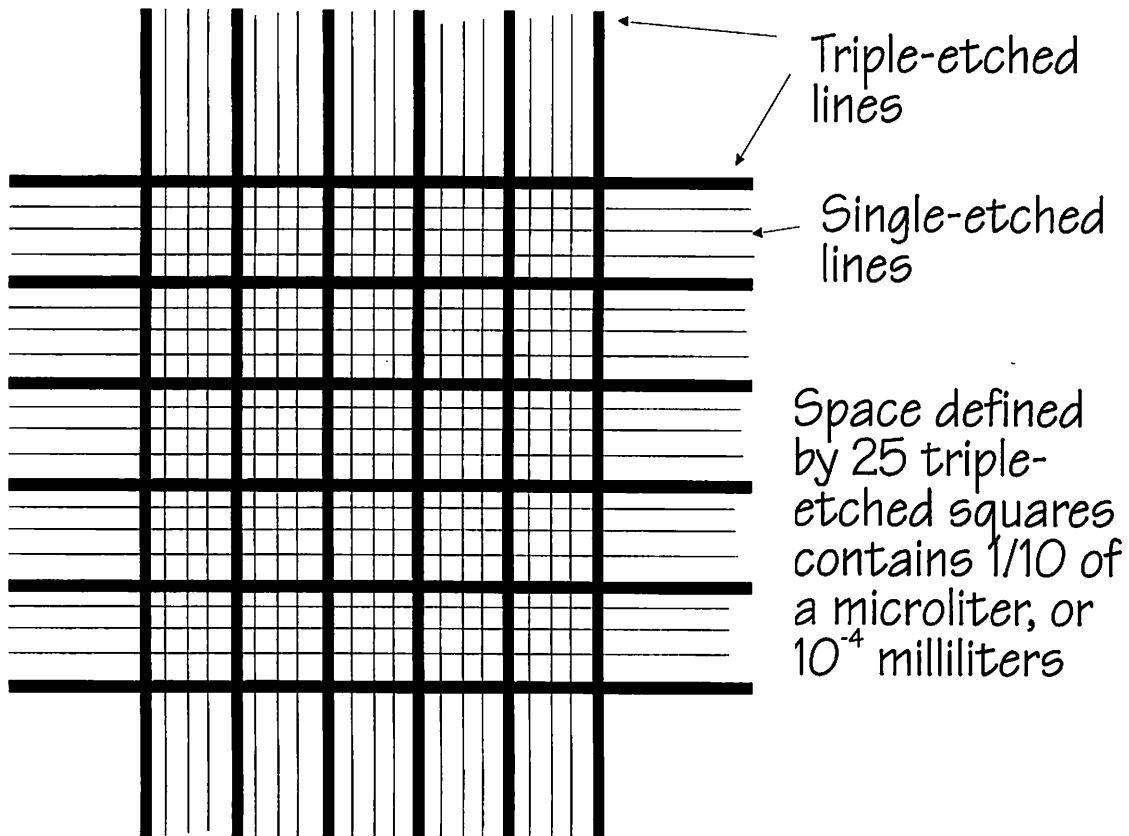
1) Cell Counting: a hemocytometer is a microscope slide designed for clinical counting of human blood cells. The principle of the hemocytometer is to define a three-dimensional space (volume) in which individual cells can be counted with a microscope; this volume is defined by a cover slip resting upon a platform above a polished glass surface with an etched grid. The etched grid defines the portion of the slide that is counted, and the cover slip limits the "depth" of water above the grid. A subsample is removed from the culture to be counted, killed with a small volume of formalin, Lugol's iodine solution, or iodine crystals (which we use so that dilution by fixing solution need not be calculated), and loaded by pipette into the hemocytometer. A compound light microscope is used to count cells at 100-400X total magnification.

Diagram of Hemocytometer
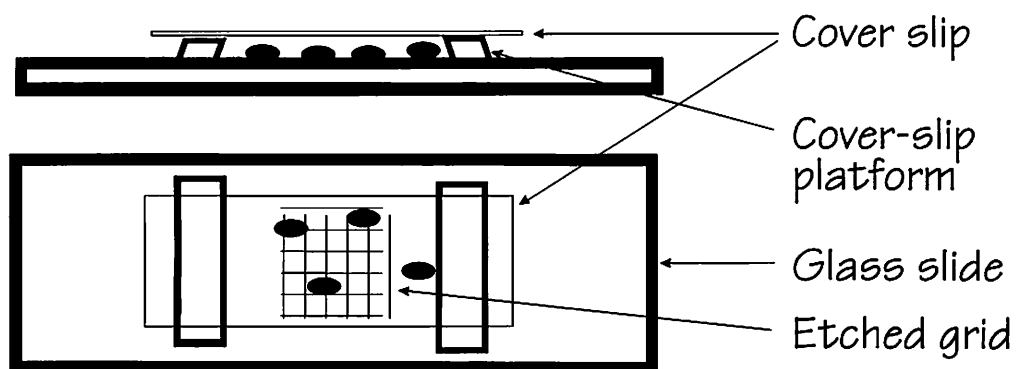
In the Improved Neubauer hemocytometer design that we use, a grid of 25 squares delineated by triple-etched lines contains 10 mL; each of these 25 squares is further subdivided by single lines into 16 squares to facilitate counting at higher magnifications. If all cells within the 25 squares are counted, then the
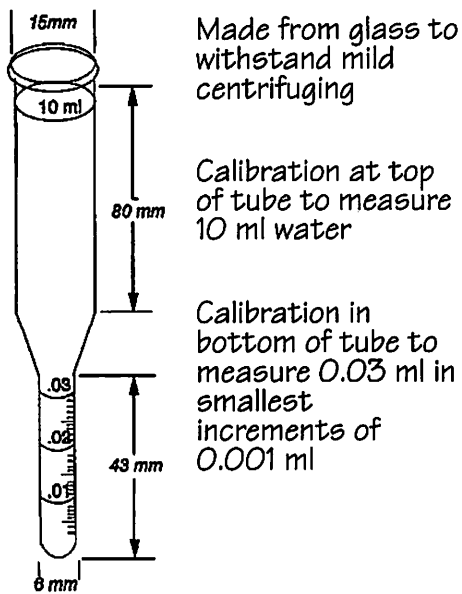
Hemocytometer Grid

Triple-etched lines

Single-etched lines

Space defined by 25 triple-etched squares contains 1/10 of a microliter, or 10^{-4} milliliters

count is multiplied by 10,000 (10^4) to obtain an estimate of the number of cells in one milliliter. In dense cultures, hundreds or thousands of cells may be present in 10^{-4} mL; in these cases, five of the 25 squares are counted and the count is multiplied by 5 X 10^4 to obtain the number of cells per milliliter. Counts between 30 and 300 recorded with a hand-held counter are considered valid. Advantages of this method include a high degree of precision and accuracy, forced microscopic evaluation of the culture for contaminants, and the ability to compare the culture with much of the published literature which generally reports algal densities in terms of cell number. The main disadvantages of counting cells are that the method is labor intensive (ca. 10 minutes per count), it requires a compound microscope, and hemocytometers are fragile and expensive to replace. Regardless of the method selected for daily quantification of algal feeds, it would be to the advantage of a hatchery operator to be able to do cell counts.

2) **Packed-cell volume (pcv):** when scallops feed on a relatively dilute suspension of microalgal cells in seawater, they essentially concentrate the cells ingested from the volume of water "filtered" to the volume of their digestive systems. Bivalve larvae will feed actively until their guts are full, spend some time digesting the "meal," expel undigested parts of the microalgae as feces, and then begin feeding again. Thus, the parameter that really defines how much a larva will eat is the volume of cells that it can pack

into its digestive system. Measuring packed-cell volume (pcv), which we define as mL of packed algal cells per 10 mL of algal culture, is the method that is most consistent with this view of its food. We employ a centrifuge tube developed to measure the volume of blood cells within a blood sample. This tube consists of a thin, capillary section with etched graduations attached to the bottom of a larger tube that holds 10 mL total volume.

Modified Hopkins tube, after Ukeles, 1973, for determining packed-cell volumes of phytoplankton cultures

Made from glass to withstand mild centrifuging

Calibration at top of tube to measure 10 ml water

Calibration in bottom of tube to measure 0.03 ml in smallest increments of 0.001 ml

15mm
10 ml
80 mm
.03
.02
43 mm
.01
6 mm

10-mL of algal suspension is measured into the tube, and the tube is centrifuged (600XG for 5 min.) so that all algal cells are "packed" into the capillary portion of the tube. The volume of cells, distinct in appearance from the now-clear medium, is read from the scale on the capillary portion of the tube. Packed algal cells are washed from the capillary using a wide-bore needle and syringe, and the tube is re-used indefinitely. Advantages of this method include the aforementioned relevance to the scallops, low labor as six or more samples can be centrifuged and read in less than 10 minutes, and an "automatic" adjustment for cell-size differences between algal strains. Disadvantages include the high cost of pcv tubes (which are no longer in medical use and must be custom-made), need for a centrifuge (although a small, inexpensive, table-top unit will do), and the inability of the method to differentiate between the alga being cultured and any particulate contaminants that may be present in the culture. We use packed cell volume measurements to adjust daily feeding rates for both larval and post-set scallops at Milford, chiefly because of the low labor required and the ability to equalize rations of algal strains of different size.

3) **Spectrophotometer/colorimeter**: it is easy to see that a culture containing more cells is "darker" than a less dense culture; light coming through a culture is absorbed and scattered by the algal cells in suspension. A spectrophotometer or colorimeter is an analytical instrument that measures the amount of light (usually a defined wavelength range) that passes through a cuvette (a specialized test tube). The simplicity of this principle is seductive, but practicality of application to production feed cultures tempers attractiveness of the method. Calibration of the instrument, calculation of useful values from numbers read on the instrument, and very high potential for interference from contaminants are drawbacks that make this method of quantifying algal-feed production cultures on a daily basis the least suitable. Those wishing to pursue this method further should consult Volume 1 of the *Handbook of Phycological Methods* (Stein, 1973) for detailed procedures.

APPENDIX B

STEP-WISE PROCEDURE FOR TESTING WATER OR FOOD FOR PRESENCE OF *VIBRIO* (SEE ALSO THE BOXED DIAGRAM)
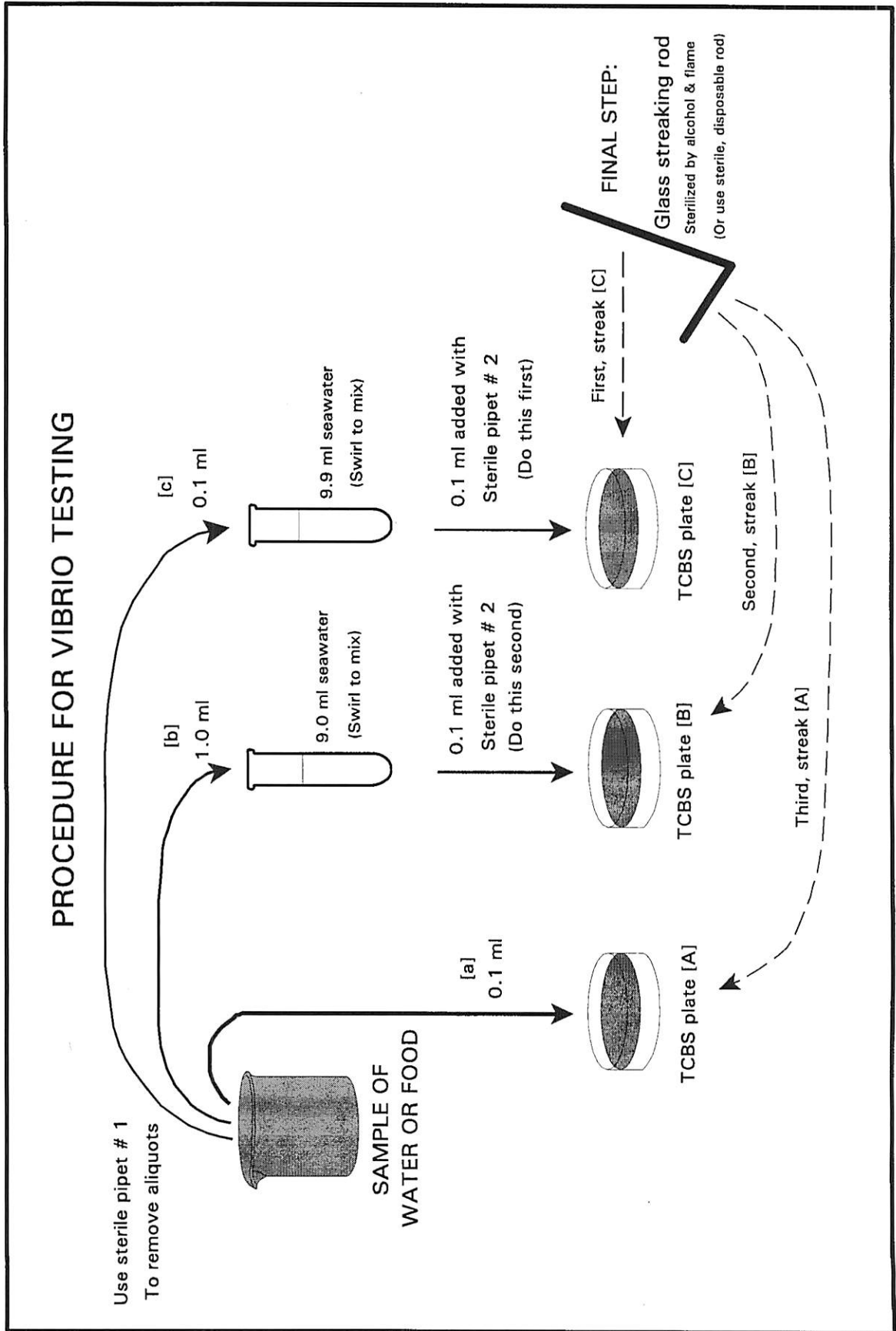
(The procedure is based on the use of a selective medium which suppresses growth of most other seawater bacteria.)

1. Prepare TCBS agar (Difco Laboratories) by dissolving the powder in distilled water that is heated to boiling according to the manufacturer's instructions; dispense into sterile Petri plates (about 10 mL per plate).

2. Allow the surface of the plates to dry overnight in a protected area, then stack the plates upside down in sealed, plastic bags and store in a refrigerator (this prevents condensation of water droplets on the lid of the plate).

3. With a sterile, 1.0-mL pipet, remove three samples (a), (b), and (c) of food or water according to the following: (a) add a 0.1-mL sample to the surface of a TCBS plate [A], (b) also add 1.0 mL to a sterile tube #1 containing 9.0-mL sterile seawater and mix the contents, and (c) add a 0.1-mL sample to a sterile tube #2 containing 9.9 mL of sterile seawater and mix the contents.

4. With a second sterile pipet, remove 0.1 mL from the tube #2 and place on the surface of a TCBS plate [C]; then with the same pipet remove 0.1 mL from tube #1 and place on the surface of a TCBS plate [B] (this is done with tube #2 first and then tube #1 because tube #2 has 10-fold fewer bacteria than tube #1); wetting the pipet first with the dilution in tube #2 will not bias the results when you then wet the same pipet with the higher number from tube #1.

5. Sterilize a bent, glass streaking-rod by dipping it in 70 - 95% ethanol and then burning it off by flaming it.

6. Cool the rod by touching it to the surface of plate [C] in an area free of the sample and then streak the sample smoothly across the surface of the plate while turning the plate. With the same rod, streak the sample on plate [B] and then on plate [A]. Note: If steps 4 and 5 (above) are not completed swiftly, the drop of sample on plate [A] may be absorbed into the medium and the bacteria will not be spread around the plate; if this happens, it is better to spread the drop on this plate with a sterile streaking rod immediately after placing it on the plate (i.e., during step 3).

STEP-WISE PROCEDURE FOR TESTING WATER OR FOOD FOR PRESENCE OF *VIBRIO*
continued from previous page

7. Cover the plates with their lids, and place them upside down in a plastic bag. After a 24-hour incubation at 68 - 78°F (20 - 26 °C), count the bacterial colonies on any plate having fewer than 300 colonies (statistically accurate counts are found only on plates containing 20 to 300 colonies). Count the clearly visible yellow or green colonies (ignore any tiny, nearly invisible colonies because these are non-*Vibrio* bacteria that are able to grow slowly on the medium despite its growth-suppressive ingredients).

8. Multiply the counts on plate [A] by 10, or plate [B] by 100, or plate [C] by 1000 to determine the number of bacteria per mL of food source.

9. Any TCBS plates containing bacteria should be autoclaved or boiled in water before they are discarded.

   Note: If the counts from step 7 (above) exceed 10,000 per mL, the food source or water may cause mortalities in scallop larvae. Some studies with other scallop species suggest that as few as 1,000 *Vibrio* per mL may cause problems. However, our experience in establishing open-tank, algal cultures suggests that initially vibrio counts in the food may reach as high as 4×10^5 per mL for several weeks. After the algal cultures become well-established, the counts drop to very low levels. Mortalities in scallops being fed *Vibrio*-contaminated food depends on the *Vibrio* strain that is present in the food. If mortalities occur, it is best to suspend feeding with that food source until *Vibrio* counts are low.

PROCEDURE FOR VIBRIO TESTING

Use sterile pipet # 1
To remove aliquots

SAMPLE OF
WATER OR FOOD

[c]
0.1 ml

[b]
1.0 ml

[a]
0.1 ml

9.9 ml seawater
(Swirl to mix)

9.0 ml seawater
(Swirl to mix)

0.1 ml added with
Sterile pipet # 2
(Do this first)

0.1 ml added with
Sterile pipet # 2
(Do this second)

First, streak [C]

Second, streak [B]

Third, streak [A]

TCBS plate [C]

TCBS plate [B]

TCBS plate [A]

FINAL STEP:

Glass streaking rod
Sterilized by alcohol & flame
(Or use sterile, disposable rod)

APPENDIX C

COMMON CELSIUS TO FAHRENHEIT EQUIVALENTS

°C	°F	°C	°F
40.0	104.0	20.0	68.0
39.0	102.2	19.0	66.2
38.0	100.4	18.0	64.4
37.0	98.6	17.0	62.6
36.0	96.8	16.0	60.8
35.0	95.0	15.0	59.0
34.0	93.2	14.0	57.2
33.0	91.4	13.0	55.4
32.0	89.6	12.0	53.6
31.0	87.8	11.0	51.8
30.0	86.0	10.0	50.0
29.0	84.2	9.0	48.2
28.0	82.4	8.0	46.4
27.0	80.6	7.0	44.6
26.0	78.8	6.0	42.8
25.0	77.0	5.0	41.8
24.0	75.2	4.0	39.2
23.0	73.4	3.0	37.2
22.0	71.6	2.0	35.6
21.0	69.8	1.0	33.8
		0	32.0

*Equivalents not shown can be calculated as follows:
$$°F = (°C \times 9/5) + 32$$