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Growing Microalgae to Feed Bivalve Larvae

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Introduction

Many bivalve hatcheries increase larval production by including an algal culture operation within the facility. Vats of dense algae provide mollusc larvae with a nutritious diet. The high quality diet accelerates growth and shortens the time to larval settlement or spat set. Production of algae in large amounts (mass cultures) is accomplished by providing a favorable environment for the species being cultured. Successful algal culture follows six basic steps:
1) sterilize the culture water
2) add nutrient enrichment
3) inoculate (or start) the new culture from a pure algal strain
4) make microscopic observations and cell counts
5) harvest algae and feed to larvae
6) maintain stock cultures

Background

Algal culture begins with a pure stock or starter culture of the algal species desired. These can be obtained from a number of sources. Commercial biological supply houses often sell algal cultures, but the species may not be suitable for use in your operation. Stock cultures of various species are often kept at government laboratories and universities. Perhaps the most common method of obtaining algae is to visit a working hatchery, since many hatchery operators will be glad to provide starter cultures to other culturists. Sources of algal species and culture supplies are listed near the end of this fact sheet.

Once obtained, starter cultures (usually transported in test tubes) are used to inoculate several new cultures. Some of these are kept as stocks for when an old culture dies, is harvested, or otherwise lost and must be restarted. The rest are used to inoculate progressively larger vessels until there is enough culture to start mass production tanks (Figure 1). The culturist must supply light, aeration, relatively stable temperature control, and sterile water with nutrients (media) to produce successful algal cultures. By avoiding major contamination from foreign algal species and microscopic predators, a continuous, dependable supply of high quality algae will be available.

The information in this guide can be used by hatchery operators to produce algal food for various bivalve organisms such as oysters, clams, scallops, or mussels.

Bivalve Mollusc Diets

Bivalves grow and develop primarily by consuming microscopic plants. These extremely small, free-floating, single-celled (unicellular) plants are called phytoplankton or microalgae. Individually, the plants are 2-10 micrometers (µm) or about 0.00008-0.00040 inches in size. They are invisible to the unaided eye. Their presence is apparent only when thousands of the algal cells come in close contact with each other and color the water green, red, or golden brown. A microscope with magnification of 400 power (400x) reveals their shape, size, abundance, and movement.

Developing Algal Stocks

There are thousands of microalgae that differ in size, color, shape, and habitat. Researchers have isolated and identified several species from natural waters that have proven to be nutritious food for growing some commercially important bivalves. *Pavlova* (*Monochrysis*), *Dicrateria*, *Thalassiosira*, *Isochrysis*, and *Isochrysis* (T-Iso strain) are used by personnel at the Frank M. Flowers & Sons Hatchery in Bayville, NY. Culturists with Ocean Pond Corporation in Fishers Island, NY use predominantly *Isochrysis* (T-Iso strain). Other species commonly used include *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Dunaliella tertiolecta*, *Nannochloris atomus*, *Tetraselmis suecia*, and some species of *Chlorella*. The University of Maryland's Horn Point oyster hatchery in Cambridge, MD has had success feeding *Isochrysis galbana* (T-Iso strain) and *Thalassiosira pseudonana* (strain 3h) to oysters and several species of clams. *I. galbana* is a five µm (micrometer) golden brown, spherical cell. It possesses two hair-like flagella at its anterior end for mo-

Stock Culture

Starter Culture 20 mLs

1 L flask

Light

7 - 14 days

500 mLs algae & medium

Five Gallon Bottle Culture

Nutrients

1 L flask

Light

17 L bottle

200,000 cells/mL

4 - 14 days

4,000,000 to 12,000,000 cells/mL

Tank Mass Culture

17 L bottle

Nutrients

Light

2 - 4 days

900 Liters available for food or inoculum

Figure 1. Culture of algae in volume.

tility. *T. pseudonana* is a 4 µm, non-motile, golden brown, barrel-shaped cell.

Algal Growth

Under favorable conditions, unicellular algae grow continuously by a process known as cell division. Each cell enlarges and divides into two daughter cells that subsequently grow and divide yielding a culture that increases exponentially (e.g., 8, 16, 32, 64...etc.).

Growth slows as the algal population becomes more crowded. Nutrients are depleted, metabolites build, and light penetration decreases because of self-shading. The cultures have reached their stationary phase (Figure 2) for the current conditions and will not increase in density. Algae harvested near this maximum density are a high quality food and provide the most efficient use of hatchery labor and space.

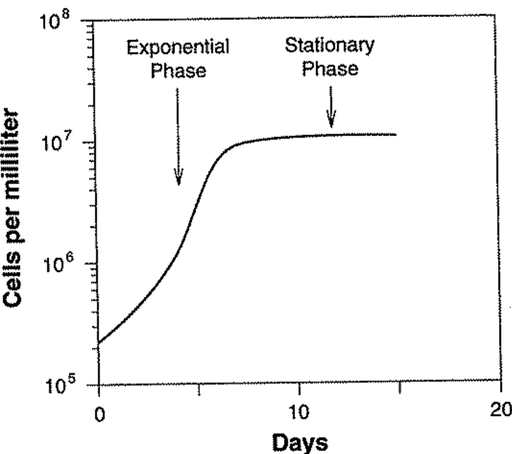


Figure 2. Algal growth when cultured.

The amount of time for algae to divide and the maximum density attained depend upon several factors:

- species
- water temperature
- salinity
- light intensity
- nutrients
- CO_2 aeration
- vessel size
- presence of microscopic, predaceous contaminants

Growth of unicellular algae can be measured in terms of cell numbers in a given volume of water. In natural waters, algal density rarely exceeds a few thousand cells per milliliter (one mL is equal to about 20 drops from an eye dropper). In a controlled hatchery environment, however, a single algal species or "monoculture" of *Isochrysis galbana* can grow from 200,000 cells/mL to four million cells/mL in three days.

Preparatory Steps for Culturing Algae

The first step in algal culture is the preparation of growth media. Media are clean, nutrient enriched, sterilized solutions of estuarine or sea water. The water should be approximately the same salinity as the water in which the

bivalves are grown. Water collected from a healthy shellfish nursery area should provide adequate media for growing algae. However, aged water (one week or more) or water from a salt water well are superior for algal culture compared to freshly collected estuarine water. It is generally not practical to store large volumes of water, but a small supply for stock cultures can be kept on hand.

To make media, water clouded with silt and debris should first be cleared by filtering or settling in a holding tank, preferably in the dark, for several days. Filter pore size is determined by the amount and size of the debris in the water column. For example, polypropylene cartridge filters with a one or two µm pore size are required to remove small suspended clay particles. Hatchery operators at many locations may find such fine filtering unnecessary. For instance, the F. M. Flower's hatchery pumps silt-free, aged water from a salt water well.

Once collected, the water should be sterilized. Small amounts (one pint or less) of saline water are sterilized in heat-resistant, loosely covered containers. A pressure cooker or autoclave is commonly used with a sustained temperature of 121° C (250° F) at 15 p.s.i. for 15 minutes. An alternative method is to boil the container, including the seawater, either in a double boiler or directly on a burner for half an hour. Either method effectively kills all living organisms present and destroys spores or resting stages. Some culturists have used microwave ovens to successfully sterilize small amounts of water. Other culturists sterilize saline water by passing it through a filter with a 0.45µm or smaller pore size into a sterile vessel. These small, sterilized containers and waters are used for stock cultures.

Boiling saline water may cause precipitates to form which inhibit algal growth. This phenomenon is usually more prevalent in higher salinity water (≥ 30 parts per thousand). A better method uses heat pasteurization to kill unwanted microorganisms. The saline water is heated to 80°C (176°F) and allowed to cool naturally to room temperature. Spores can be killed by heating and cooling the water a second time. If the water is not used soon after cooling care should be taken to insure against contamination. Hatcheries in locations where heavy silt loads are not a problem may use a filtration system to sterilize water mechanically. Filters with an absolute pore size of one or two µm should be used. Filter sterilization could eliminate heating and chemical costs.

A more practical sterilizing technique for large volumes (one gallon or more) of saline water requires the use of common chemicals. Either laundry bleach (sodium hypochlorite) at 0.5 milliliters per liter (mLs/L) of culture water or hydrochloric acid, also called muriatic acid, can be used (Table 1). Once combined, the water and chemical mixture is left for 6-24 hours. Treatment with bleach or acid renders the water "clean" enough for an algal inoculum.

The acid is deactivated with sodium bicarbonate (baking soda; Table 2). The bleach is deactivated with 0.1-0.15 mLs (for each liter of chlorinated water) of a sodium thiosulfate (248 grams/L) solution and aerated for two or more hours. The presence of chlorine should be checked. Inexpensive test kits are available from pool supply stores.

Table 1: Acid Sterilization Schedule

Amount of Water in Vessel Solutions of	Concentrated Acid Added: Milliliters	Concentrated Acid Added: U.S.Measure
1 Liter (1 Quart)	0.2	4 Drops
5 Liters (1.3 Gallons)	0.9	18 Drops
17 Liters (4.5 Gallons)	3.0	1/2 Tsp.
190 Liters (50 Gallons)	33.5	2 Tbsp.
380 Liters (100 Gallons)	67.0	2.3 Fl. Oz.
900 Liters (240 Gallons)	160.0	5.5 Fl. Oz.

Table 2: Chemical Neutralization Schedule for Two Algae

Amount of Acidified Water in Vessel	Sodium Bicarbonate Added to Cultures			
	T. pseudonana		*I. galbana*	
	Grams	U.S. Measure	Grams	U.S. Measure
1 Liter	0.4	1/8 Tsp.	0.9	1/8 Tsp.*
5 Liters	1.3	1/4 Tsp.	4.5	1 Tsp.
17 Liters	4.3	1 Tsp.	15.5	1 Tbsp.
190 Liters	47.5	3 Tbsp.	171.0	3/4 Cup
380 Liters	95.0	1/3 Cup	342.0	1 1/3 Cups
900 Liters	250.0	1 Cup	950.0	3 1/2 Cups

*Heaping

Acid sterilization lowers the pH of water to 3.6 or less. Occasionally a bicarbonate residue is left on the walls of the vessel from a previous culture. This requires an increase in the amount of acid, and a check with a pH meter to ensure the desired pH is attained. To avoid contamination, the treated water and interior of the container must not contact anything except the seed algae and sterile nutrients.

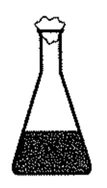
After sterilization, the water is enriched to provide the cultured algae with nutrients needed for rapid growth to high densities. There are numerous recipes available for the preparation of algal nutrients. The one used depends on nutrient levels in local, ambient water and the algal species cultured. Many algae grow well with Guillard's f/2 nutrients (Figure 3). Guillard's f/2 nutrients are used at the rate of 2 mLs for each liter of algae cultured. The trace metal and vitamin solutions are saved to spike 10 batches of primary f/2 solution as needed. Guillard's f/2 and other nutrients can also be purchased ready-to-use from suppliers listed near the end of this guide.

Experienced culturists can modify a recipe to increase growth or reduce cost. For example, vitamins, an expensive ingredient, can sometimes be omitted from mass tank cultures. Some hatcheries have used off-the-shelf fertilizer, a practice that may not produce consistently reliable growth. Fertilizers should be compared and those containing high concentrations of heavy metals should be avoided. Diatoms, including *T. pseudonana*, are enhanced in some locations with the addition of 0.03 grams of sodium silicate per liter of media. Typically a separate solution (30 grams/L) of sodium silicate is mixed and added to di-

1. Primary solution

To 1 liter of distilled or tap water, add:

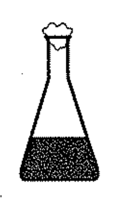
Chemical	*Grams*
sodium nitrate ($NaNO_3$)	75.0
sodium phosphate (NaH_2PO_4)	5.0
iron sequestrene (Fe-EDTA)	5.0
boric acid (H_3BO_3)	2.5



2. Trace metal solution

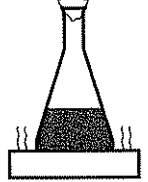
To 1 liter of distilled or tap water, add:

Chemical	*Milligrams*
cupric sulphate ($CuSO_4$)	98.0
cobalt chloride ($CoCl_2$)	10.0
manganous chloride ($MnCl_2$)	1800.0
sodium molybdate (Na_2MoO_4)	63.0
zinc sulphate ($ZnSO_4$)	22.0



3. Add 10 mLs of **trace metals solution** to primary solution.

4. Heat sterilize and cool mixture to room temperature.



5. Vitamin solution

To 10 mLs of distilled or tap water, add:

Chemical	*Milligrams*
vitamin B12	1.5
biotin	1.5
thiamine hydrochloride	167.5

6. Add 1 mL of **vitamin solution** to primary solution.

Figure 3. Guillard's modified f/2 nutrient enrichment.

atom cultures (15mLs/5 gallons). *I. galbana* mass cultures grow well on Stern's Miracid™ (plant food) alone, used at the rate of 1.3 oz. per 240 gallons (0.04 grams/L).

The algal inoculum or seed is added soon after the nutrients and chemical neutralizer. The algae are not harmed even though the baking soda will not be completely dissolved for several hours. Ordinarily enough algae are added to produce a strong tint in the water (100,000-200,000 cells/mL). More inoculum is used if peak density is desired quicker than usual. The culture is illuminated and may be aerated.

Sunlight, fluorescent, VHO fluorescent, and metal halide lights can be used either singly or in various combinations to provide light. Special "plant grow" lights do not increase algal production. A tank eight feet long, four feet wide, and one foot deep can accommodate 16, eight-foot fluorescent tubes, and yield dense cultures. Several smaller cultures in pint, quart, or five gallon bottles can be placed in front of four-foot, two or four bulb (40 watt each) fluorescent tubes. Generally, the more light the greater the final peak algal densities. Decreasing the depth of algae in a mass tank culture can have the same effect as higher light intensity. Low density cultures, such as fresh inoculations of *I. galbana* , (less than approximately 50,000 cells/ mL) require much lower light levels initially. This can be accomplished by using window screening to shade some sunlight, by turning off some bulbs for the first 24 hours, or increasing the distance between the tubes and the cultures. Fluorescent 2-bulb light fixtures are commercially available in various lengths (2, 4, and 8 feet). They can be purchased according to the size tanks that are to be illuminated.

Stock Culture Maintenance

Stock cultures are kept on hand for initial start-up and as a source of "clean" algae when the mass tank cultures or the smaller intermediate cultures become contaminated, die, or are harvested.

Stock cultures are usually kept in heat-resistant, transparent test tubes or conical flasks. Flasks are available in sizes ranging from 1.7 fl. oz. to 4 quarts (50 to 4,000 mLs). The culturist can choose the size that is most convenient and practical for his operation. An adequate volume (usually 250 mLs or more) of stock culture must be available for the next step in vessel size. Air is not bubbled into stock flasks, thus eliminating one potential source of contamination. Each flask is half filled (or less) with media. Partially filled flasks provide a larger surface area for gas exchange than full flasks. Good gas exchange increases the prospect of stable, long-lived, and healthy stock cultures.

Many hatcheries keep back-up stock cultures. Some are kept on-site in duplicate culture chambers. Others are kept at locations remote from the hatchery. In this way problems with equipment, power failures, unexplained crashes, and other unforeseen difficulties can be minimized.

By following a strict transfer regime, pure algal monocultures can be kept healthy and used indefinitely to seed new cultures. For example, once a week, one drop of *T. pseudonana* inoculant is aseptically transferred to media in a newly sterilized, 1-liter flask. A *I. galbana* inoculant is typically transferred once every two weeks. The amount transferred and frequency depends on the needs of the hatchery.

Mass Cultures

Stock cultures are used to inoculate intermediate-sized cultures. Many hatcheries use five gallon bottle (19 L) cultures as a step between flask stock cultures and large mass tank cultures. These bottles are available in either plastic (polycarbonate) or glass. Glass bottles are heavy and breakable, but can be autoclaved without ill effects. Light-weight plastic bottles have seams that could develop leaks, particularly if autoclaved. Other intermediate-sized vessels such as translucent 2.5 gallon bottles, plastic one gallon milk jugs or polyethylene bags can be used. Polyethylene material for constructing bags can be purchased as tubing (diameters from 1.5 - 58 inches). The ends can be heat sealed to any convenient length. They require external support, but are disposable and don't need to be sterilized when first used. Many hatcheries are using this technique to mass culture algae.

For a small, laboratory-sized hatchery, these containers may provide enough algae to satisfy production needs. Commercial-size bivalve hatcheries require mass tank cultures containing 25-5,000 gallons (100-20,000 L) or more of quality algae. In these operations, mass tank cultures are typically started from healthy bottle cultures.

Tank and bottle cultures should be provided with the most favorable environment possible. Deviating from ideal conditions will reduce algal growth rate. Lower light levels or reliance on natural sunlight could be used to reduce electrical costs, but with a concurrent decrease in production. Some hatcheries eliminate aeration to reduce the spread of biological contaminants. Departures from optimal conditions may be necessary for other reasons. For example, if several algal species are cultured in one room, a compromise in environmental conditions may be necessary. A cooler temperature, such as 65° F, would increase the time needed to attain peak density for some species, but may eliminate a biological contaminant.

Inoculum sufficient for a bottle produces a visible tint in the water. For example, one or two pints (500-1,000 mLs) would be an adequate inoculum for a five gallon bottle. Under favorable conditions, a newly inoculated *I. galbana* culture in a five gallon (19 L) bottle takes 10-14 days to reach peak density (10-12 million cells/mL). The faster growing *T. pseudonana* reaches its peak (four million/mL) in about three days. Once maximum density is attained, healthy *I. galbana* bottle cultures are stable for two additional weeks. Cultures of *T. pseudonana* will deteriorate within five days once reaching maximum density. Growth rate and culture stability of most other algae fall between these two.

Using a larger volume of inoculum will decrease the time for a culture to reach peak density. For example, a half gallon (2 L) inoculum instead of one pint (500 mLs) would shorten by 30-50% the time a five gallon (19 L) bottle needs to reach peak density. Algal growth could be increased by bubbling in a mixture of air and 10% carbon dioxide instead of air alone. Fast growing cultures could be enhanced with carbon dioxide pulsed in several times a day, keeping the pH below 10. Carbon dioxide is available in pressurized bottles from bottled air suppliers in most communities.

A dense (10 million cells/mL), healthy five gallon (19 L) bottle culture contains enough inoculum to seed a 240 gallon (900 L) mass tank culture. At lower densities (five million cells/mL, for example), two or more bottles would be necessary to produce the same amount of algal inoculum. Larger tank cultures would benefit from a proportionally larger inoculum. Mass cultures in 240 gallon tanks (8'x 4'x 1') should reach peak density in 2-3 days when configured as follows:

1) five gallons of inoculum
2) six air outlets @ 3 L/minute
3) ten 8-foot cool-white fluorescent lights
4) 73° F temperature
5) 36 grams Stern's Miracid™

Table 3 compares the general growth parameters for *I. galbana* and *T. pseudonana*. The mass tank cultures should be harvested near peak density and before biological contamination becomes a problem. Some contaminants, as explained in a later section, will quickly destroy the algae.

Two algal harvesting methods can be used: 1) batch and 2) semi-continuous. With the batch method, a total harvest occurs at once or over several days. The tank or bottle is then cleaned, refilled and prepared for a new inoculum. In the semi-continuous method, part of the algae remains in the vessel and serves as an inoculum. New media is added to replenish the amount of algal suspension removed. The semi-continuous technique works well with diatoms (*T. pseudonana*, *Chaetoceros* spp.). Diatoms outgrow foreign algae and many other biological contaminants. The most appropriate harvest method will depend on the water quality and the algal species cultured. A salt water well typically provides the best media for the semi-continuous method.

Table 3: Comparison of Several Growth Parameters for Two Algal Species

T. pseudonana	*I. galbana*
73° - 85° F	73° - 85° F
Guillard's f/2 or equivalent	Stern's Miracid™ and vitamins
sterilize — 0.18 mL/L HCL	sterilize — 0.18 mL/L HCl
neutralize — 0.25-0.28 g/L sodium bicarbonate	neutralize — 0.89-1.2 g/L sodium bicarbonate
constant high light intensity	low light for weak inoculum (<50,000 cells/mL)

Determining Algal Densities

Monitoring algal growth in mass tank cultures is essential for successful production. Cultures that have stopped growing are either:

1) significantly contaminated with microscopic predators and/or competitors, or

2) have reached maximum cell density under the present conditions.

A general gauge of algal growth is the daily darkening color as the cells multiply. A more accurate measure of growth can be made by comparing actual cell numbers with the previous day's count.

The number of cells can be determined by studying a sample from a culture under a 400X microscope. The sample is placed on a special slide called a hemacytometer. These slides are available from scientific or medical supply stores and come with instructions. To use the hemacytometer, place a pipet filled with a sample from the culture tank against the "V" groove of the slide (a cover slip must be in place). Withdraw the pipet before the sample runs off the flat grid area, but not before the grid area is completely covered with the algal suspension. Wait about five minutes for the cells to drift onto the grid. The grid area resembles the pound symbol (#) inside a square border so that there are nine equal squares. Each of the four corner squares are further divided into 16 smaller squares. The total number of algal cells in all 16 small squares in the four corner areas (64 total small sqs.) are counted. Divide this number by four, for an average, and multiply by 10,000 (hemacytometer volume factor). The product is equal to the number of cells per mL in the sample. Motile algae like *I. galbana*, must be killed before counting. A weak toxin such as acetic acid or ethanol may be used (one drop into 20-30 mLs of algal sample). Dense cultures (>3 million) should be diluted 1:10 to facilitate counting and to reduce error. Remember to multiply by 10 (for a 10:1 dilution) or by the appropriate correction factor if a different dilution is made. There are numerous other methods to quantify algal densities, however using a microscope and hemacytometer, will reveal the presence of biological contaminants.

Peak or maximum density (for existing conditions) is attained when, on consecutive days, the cell count does not increase by a factor of two (Figure 2). With practice, approximate cell counts can be estimated by observing the gross color of each culture. These estimates are based on the tint of the water caused by the algal cells and comparing it to the actual cell count. Tedious and time consuming cell counts (or other growth measurements) can be eliminated from the daily routine. Cell counts should be made periodically as a check on the gross estimate.

Feeding Bivalve Larvae

As the cultures reach their peak density and are used, new cultures are inoculated as replacements. A total of 15 bottles (five gallon) of *I. galbana* cultured over three weeks are needed to grow five million oyster larvae from eggs to "setting." This assumes a peak algal density of 10-12 million cells/mL and a larval density of 5/mL. Many factors including food, salinity, brood stock, disease, and water quality affect the growth and survival of each brood.

Ideally, larvae are provided with a continuous supply of cultured microalgae until they are ready for setting. (At setting the bivalve larvae metamorphose into the adult form.) Feeding levels should be adjusted according to the density and grazing activity of larvae. Larvae (5-10 organisms/mL) up to one week old, grow well when initial algal concentrations in the tank proximate 50,000 cells/mL. Doubling or halving this density during week one produces no discernible growth differences. A daily adjustment to approximately 100,000 algal cells/mL for larvae over one week old is adequate. Near the onset of setting, feed requirements of bivalve larvae increase. Algal densities are raised to 200,000 cells/mL. Higher algal densities do not increase growth and may be detrimental to the larvae. A mixture of several algal species is frequently used to enhance growth. Generally, sufficient algae is added so the tank will not be completely cleared in 24 hours.

Algal concentrations in the larval tanks can be monitored with a hemacytometer as described above. With experience, feeding can be accomplished by the visual or "eyeball" method (observance of the water tint caused by the addition of algae). Novice culturists can use the following formula as a guide to determine the volume of algae (5μm algal cell size) to add:

$$\text{Liters to feed} = \frac{TD \times V}{CD}$$

Where TD equals the algal Target Density in the larval tank (in thousands of cells/mL); V is the Volume of the larval tank (in thousands of L); CD is the Cell Density of the algae feed tank (in millions of cells/mL).

Example 1. To feed a population of larvae 50,000 cells/mL in a 6,000 liter tank from an algal culture with 3.5 million cells/mL would require how many liters of algae?

$$\frac{50 \text{ cells/mL} \times 6 \text{ L}}{3.5 \text{ cells/mL}} = 86 \text{ L}$$

Example 2. To feed larvae at the rate of 200,000 cells/mL in an 8 L container from an algal culture with 12 million cells/mL would require how much algae?

$$\frac{200 \text{ cells/mL} \times 0.008 \text{ L}}{12 \text{ cells/mL}} = 0.133 \text{ L}$$

Trouble Shooting Biological Contamination

Contamination of cultures by live microscopic predators and competitors is a problem that cannot always be controlled successfully. The 2.5-5 gallon bottles and small stock cultures can be isolated from live, airborne contaminants (such as alien algae, bacteria, and predatory protozoans). A cotton plug, aluminum foil, plastic wrap, or a

screw cap can be used to seal the top. The large, open mass tank cultures cannot be covered as readily as small-mouth containers. Mass tank cultures are easily contaminated from non-sterile containers or splashed water from buckets, hoses, or workers hands. Another source of contamination may be in the media itself. Chemical sterilization does not kill the resting or spore stage of all microscopic organisms. When the media is initially readied for the algae, the environment also becomes favorable for the hardy spores of any organism that is present. They begin to proliferate along with the inoculated algae. For this reason autoclaved water is the preferred sterilizing technique for stock cultures.

A quick microscopic examination of suspect, "different looking" cultures enables the culturist to detect biological contaminants. The goal is to determine if an algal die-off (crash) is likely. Knowing the status of the growing algae helps the culturist to decide when the next mass culture should be started. Common contaminants include unwanted algal species, bacteria, and protozoans. Many flagellated protozoans can have a devastating effect on an algal culture. They engulf and consume individual algal cells, while their population grows exponentially (eg., 2, 4, 8, 16, ... etc.), similar to algae.

An algal culture that is contaminated with certain species of protozoans (such as *Monas* spp.) is likely to be completely destroyed within 12 to 18 hours of first detection. Diligent monitoring of the culture with a 400 power microscope is the only way to be certain of its presence. Even with 90% of the algal cells destroyed, the culture can retain a brown tint similar to a healthy algal culture. An algal "crash" caused by this protozoa renders the culture useless as larval food. If inadvertently used as mollusc food, the larval growth rate will be severely hindered. Not only are the protozoa very efficient at eliminating the algae, they also offer little or no nutritional value to the larvae. Algal cultures heavily infested by *Monas* are best dumped.

Although *T. pseudonana* cells are attacked by these protozoans, they are not as susceptible as *I. galbana* cells. An algal culture with a protozoan invasion should be discarded or used as larval food only as a last resort. Inoculating a new culture with a protozoan-contaminated culture is counterproductive.

The *Monas* culprit is identified by its pale color, larger size (11µm, spherical, or pear shape), and slower, sometimes circular movement. One or more algal cells can usually be seen within its cell membrane.

Cultures that become contaminated with foreign algae, bacteria, or organisms other than protozoans do not usually suffer a complete algal "crash." These cultures grow slower and reach a lower final density than that of pure monocultures. Such cultures should not be used as an inoculum, but are suitable as larval food with a somewhat reduced nutritional value. Slow growing foreign algae, causing minor contamination, can generally be ignored. Cultures that become overrun with blue-green algae, which offer little nutrition to immature molluscs, should be discarded to make room for new, pure cultures. Diatoms and dinoflagellates larger than about 25 µm are too large for most bivalve larvae to ingest.

Bacteria, present in small numbers, are unavoidable and normally do not adversely affect the algae or larvae. Ingested bacteria probably play a positive role in the nutrition of the larvae. Large bacterial populations are associated with senescent (old, non-growing) algal cultures. Two methods are effective in avoiding senescent cultures: 1) adding fresh media to the culture or 2) transferring some of the algal solution to new media in a separate, sterile vessel.

A vigorous effort to avoid contamination should be the top priority of the mass algal operation. This is accomplished by keeping all equipment meticulously clean (chemically and biologically) and by avoiding introduction of any foreign substance into the cultures. The rule is: start with a pure inoculum, grow the algae under the most ideal conditions possible, and use the algae before biological contaminants become established. Early detection of protozoans by microscopic examination enables the culturist to avoid the unwitting use of contaminated algae.

Summary of Steps to Culture Microalgae

Step 1. Collect, filter and sterilize water and culture vessel
Step 2. Neutralize the water (for chemical sterilization)
Step 3. Prepare nutrients and add to the water (media)
Step 4. Inoculate the media with appropriate algae
Step 5. Monitor algal culture for live contaminants and peak density
Step 6. Harvest algal food or use as inoculum for a new culture
Step 7. Re-bloom culture or clean, re-sterilize, and inoculate

Equipment & Supplies

The basic equipment and resources required to culture microalgae successfully include:

- numerous culture vessels of different sizes
- light
- pure algal stock
- seawater (or brackish water)
- seawater pump
- blower or other aeration equipment
- temperature control
- chemicals
- balance
- hot plate or autoclave
- salinometer, hydrometer, or refractometer
- pH meter
- hemacytometer
- microscope with 400 power magnification
- suitable space
- knowledgeable staff

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Smith, W.L. and M.H. Chanley (eds.). 1975. Culture of Marine Invertebrate Animals. Plenum Press, New York.

Stein, J.R.. 1973 Handbook of Phycological Methods, Cambridge University Press, Cambridge, England.

Sources of Supplies and Information

Aqua Fauna Bio-Marine, Inc
P.O. Box 5
Hawthorne, CA 90250
213-973-5275
general lab supplies, chemicals, f/2 nutrients

Bradley's Plastic BAg Co.
9130 Firestone Blvd.
Downey, CA 90241
800-322- 8775
Polyethylene tubing and liners

Carolina Biological Supply Company
2700 York Rd.
Burlington, NC 27215
919-584-0381
algal cultures, media recipes

Center for Culture of Marine Phytoplankton
Bigelow Laboratory for Ocean Sciences
West Boothbay Harbor, ME 04575
207-633-2173
algal cultures

CIBA-GEIGY Corporation
Agriculture Division
P.O. Box 18300
Greensboro, NC 27419
800-334-9481
NaFe Sequestrene

Florida Aqua Farms
5532 Old Saint Joe Rd.
Dade City, FL 33525
904-567-8540
algal cultures, f/2, general lab supplies, culture manual

Frank M. Flower & Sons, Inc
Att. David Relyea
P.O. Box 1436
Bayville, NY 11709
516-628-2077
algal cultures, trouble shooting

Fritz Scientific
P.O. Drawer 17040
Dallas, TX 75217
800-527-1323
Guillard f/2 nutrients, general lab supplies

National Renewable Energy Laboratory
1617 Cole Blvd.
Golden, CO 80401
303-231-1000
algal cultures

University of Maryland
Horn Point Environmental Laboratory
Att. Garry Baptist
P. O. Box 775
Cambridge, MD 21631
410-228-8200
algal cultures, trouble shooting

US Department of Agriculture
National Agricultural Library
Beltsville, MD 20705-2351
301-344-3755
mollusc culture, bibliography

VWR Scientific
general lab materials
P. O. Box 8188
Philadelphia, PA 19101
609-467-3333
chemicals, microscopes

Waco Associates Inc.
12th & Forest Street
Conshohocken, PA 19428
215-825-3300
filter cartridges, filter housings