CBCS 3RD SEM SEC : CODE-ZOO-SE-3014 ORNAMENTAL FISH AND FISHERY

UNIT:10 PURE CULTURE OF PLANKTON

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PLANKTONS ARE MAINLY 2 TYPES:

1. **PHYTOPLANKTONS (PLANT)**
2. **ZOOPLANKTONS:(ANIMALS)**

Phytoplankton Culture for Aquaculture Feed

Phytoplankton consists of one-celled marine and freshwater microalgae and other plant-like organisms. They are used in the production of pharmaceuticals, diet supplements, pigments, and biofuels, and also used as feeds in aquaculture. Phytoplankton are cultured to feed bivalve molluscs (all life stages), the early larval stages of crustaceans, and the zooplankton (e.g., rotifers, copepods) that are used as live food in fish hatcheries.

Flagellates and diatoms are two important types of phytoplankton at the base of the food chain. They manufacture cellular components through the process of photosynthesis, taking up carbon dioxide and nutrients from the water and using light as an energy source.

The microalgae used as feed in hatcheries vary in size, environmental requirements, growth rate, and nutritional value (Fig. 1, Tables 1 and 2) (Helm et al., 2004). When selecting a species for culture, it is important to take all of these parameters into consideration. Most hatcheries grow a variety of species that serve different needs throughout the production cycle with respect to size, digestibility, culture characteristics, and nutritional value (Muller-Feuga et al., 2003).

Culture conditions can vary widely—from outdoor ponds or raceways with nutrients added to promote a bloom of the natural microalgae, to monocultures reared indoors under controlled environmental conditions. This paper focuses on the monoculture of microalgae under clearly defined environmental conditions and production protocols.

Cell Volume, Organic Weight, and Gross Lipid Content of Some Commonly Cultured Phytoplankton Species used in Bivalve Mollusc and Fish Hatcheries (Helm et al., 2004).

Species	Median cell volume (μm^3)	Organic weight (pg)	Lipid content (%)
FLAGELLATES			
Tetraselmis suecica	300	200	6
*Dunaliella tertiolecta**	170	85	21
Isochrysis galbana (T-ISO)	40 – 50	19 – 24	20 - 24
DIATOMS			
Chaetoceros calcitrans	35	7	17
Chaetoceros gracilis	80	30	19
Thalassiosira pseudonana	45	22	24
Skeletonema costatum	85	29	12
*Phaeodactylum tricornutum**	40	23	12

Micro algal culture facilities typically use seawater enriched with nutrients—primarily nitrates, phosphates, essential trace elements, vitamins, and, in the case of diatoms, silicates. Water used to culture microalgae should have similar chemical composition to that used to culture the animals, and it should be pretreated. Some laboratories use synthetic seawater for small-scale cultures, but it is prohibitively expensive for large-scale production in commercial hatcheries.

Temperature, Light, and Salinity Ranges for Culturing Selected Microalgae Species (Hoff and Snell, 2008)

Species	Temperature (°C)	Light (Lux)	Salinity (ppt – ‰)
Chaetoceros muelleri	25 – 30	8,000 – 10,000	20 - 35
Phaeodactylum tricornutum	18 – 22	3,000 – 5,000	25 – 32
Dicrateria sp.	25 – 32	3,000 – 10,000	15 – 30
Isochrysis galbana	25 – 30	2,500 – 10,000	10 – 30
Skeletonema costatum	10 – 27	2,500 – 5,000	15 – 30
Nannochloropsis oculata	20 – 30	2,500 – 8,000	0 - 36
Pavlova viridis	15 – 30	4,000 – 8,000	10 – 40
Tetraselmis tetrathele	5 – 33	5,000 – 10,000	6 – 53
Tetraselmis subcordiformis	20 – 28	5,000 – 10,000	20 - 40
Chlorella ellipsoidea	10 – 28	2,500 – 5,000	26 - 30

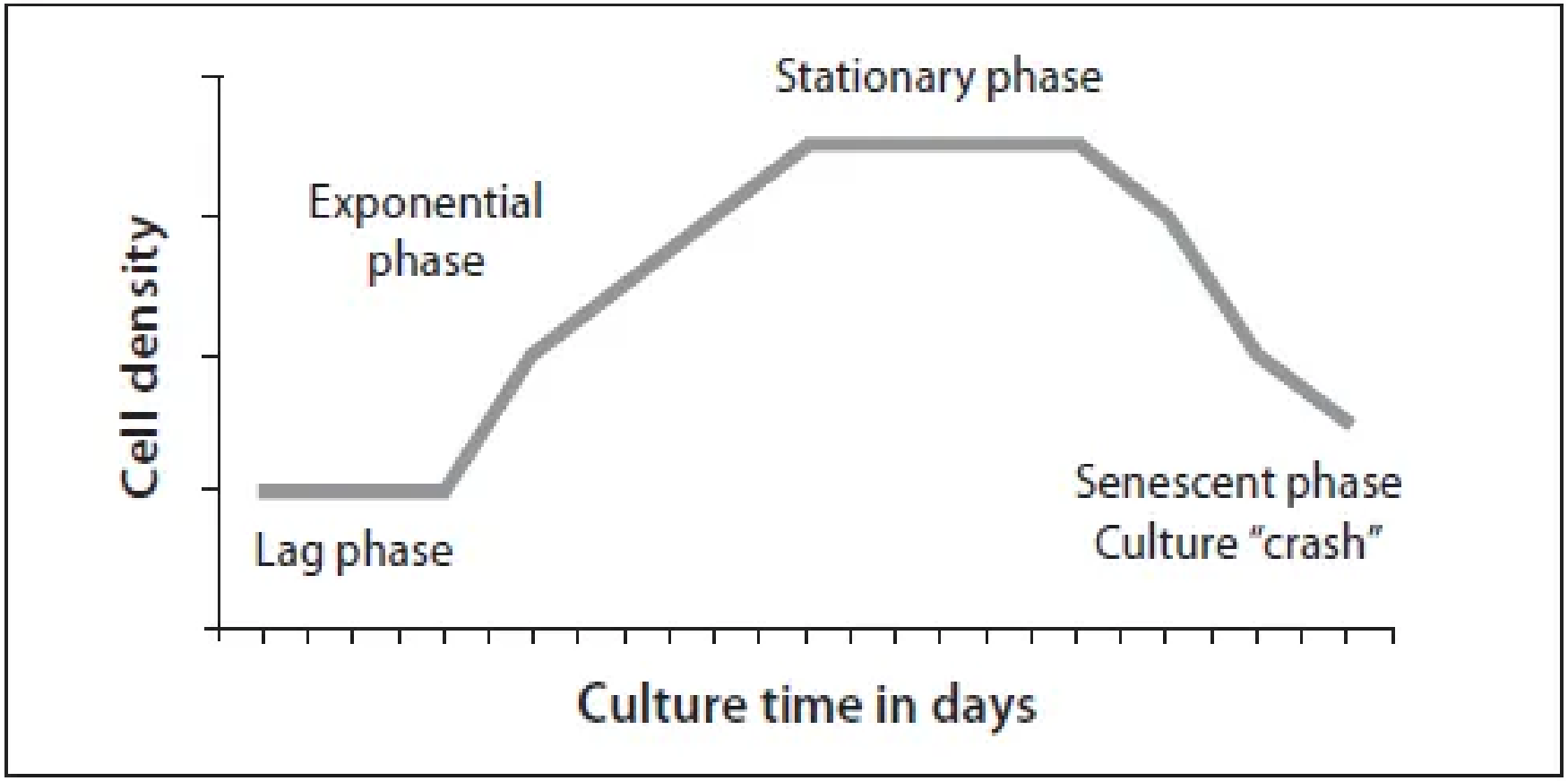
Population Dynamics

Algal cells from a starter culture are inoculated into a larger volume of treated, enriched water to reach an initial low density of about 30 to 100 cells/?L. For the first 2 to 3 days the cells become acclimated to the new medium, grow, and begin cell division. This phase, termed the lag phase, varies in length depending on the amount of inoculum used (initial cell density), alga species (inherent division rate), irradiance, and temperature (Fig. 2).

Once acclimated, the algal cells divide at an accelerating rate, and the population increases logarithmically; this exponential growth phase lasts 4 or more days. The cells are usually harvested for feeding during this phase. The exponential growth phase is followed by the stationary phase, when cell division declines and there is no further increase in cell density.

This decreased growth is the result of changes in the concentration of nutrients, self-shading (high cell density reduces the amount of light available to algal cells), and changes in the culture medium, such as increasing pH and the build-up of metabolic waste products or substances called autoinhibitors that are secreted by some species (mostly diatoms). As the culture ages, the stationary phase is followed by a senescent phase in which the density of the culture will decline.

Stationary phase algae should not be used for larviculture because although the algae may be nutritious, as they die the cells rupture and bacteria can proliferate (including some pathogenic bacteria such as Vibrio spp.). The wise culturist knows that the line between feeding larvae and poisoning them can become blurry as algal cultures age.



Phases in the growth of algal cultures

The Culture Environment

When designing a microalgal production system, consider which species is most appropriate for the intended use (e.g., size and nutritional characteristics). Also consider yield, operating costs, and reliability. Microalgal culture is the most expensive and technically challenging aspect of all hatchery operations. The cost of producing microalgal feed ranges from $100 to $400 per dry kilogram ($45 to $180 per pound) of microalgal biomass (Wikfors, 2000). Algal culture accounts for about 40 percent of the cost of rearing bivalve seed to a shell length of 5 mm in a land-based hatchery (Ukeles, 1980).

Hatcheries use either intensive indoor culture with artificial lighting or extensive outdoor culture in large tanks, raceways, or ponds with natural lighting. Some hatcheries use a combination of the two.

Intensive indoor systems are expensive and labor intensive, but they are more reliable and more productive (relative to space requirements) than outdoor systems. Open ponds and raceways are also more prone to biological contamination or other water quality problems. As one might imagine, the potential for "culture crashes" increases as the degree of control over environmental factors such as temperature, illumination, nutrient availability, pH, and potential contamination decreases.

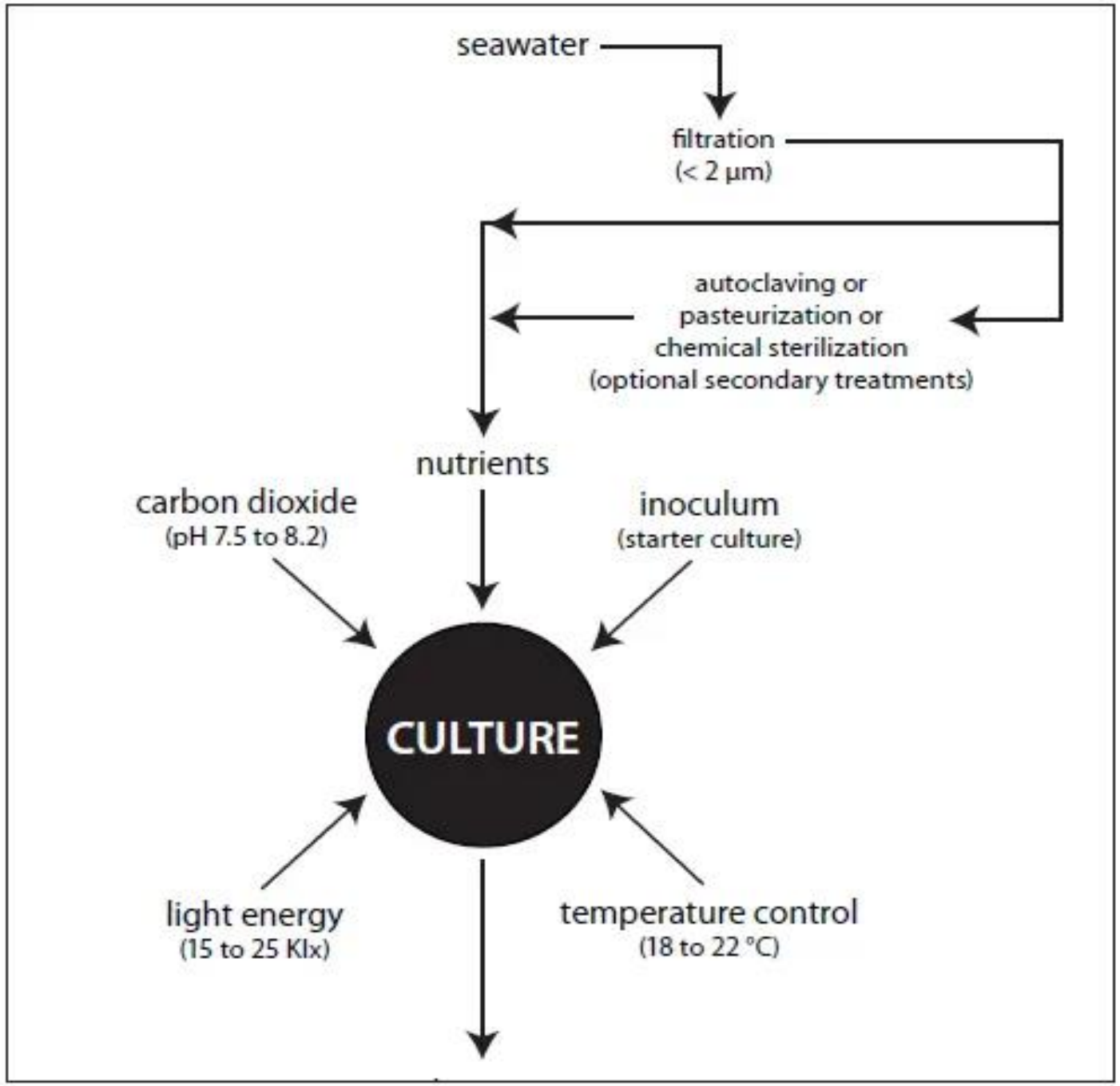
The nutritional value of algae is affected by culture age and growth phase, light characteristics and intensity, nutrient limitation and source, and cell density (Depauw and Persoone, 1988). Whether intensive or extensive, microalgal culture requires filtered and treated water, nutrients, a light source, aeration and mixing, temperature/ salinity control, pH control, and a high-quality inoculum to ensure a satisfactory yield (Fig. 3).

Filtered and Treated Water

Pretreatment of water, whether saltwater or freshwater, is one of the most important steps in successful microalgal culture.

Culture water should be free of suspended solids, plankton (e.g., protozoans, ciliates and other algae species), bacteria, unacceptably high concentrations of dissolved organic compounds (DOC), dissolved metals, and pesticides.

Input requirements and environmental criteria for microalgal cultures. Optimal temperature, salinity, and light intensity may vary with species (e.g., tropical vs. temperate microalgae). Although tropical species grow more rapidly at warmer temperatures (25 to 30 °C), stock cultures are kept cooler to inhibit bacterial growth (Helm et al., 2004).



Pretreatment typically includes mechanical and chemical filtration, sterilization or disinfection, and nutrient enrichment. The choice of treatment should be based upon species cultured, volume requirements, and cost.

Mechanical filtration. Mechanical filtration removes suspended solids, plankton and bacteria and is usually used with the other forms of treatment described below. The type of mechanical filtration used depends on the condition of the incoming water and the volume of water to be treated.

A mechanical filter usually consists of a series of filters that remove increasingly smaller particles—sand filters or polyester filter bags (20- to 35-?m), followed by cartridge filters (10-, 5-, 1-?m) or diatomaceous earth (DE) filters. Small volumes of seawater can be filtered to remove bacteria using 0.22- or 0.45-?m membrane cartridge filters.

Chemical filtration.

Dissolved inorganic and organic compounds (DOC), metals, pesticides, and other contaminants can prevent or retard microalgal growth, although detecting them can be complicated and costly. Activated carbon (charcoal) filtration is helpful in reducing DOC, while deionization resins are effective in removing metals and hydrocarbons.

Heat sterilization. Pre-filtered seawater can be sterilized by autoclaving at 1.06 kg/cm2 for 20 minutes. Autoclaving is most suitable for small volumes, while batch or continuous pasteurization at 65 to 70 °C is used for large volumes. Pasteurization at 50 °C for 8 to 10 hours is also effective; a glass-lined water heater or 500- to 1,000-W submersion heater can be used. Microwave sterilization is useful for small volumes of pre-filtered seawater (1 to 5 ?m for 8 to 10 minutes per 1 to 1.5 L using a 700-W unit). Nutrients can be added before microwaving since the temperature will not exceed 84 °C (181 °F) (Hoff, 1996). Bellows and Guillard showed that using a 1.2-ft3, 700-W microwave on high power would effectively kill microalgae in 5 minutes, bacteria in 8 minutes, and fungi in 10 minutes in a volume of 1.5 L of filtered (and unfiltered) seawater (Table 3).

Chemical sterilization.

Chlorination is the simplest and most common method of chemical sterilization for culture volumes of at least 4 L. Pre-filtered seawater can be sterilized with sodium hypochlorite solution at 2.5 mg/L free chlorine by adding 1 to 5 mL of household bleach (5% sodium hypochlorite) per liter of seawater. Granular swimming pool chlorine is also effective; a dosage of 1 ounce (28 g) to 500 gallons (1,875 L) yields a similar concentration of chlorine as liquid bleach. Sterilization occurs in a short period of time, usually 10 to 30 minutes, although many culturists suggest a longer time (12 hours or overnight) for a margin of safety. Before use, neutralize the residual chlorine by adding an excess of sodium thiosulphate solution ($Na2S2O3 \cdot 5H2O$). If 250 g of sodium thiosulfate is dissolved in 1 liter of water, then 1 mL of the sodium thiosulfate solution added for every 4 mL of bleach used is sufficient to eliminate residual chlorine (dechlorination). Common swimming pool chlorine test kits can be used to determine the presence of residual chlorine, but they do not give a precise measure of chlorine concentration; as a general practice, additional sodium thiosulfate solution should be added if there is any indication of residual chlorine.

Sterilization method	Effective method	Application	Limitation
Autoclaving	121°C at 29.4 psi (2 atm) @ 10 minutes for test tubes @ 1 hour for 10 liters liquid	Liquids and agar, glass and metal vessels	Avoid non-heat-resistant materials; pH change, metal contamination
Pasteurization	65–80 °C followed by quick cooling (4–10 ° cooling) 50 °C for 8–10 hours using glass-lined water heater, 500- to 1000-W immersion heater	Liquids with heat labile components	Not complete sterilization
Microwave	10 minutes/L of liquid with 700-W microwave; 20 minutes at 600-W, 45 minutes for dry goods	Small volume liquids (including media), dry goods, and vessels	Limited volume capability

Summary of Heat Sterilization Types, Effective Methods, Application, and Limitations (Kawachi and Noël, 2005)

Ultraviolet irradiation (UV) and ozone (O3) disinfection.

Either UV or ozone can be used to disinfect culture water, although both are most effective after mechanical filtration has removed suspended particulates.

It should be noted that "sterilization" is defined as the absolute destruction of all microbial organisms (including bacterial spores), while "disinfection" does not eliminate all microbes but reduces their numbers to a level where the risk of infection is small enough to be acceptable.

UV is the more common of the two, largely because it does not leave concentrations of hazardous by-products.

Ozone at high levels can produce chloramines, which are toxic to marine animals. Ozone released into the air can be a safety hazard (if you can smell a faint chlorine smell, residual ozone is present and may be hazardous to your health).

Ozone is a strong oxidizing agent that is particularly effective in removing dissolved organics, pesticides, color and nitrates. It is highly unstable and quickly reverts to O2, but it is also highly corrosive and must be handled with special materials. In-line ozone generators are the most common and usually have monitors/controls to provide an adequate level of ozone yet avoid residual build-up. However, because there is a risk of introducing ozonated water into the culture system, as well as safety concerns for hatchery staff, ozone is not recommended for operators who lack experience and the monitoring equipment to properly manage ozone levels.

Nutrient Enrichment

The objective of culturing microalgae is to obtain the highest cell densities in the shortest period of time, and natural concentrations of nutrients in freshwater and seawater are usually insufficient to support high algal yields. Although trace elements are usually found in sufficient quantities, macronutrients are in short supply (usually phosphorus in freshwater and nitrate in saltwater). Several nutrient enrichment media containing soil extract, nitrates, phosphorus, trace elements, and vitamins have been described for freshwater and saltwater (Creswell, 1993). Of the nutrient media formulations used to culture marine microalgae in laboratories and hatcheries, Guillard and Ryther's F/2 media is the most widely used, and a pre-mixed solution is available from a variety of vendors (Table 4). There are dozens of culture media recipes, many of which were formulated specifically for certain types/species of microalgae and cyanobacteria. A good reference is Algal Culturing Techniques, edited by R. A. Anderson. Table 5 lists services that have culture medium recipes on their websites.

Guillard's F/2 Media used to Culture Marine Microalgae (Guillard, 1975)

Major nutrient	Chemical formula	Concentration (gram/liter)
# 1. Nitrate	$NaNO_3$	75.0 g/L
# 2. Phosphate	$NaH_2PO_4 \cdot H_2O$	5.0 g/L
# 3. Silicate	$Na_2SiO_3 \cdot 9H_2O$	30.0 g/L
# 4. **Trace Metals**		
	$FeCl_3 \cdot 6H_2O$	3.5 g
	Na_2EDTA	4.36 g
Dissolve in 900 ml of distilled H_2O. Add 1 ml of each of the following trace metal solutions.		
	$CuSO_4 \cdot 5H_2O$	0.98 g/100 ml
	$ZnSO_4 \cdot 7H_2O$	2.20 g/100 ml
	$CoCl_2 \cdot 6H_2O$	1.00 g/100 ml
	$MnCl_2 \cdot 4H_2O$	18.00 g/100 ml
	$Na_2MoO_4 \cdot 2H_2O$	0.63 g/100 ml
Make up the volume to 1 liter with distilled H_2O. Add 1 ml per liter of seawater of the above solutions # 1–4.		
# 5. **Vitamins**		
	Biotin	1.0 mg
	B_{12}	1.0 mg
	Thiamin HCl	20.0 mg

Dissolve vitamins in 1 liter of distilled H2O. Store frozen. Add 0.5 ml of vitamin solution for every 1 liter of seawater

Light Source

Light is the energy source that drives photosynthesis to convert nutrients into algal biomass. Maximum culture depth and cell density are the primary variables regulating the efficient use of light (Richmond et al., 1980).

Light intensity, spectral characteristics, and photoperiod are the components of an illumination regime. Indoor microalgal facilities usually use fluorescent "cool white" bulbs (2,500 lux), while outdoor systems and greenhouses use ambient sunlight in combination with fluorescent or metal halide bulbs to provide evening illumination.

The spectral characteristics of "cool white" bulbs are not ideal for intensive microalgae production; bulbs with enhanced red and blue wavelengths (Gro-LuxTM) support higher yields.

The age of the bulb is also important, as the spectral characteristics and luminosity change over time; bulbs should be replaced at least annually.

Although most commercial light meters measure “lux,” many references in the literature related to light requirements for phytoplankton culture prefer to express optimum irradiance in terms of “Photosynthetically Active Radiation” (PAR), which is expressed as mol photons · s-1 · m-2, radiation within wavelengths of 400 to 700 nm. Converting lux values to PAR depends on the type of lamp and its spectral characteristics. Multiply lux by the following conversions for PAR:

incandescent = 0.019
metal halide = 0.014
cool white flourescent = 0.013
daylight flourescent = 0.014
GRO flourescent = 0.029
clear day sunlight = 0.018

Temperature Control

Because most of the microalgae species preferred by culturists are tropical/subtropical, most strains grow best at temperatures ranging from 16 to 27 °C (60 to 80 °F).

The optimum is about 24 °C (75 °F). Ukeles (1976) compared the growth response of several microalgae species to temperature (Table 6).
The optimum temperature for growth will vary with species, and to some extent is a complex factor that depends on other environmental conditions.

Cultures should be maintained at the lowest temperature that is consistent with good yield to avoid encouraging bacterial growth.

When considering temperature characteristics for an enclosed culture room, one should consider: 1) the size of the room, 2) heat sources (such as lights and ballasts), and 3) the volume and temperature of air pumped into the culture vessels.

Growth Response of Different Microalgal Species to Various Temperatures (°C). Growth Rates are Relative to Performance of a Control Cultured at 20.5 °C (Ukeles, 1976).

Species	No growth	Growth less than control	Growth equal to control at 20.5 °C	Growth less than control	No growth
Monochrysis lutheri	8–9	12	14–25	27	29–5
Isochrysis galbana	8–9	12	14–22	24–25	27–35
Phaeodactylum tricornutum	–	–	8–24	27	29–35
Dunaliella euchlora	8–9	–	12–35	–	39
Platymonus (Tetraselmis sp.)	–	8–9	12–32	–	35
Chlorella sp. (isolate # 580)	8–9	12	14–35	–	–
Chlorella sp. (UHMC isolate)	8–9	12	14–29	–	32–35

Aeration and Mixing

Aeration is important for microalgal culture because:

1) air is a source of carbon (from CO2) for photosynthesis;
2) 2) CO2 provides essential pH stabilization; and

3) 3) physically mixing the culture keeps nutrients and cells evenly distributed, reduces self-shading and/or photoinhibition (a decrease in photosynthesis due to excess light), and avoids thermal stratification in outdoor systems. Air diffusers (airstones) create small bubbles that maximize oxygen/ CO2 transfer, and they are frequently used for smallvolume cultures.

4) In larger culture containers, fine bubbles from air diffusers create spray and foam that can promote bacterial growth; larger bubbles (no airstones) actually do a better job of mixing the culture with minimal foaming. Common alternatives for mixing larger volume cultures include jet pumps, paddle wheels, continuous recirculation, and air-lift pumps (Persoone et al., 1980).

Carbon dioxide (CO_2) source and pH control

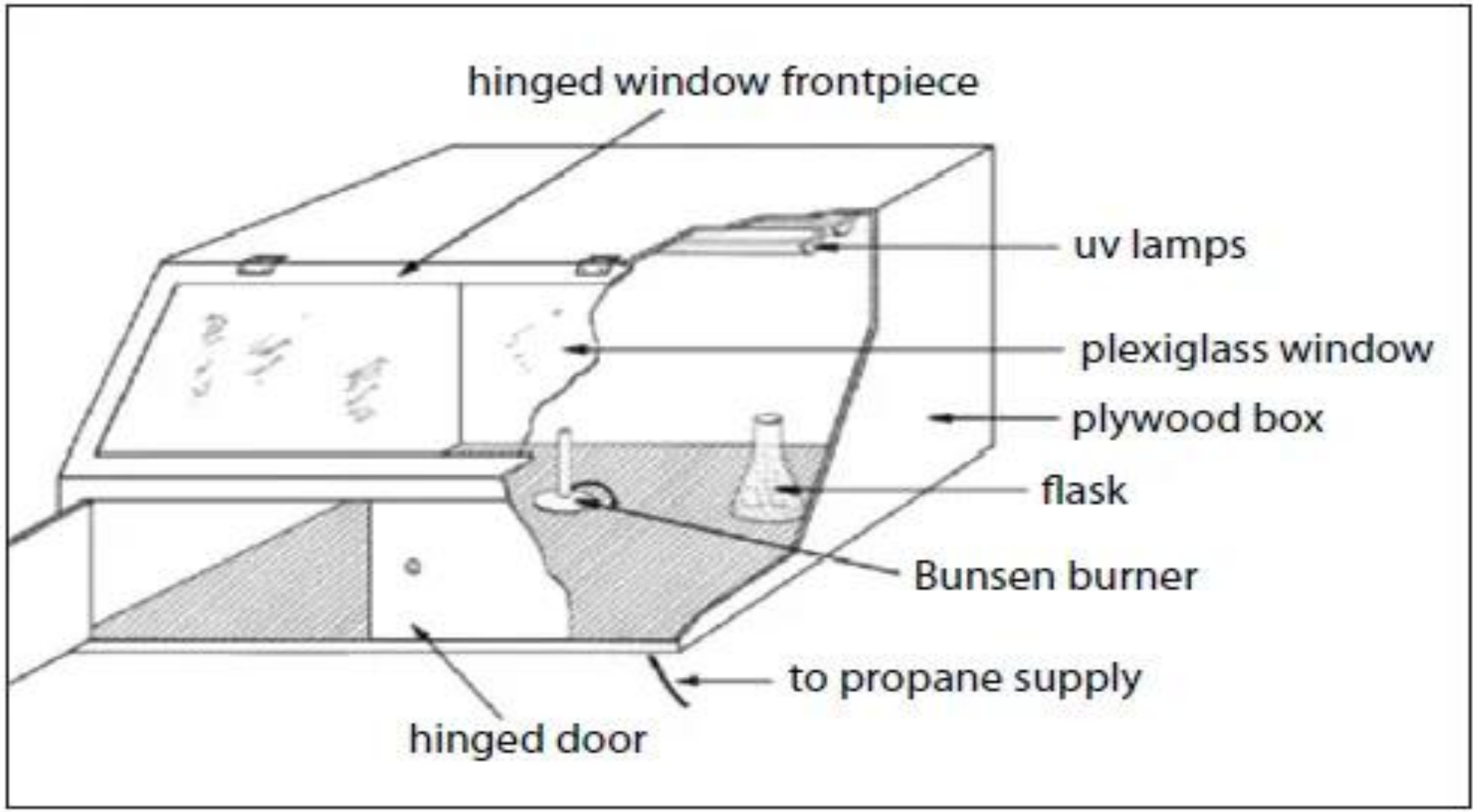
Carbon dioxide plays a dual role in microalgal culture. It provides a source of carbon to support photosynthesis, and it helps maintain pH at optimum levels (7.5 to 8.2 for marine species). As culture density increases, more carbon is consumed through photosynthesis, reducing CO_2 concentration and causing the pH to rise. At about pH 10 some nutrients will precipitate, algal growth will be retarded, and the culture could completely collapse. This can be prevented if the pH is maintained by introducing CO_2 into the air delivery system. This can be done manually (while the cultures are illuminated), pulsed intermittently using a timer and solenoid valve, or, most effectively, by using a pH monitor/controller.

Most hatcheries will culture several species of microalgae to provide live feeds with different sizes and nutritional characteristics, depending on the animal being cultured and its life stage. The culture protocol for each species will be dictated by the characteristics of the microalgae (e.g., growth rate and environmental requirements), harvest yields, and use requirements.

Maintaining and Transferring Stock and Starter Cultures

Stocks of monospecific (uni-algal) cultures can be obtained by collecting local species, separating them by size (filtration) or density (centrifugation), and inoculating agar plates containing enrichment media. From these multi-species algal cultures, individual colonies are selected through agar streaks, micropipette isolation, liquid dilution, or flow cytometer cell sorters (Fulks and Main, 1991). Culturing algae in highly filtered, autoclaved, enriched seawater in the presence of antibiotics allows bacteria- and protozoanfree pure cultures (axenic) to be isolated. However, the isolation and screening of local species is laborious and mono-specific cultures of most microalgae species used for aquaculture are readily available from research laboratories, commercial hatcheries, and vendors.

A schematic diagram of a stock culture transfer chamber (Helm et al., 2004).



The sterile procedures described below should be followed.

Wipe all inner surfaces of the inoculating hood and working surfaces with 70 percent ethanol.

Place all flasks that will be used in the hood, including flasks to be transferred from (the transfer flask) and flasks containing sterilized media that will be inoculated under the culture transfer hood.

Irradiate flasks to be inoculated with an ultraviolet lamp for at least 20 minutes. Be sure the hood has a dark cover over the viewing glass (UV radiation can be damaging to the eyes).

Switch off the UV lamp; ignite a small Bunsen burner; remove caps from one transfer and one new flask; and flame the neck of each flask by slowly rotating the neck through the flame.

Tilt the neck of the transfer flask toward the new flask. In one motion, remove both stoppers and pour an inoculum into the new flask.

Transfer approximately 50 mL for diatom species and 100 mL for flagellates. Avoid touching the necks of the two flasks. Never touch the portion of the stopper that is inserted into the flask. Once the inoculum is added, replace the stopper in the transfer flask. Slowly flame the neck of the new flask before replacing its stopper.

Replace the cap over the neck of the new flask and use a waterproof marker pen to label the new flask with the algal species inoculated and the date of transfer.

After all inoculations are completed, turn off the burner and transfer all new flasks to an algal incubator or a well-lit area in the algal culture facility. The remaining inoculum in the transfer flasks can be used to inoculate larger cultures such as 4-L flasks or carboys.

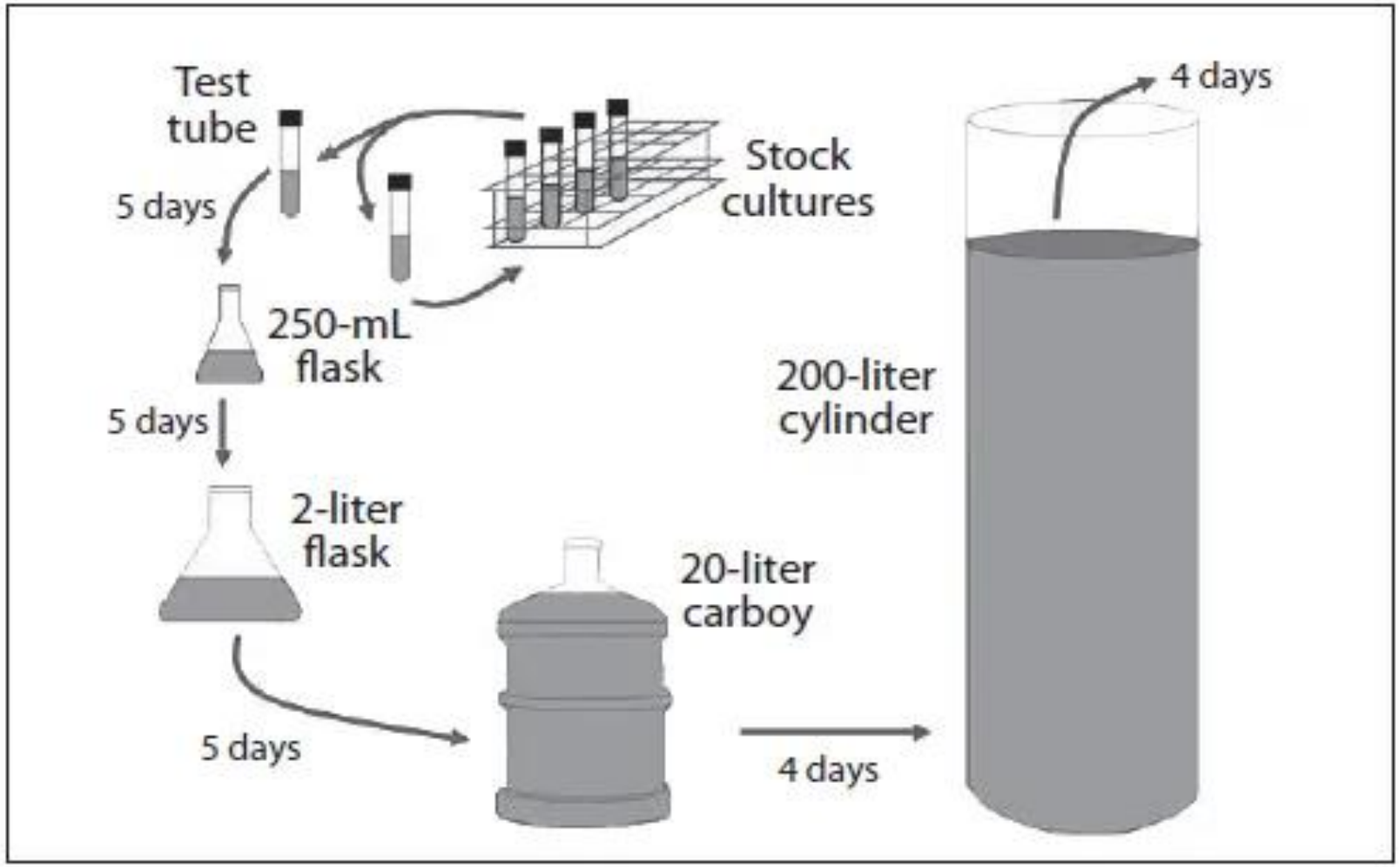
Empty test tubes, flasks, stoppers and/or caps should be removed, thoroughly washed, and sterilized or discarded.

Remove all materials from the working area and wipe the surface with 70 percent ethanol.

Progressive Batch Culture

The quantities of algae cells required for feeding mollusc larvae and other zooplankton are produced through a process called progressive batch culture (transferring small-volume cultures of concentrated inoculum into larger volumes of treated, enriched water). Starting with cells taken from an axenic stock culture (test tubes), microalgae are cultured in an enriched medium through a series of culture vessels of increasing volume (Fig. 5). The algae grown in each culture vessel serves as the inoculum for the next larger vessel, until the quantity of cells required for feeding is reached. This is a typical series for large-scale production:

25-mL test tubes (10-mL stock culture) inoculates….
500-mL flasks (250-mL starter culture) inoculates….
2.8- to 4-liter flasks (1,000-mL culture) inoculates….
20-liter carboys (16-liter culture) inoculates….
250-liter cylinders (180-liter culture) inoculates….
12,000-liter tanks (10,000-liter culture) inoculates….



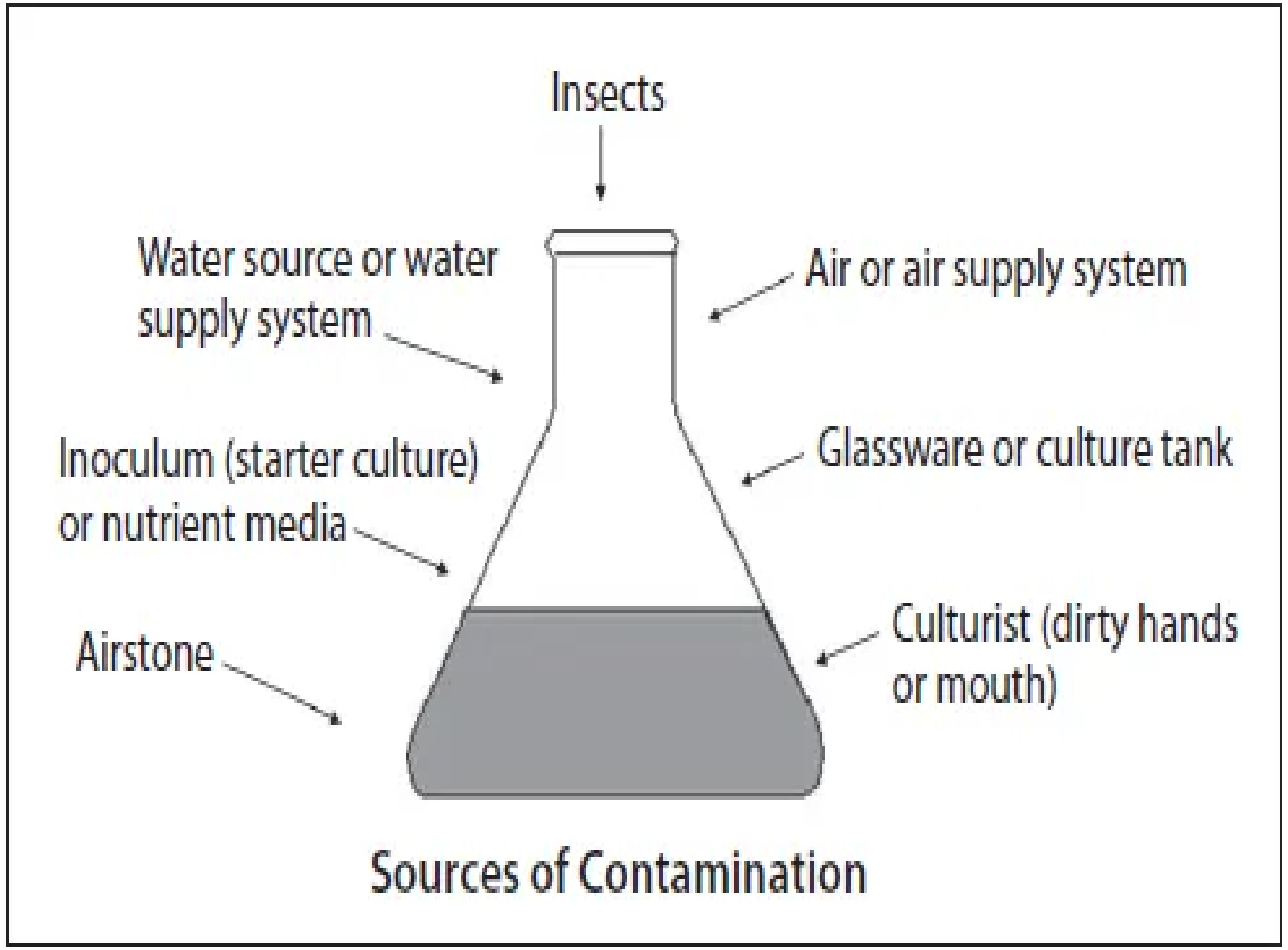
Serial dilution of microalgae from stock culture (test tubes) to 200-L fiberglass cylinders with the approximate duration between each transfer (courtesy P. van Wyk, Harbor Branch Oceanographic Institute

Starter cultures are used to inoculate “intermediate cultures” (2 to 25 L), which are used to inoculate even larger volume cultures for final production before harvest and feeding. Similar to stock cultures, starter cultures can be grown in 500-mL flasks with 250 mL of sterile medium; about 50 mL of the starter culture is transferred to similar volume flasks to maintain the line, while the remaining 200 mL are used to inoculate intermediate culture containers (typically from 4-L flasks to 20-L carboys) (Fig. 6).

Throughout the scale-up process, contamination is a constant threat and cleanliness and attention to detail are critical.
Contaminants may be chemical or biological and they can originate from one or several sources.

A common chemical contaminant is residual chlorine from the sterilization process, while biological contaminants might include: 1) excessive levels of bacteria (indicated by cloudy water), 2) protozoans or rotifers (culture water turns off color and clears), 3) competing microalgae (color change or crust attached to culture vessel walls), and 4) macroalgae (green or brown strands attached to culture vessel walls).

Identifying bacterial and microalgal contaminants usually requires 100X to 400X magnification, while protozoan contamination can be observed under 15X to 40X magnification (Hoff and Snell, 2008). Possible sources of contamination are shown in Figure 7.



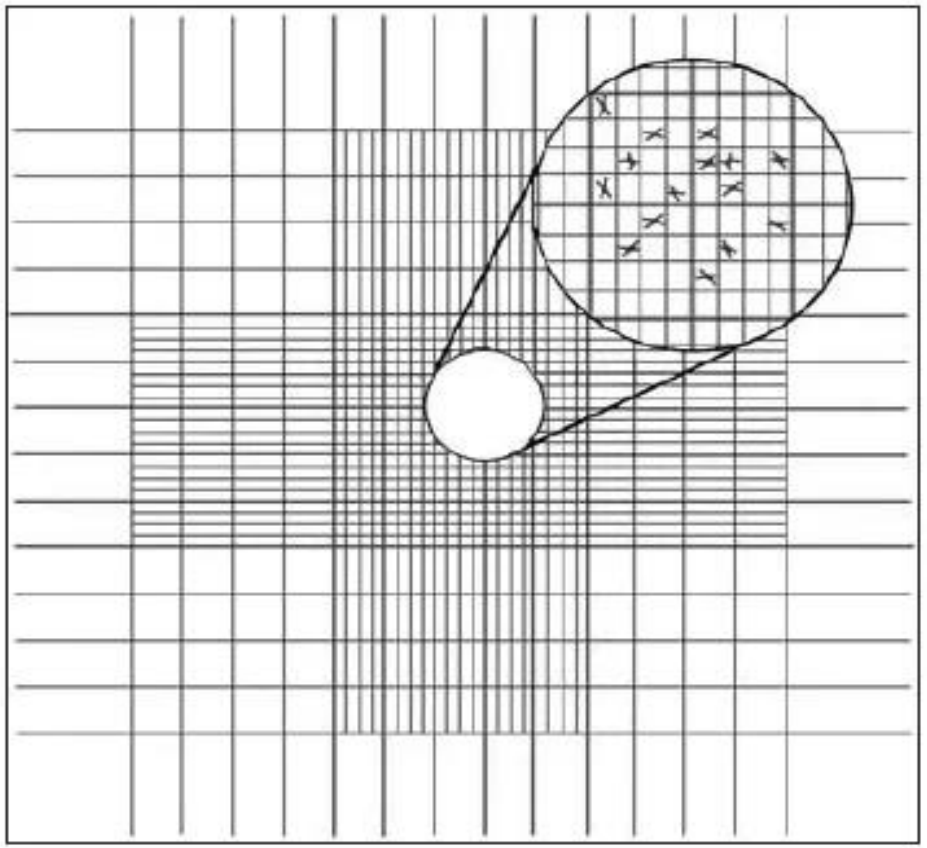
Sources of Contamination

Estimating Algal Density

Estimating algal density is an inherent part of any algal production system. Algal biomass is the criterion used to determine when to transfer inoculum through serial dilution to larger volume cultures and to determine harvest volumes of cultures in production.

For stock and starter cultures, the most accurate measurement of cell density can be made using a Palmer-Maloney slide or a hemacytometer.

A hemacytometer used to count microalgae cells (Helm et al., 2004).



Intermediate Culture

Intermediate culture volumes, typically 4-L flasks to 20-L carboys, are used to inoculate larger vessels, typically 100- to 200-L translucent fiberglass cylinders or polyethylene bags, or even larger fiberglass tanks and raceways.

Carboys (20-L) are "intermediate" cultures that will be used to inoculate 200-L fiberglass cylinders or fed directly to bivalve larviculture tanks (courtesy J. Scarpa, Harbor Branch Oceanographic Institute).

Translucent fiberglass cylinders (200-L) are commonly used for production of microalgae up to harvest and feeding. For large-scale algae production, they can be used to inoculate 2,000- to 5,000-L mass culture tanks.

Batch cultures are inoculated with the desired species that will grow rapidly under optimal conditions until the rate of cell division begins to decline, indicating the transition from the exponential phase to the stationary phase

Semi-continuous cultures begin much the same way as the batch cultures, but instead of harvesting the entire volume, 25 to 50 percent of the volume is harvested at the point when light has become a limiting factor (late exponential phase). The harvested volume is then replaced with freshly prepared culture medium and the remaining algal cells serve as inoculum. Semi-continuous cultures grow rapidly and can be harvested every 2 or 3 days.

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Culture in Polyethylene Bags

Heavy-gauge polyethylene tubing can be cut to a suitable length and one end heat-sealed to form a sterile, flexible culture container that is either a cylinder or an oblong bag (Baynes et al., 1979; Trotta, 1981). The culture vessel design is based on that used by SeaSalter Shellfish Company Ltd. (Farrar, 1975). Containers formed in this way can be strengthened by supporting them within a plasticcoated, steel-mesh frame (Fig. 11). Or, the cylinders can be suspended, with or without lateral support mesh, if the diameter of the bag is less than 30 cm and the height less than 200 cm.

Large-scale outdoor bag cultures are often positioned horizontally to maximize sunlight penetration (Fig. 12). Such large-volume systems are often used to induce multi-species blooms that are best suited for feeding juvenile shellfish in nursery systems or adult shellfish in broodstock systems, rather than for hatchery production. The rate at which a bloom develops is related to the species composition; the volume and cell density of the inoculum; the quantity, quality and duration of light; nutrient levels; and temperature.

Concentrating Algal Biomass

In most hatcheries, microalgae are fed in liquid form directly to the animal culture tanks. Recently, though, there has been an interest in concentrating algae to reduce the volume of microalgal culture water (and possible contaminants) introduced into culture tanks. The use of "algal paste" or concentrate has gained popularity because during periods of excess production, the concentrate greatly reduces the physical space required, can be refrigerated until needed, and can be diluted when used. However, the nutritional quality of the microalgae may be a concern if the concentrate is stored for extended periods. Although this concept is not new to aquaculture (Barnabe, 1990), algal paste in preserved or fresh forms recently has become commercially available.

Advanced Algal Production Systems

There are several new microalgal production systems on the market; they are collectively termed "AAPs" (Advanced Algal Production Systems) or photobioreactors. Photobioreactor systems can provide higher algal densities, more efficient space usage (a smaller footprint), continuous or semi-continuous production, longer production cycles with less contamination, and lower labor requirements (Ellis and Laidley, 2006). In general, three types of systems are under production: 1) tubular photobioreactors, 2) column or cylinder photobioreactors, and 3) flat-panel or plate photobioreactors (Tredici et al., 2009).

Conclusion

Phytoplankton culture is a multi-faceted activity, and the task of designing a large-scale microalgal production system can be complex. The size, location, and engineering specifications should depend on the type of culture to be practiced (e.g., batch, semi-continuous, continuous), the site characteristics (such as water quality and temperature), illumination, the requirements of the target species, and production goals. These considerations are interdependent, and each should be treated as a part of the whole. The principles and methods described here represent only a few of many approaches to growing phytoplankton. The reader is advised to modify these guidelines as needed to suit specific hatchery conditions.

Zooplankton culture

Two 36-watt daylight fluorescent tubes were suspended 60 cm above a series of 5 - 60 L aquaria to illuminate a wooden cupboard of 120 × 60 × 80 cm 3 (0.6 m 3).Room temperature was kept at 28 ± 1°C and aeration using two 1.5 hp pumps was continuous.

The pure zooplankton cultures received 50 mgl-1 of baker's yeast (Saccharomyces cerevisiae)or 1.75 mgl-1 of inorganic fertilizer (N: P: K) (20:10:10) every other day to release the macronutrients needed for zooplankton growth, whereas 1.0 ml of nutrient salt solution A and 0.1 ml of solution C (Laing and Ayala, 1990) were administered every other day to the pure algal cultures (Ajah, 1995).

Outdoor cultures :

Fertilization of outdoor cultures was made possible using pig manure supplied regularly by 20 weaned pigs that were fed daily on 10% body weight with pig mash (Akpan and Okafor, 1997). The chemical composition of samples of pig manure was determined and found to contain the essential nutrient elements for algal growth. One (1.0) kg of pig manure was introduced into each outdoor tank during the dry season and 2 kg during the wet season for the first two consecutive days. Thereafter, 1 kg perr tank was administered every three days and every other day, for the dry and

wet seasons, respectively.

50 ml of each axenic algal monoculture, namely, C. vulgaris, S. quadricauda and E. elegyears ,from the laboratory, were inoculated into the respective tanks at cell densities of 2.6 × 106, 1.68 × 106 and 0.12 × 106 cells/ml.

Five litres each of 4 ind ml-1 from pure B. quadridentatus cultures (Ajah, 1995) were introduced into each 10 m3 tank.

The feeding duration of B. quadridentatus with each of the three algae lasted for an average of 18 days (range 15 - 21 days).

Each culture was repeated three times during both the dry season (November to March/ April) and wet months (May/ Ja to October).

The batch culture trials continued for a period of three years amounting to a total of eighteen trials, nine per season (Ajah, 1995, 1997, 1998).

Cell counts A 1 l plastic funnel was used to scoop samples from the thoroughly mixed plankton cultures.

The samples filtered through a 56 µm-plankton sieve to collect and retain both phyto- and zooplankton.

Four cell counts of 1 ml each using a haemocytometer and four zooplankton counts using a one ml counting chamber (model: AJAH001) (Dad, 1995) were carried out every day or every two days, and average values were recorded.

Physicochemical parameters:

The physical and chemical factors were monitored throughout the experimental period. Dissolved oxygen in the pond was determined using Lectron 5509 DO meter and temperature was read using standard thermometer.

Chlorine was assessed by the chlorosity method of Rump and Christ (1988).

Nitrite (NO2--N) by the diazotization (spectrophotometric) method; nightrate (NO3-N) by the cadmium reduction/ diazotization method, ammonium (NH 4 + -N)level by the Nesslerization (spectrophotometric) method, phosphate by the molybdenum blue method (spectrophotometric) (Parsons and al., 1984), conductivity was read using a HACH 3000 spectrophotometer and turbidity by secchi disc.

Single classification analysis of variance (ANOVA) was used following Sokal and Rohlf (1981) to compare means of population density of zooplankton counts from the replicates.

Correlation coefficients between B. quadridentatus and each primary producer as well as the coefficients of determination were also calculated using SPSS.

Descriptive statistics were used to compare the means.
The intrinsic rate of natural increase (r) and
the population doubling time in days (tD) were calculated as follows:

$r = (\ln Nt - \ln No) / t = D'/t$ or $2.3026(\log Nt - No)/t$ (James and Dias, 1984).

Where: D' = The dilution time in days.

$Nt = No.e^{rt}$ (James and Dias, 1984).
Nt = Final number of individuals.
No. = Initial number of individuals,
and t = time in days .
e (exponent) = 2.7183.
r = The intrinsic rate of natural increase.
$tD = 0.6931/r$ (James and Dias, 1984).
tD = Doubling time of the population in day

CONCLUSION:

The physicochemical factors and nutrient load were within the acceptable limits required for the proper management of earth ponds. Physicochemical values in this report that fell within the optima required for plankton growth included amapprox others temperature , DO (Banerjea, 1967), the electrolyte conductivity, pH and the DO (Dad, 1995, 2008), turbidity (Dad, 1995, 2008), nitrate (Sachidanandamurthy and Yajurvedi, 2004) and phosphate (Sawyer, 1947; Screenivasan, 1965).

THANK YOU