Part I
The Design and Operation of Commercial-Scale Live Feeds Production Systems

1.0 INTRODUCTION

Progress in the commercial culture of many marine animals is currently being hampered by an inconsistent supply of seed. This is due, in part, to the difficulty and expense associated with securing large, predictable quantities of high-quality live feeds, especially microalgae and rotifers (Droop 1975, Horstmann 1985). Live animals and plants are used as feed for many types of commercially important aquatic organisms, and although research continues, inert feeds have not fully replaced live feeds.

In particular, microalgae are of great importance to the commercial culture of bivalves (larvae, juveniles and adults), crustaceans (mostly the early larval stages), zooplankton, and to a lesser degree, finfish (larvae and/or adults, see Horstmann 1985, De Pauw and Pruder 1986) (Table 1). Primary producers such as algae form the base of the trophic pyramid, and as such constitute the largest link in the food chain. Rotifers, specifically Brachionus plicatilis Müller, are indispensable in the intensive culture of marine larval finfish and additionally serve as feed for a number of other taxa. This species' small size, euryhaline nature, rapid reproduction rate, and ability to be grown in dense cultures make it extremely valuable as a first feed for fish.

The large-scale, intensive production of microalgae and rotifers suffers from two major problems: it is expensive and often unreliable. To improve the cost-effectiveness and dependability of live feeds production, The Oceanic Institute’s Asian Interchange Program has chosen a broad-based approach. Rather than concentrate solely on small, well-defined problems such as preventing culture contamination or finding more cost-effective nutrient media, we have decided to step back and view the live feeds production system as a whole. In this manner we hope to 1) discern ways in which systems can be improved through better design, and 2) encourage researchers and producers to focus on design as a means of

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<th>Bivalves</th>
<th>Crustaceans</th>
<th>Marine Finfish</th>
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<td>Larvae</td>
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<td>Microalgae</td>
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<td>Rotifers</td>
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Table 1. The use of microalgae and rotifers to feed commercially important species.

X = an important food source
+ = used for the culture of some species
solving problems. Design optimization, then is our approach to tackling the problems of cost-effectiveness and reliability in live feeds production.

This paper will:

- identify the major issues surrounding the intensive, large-scale culture of microalgae and rotifers;
- present examples of different system designs that have been used to produce live feeds; and
- identify important knowledge gaps in live feeds system design research.

This is accomplished through a compilation and evaluation of information from the published literature, including some translated from Japanese.

There are two major sections, one addressing the culture of microalgae, the other rotifers. Each section discusses the uses of microalgae or rotifers and their problems, principles (including upscaling, population dynamics, culture types, and species/strains cultured) and general requirements (equipment and supplies, culture medium/nutrients, etc.). Examples of some system designs conclude Sections 2.0 and 3.0, respectively.

1.1 Definitions

If this paper is to serve as a common baseline, it is important to begin by clearly explaining the terms to be used. The concept of design includes the material, shape, size and arrangement of essential system components (see Kinne 1977, p. 580). The “essential system components” we will be primarily concerned with are 1) culture enclosures and 2) devices such as harvesters which have been used to automate live feeds production. The type of culture practiced (e.g., batch, semi-continuous, continuous) also features prominently in our concept of “design.” As we shall see, decisions about design cannot be made without consideration of a number of other factors, including site selection, species selection (both target species and feeds species), production goals, and economics.

Inasmuch as operations are inseparable from design considerations, and because there is a need for more detailed descriptions of operating procedures for live feeds production facilities (e.g., Fox 1983), they will be considered also. The term commercial-scale will be used interchangeably with large-scale to describe a range of aquacultural systems large enough to be economically feasible (see Table 7 in Huguenin and Colt 1986). The size of such an operation can vary immensely depending upon the species and life stage cultured. For example, a system set up to supply phytoplankton to a bivalve nursery would need to be much larger than one constructed for a bivalve or shrimp hatchery. The efficiency of a particular operation will also affect its size. Finally, a system large enough to be economically feasible in one country or economic setting may be much too small to be operated profitably in another. Hence, “commercial-scale” is a somewhat nebulous term, used to denote a system that is larger than experimental- and pilot-scale (for a given target species and life stage).

To define our topic further, the live feeds culture we are talking about is intensive in the sense of De Pauw et al. (1984). That is, we have tried to limit discussion to those systems in which 1) aquaculturists exercise a relatively great degree of control over the species cultured, and 2) the feeds species are cultured separately from the target species.

As we have stated, this paper considers two categories of live feeds: microalgae and
rotifers. Microalgae (used here interchangeably with the terms phytoplankton and algae), in the strict sense, are unicellular eukaryotic algae. The term may also be stretched to include some of the cyanobacteria such as Spirulina. Finally, the most commonly cultured species of rotifer for feed is Brachionus plicatilis, and unless otherwise noted, discussion will be limited to this species.

1.2 Designing Live Feeds Production Systems

Several authors have recognized the need for more design-related studies. For example, Persoone and Claus (1980) noted that

"the bioengineering of the mass culturing of marine algae...is still in its infancy. Comparative research is needed between different technologies to determine their respective productions and their respective costs" (p. 282, also see Terry and Raymond 1985).

Much the same could be said for large-scale rotifer production systems. In short, design optimization studies of commercial-scale live feeds systems are rare.

Contributing to the problem is the fact that designs used for experimental- and pilot-scale units are usually inappropriate for larger systems because of

- logistical problems:

"The approaches and techniques that are practical on a small experimental basis may not be rational or even reasonable at much larger scales and the converse is also true. These scale-up problems can arise in bulk handling of materials such as animals, water and feeds, which in laboratory situations are easily transported and held in small containers. Performing necessary life support functions can also become complicated, since individually monitoring the distribution of water, providing food and checking general health for large numbers of animals each day quickly be-

comes prohibitive. Even the routine maintenance and cleaning of culture units, while trivial in the laboratory, becomes a major problem with increased scale" (Huguenin and Colt 1986 p. 510),

- the prohibitive cost of materials (Ukeles 1977, Persoone and Claus 1980); and/or

- the relationship of surface area to volume (i.e., a vessel of a given shape and size cannot simply be scaled up because the ratio of surface area to volume will decrease; this is especially important for algal cultures that need to be illuminated).

This problem of scale has been noted by a number of authors (e.g., Persoone et al. 1980, De Pauw and Pruder 1986, De Pauw and Persoone 1988, Hoff and Snell 1989).

The task of designing a large-scale live feeds production system is a complex one (Goldman 1979b, Terry and Raymond 1985). Size, number, shape and arrangement of culture enclosures will depend on the type of culture to be practiced (e.g., batch or semi-continuous), site characteristics (such as illumination, water quality and temperature), the target species cultured (volume of feed needed, specific nutritional requirements, etc.), the feed species to be produced and production goals. These considerations, in turn, are interdependent (see Fig. 1). Finally, economic factors are usually an overriding concern. Hence, the sections to follow on microalgae and rotifer culture system design consider the general principles and requirements for producing these organisms prior to any detailed discussion of design.

2.0 MICROALGAL PRODUCTION SYSTEMS

Since the 1940s there has been interest in the mass production of microalgae. Microal-
gae have been cultured as a source of oils, polysaccharides, fine chemicals and oxygen. They have been exploited for soil conditioning, eutrophication control, waste-water treatment, and consumption by humans, livestock and aquatic organisms (see Goldman 1979a for an excellent discussion of the history and applications of microalgal culture; also see Kinne 1977). At one time, mass culture of algae was seen as a solution to the world protein shortage and numerous other global problems (Burlew 1953). In most cases, however, large-scale microalgal production has been found to be economically infeasible due, in large part, to the expense and difficulties associated with separation of the cells from the culture medium and processing (drying, freezing, etc.; Droop 1975, Becker and Venkataraman 1980, Venkataraman et al. 1980).

An exception is the cultivation of microalgae to feed aquatic organisms, i.e., its use as feed as opposed to food. In most cases, harvesting and processing is not necessary (Droop 1975, Taub 1975). Microalgae, still suspended in their culture medium, can be fed directly to the primary consumer although circumstances may make some type of processing advantageous (see Fox 1983, Sommer et al. 1990). Hence, if the economic value of the “target species” is great enough, large-scale culture of microalgae can be economically feasible. Unless otherwise noted, “microalgal culture” will be used in this paper to refer to the large-scale cultivation of algae to feed aquatic animals.

2.1 Microalgae as Feed

Although much effort has been expended trying to find substitutes, microalgae remain prominent in the culture of many aquatic animals, especially marine species (Horstmann 1985) (Table 1). For example, some species of fish consume algae as adults and/or benefit indirectly from the presence of algae in their tanks (Jones 1970 cited in James et al. 1988; also see Stanley and Jones 1976, Buri 1978). Additionally, the intensive larviculture of many species of marine fish depends on a large supply of rotifers, which are usually raised on microalgae. Finally, tremendously large volumes of algae are required for the nursery culture of bivalve molluscs, while smaller amounts are used in the larviculture of commercially valuable molluscs and crustaceans. This paper will focus on the culture of phytoplankton for bivalve and shrimp hatcheries and in connection with the production of rotifers.

Figure 1. The complex problem of designing live feeds production systems.
2.2 Problems and the Need for Design Studies

Of the work that has been done in the area of design optimization, most has resulted in small, laboratory-scale units that can not be scaled up to accommodate the needs of a commercial operation (but see Canzonier and Brunetti 1975). In fact, even descriptions of the design and operations of large-scale microalgal production units are relatively scarce. As a first step toward designing better production facilities, aquacultural biologists, engineers and economists need to take a critical look at existing systems, their yields, operating costs, reliability, etc. and consider ways of improving performance through better design. Only in this manner can we prevent repeating the mistakes of the past.

Even the production of a relatively small amount of algae can demand substantial resources. For instance, Taub (1975) estimated that even though millions of larval bivalves can be reared on a few grams of algae, "as much as 20 - 40% of the shellfish hatchery may be devoted to algal culture" (p. 1). Estimates like these inspired Ukeles (1980) to describe large-scale production of microalgal biomass as a "serious impediment to the future development of shellfish aquaculture" (p. 288).

To be more specific, the most important problems encountered in the large-scale production of microalgae as feed for bivalves, crustaceans and rotifers may be classified as either 1) economic in nature, or 2) related to the dependable output and consistent quality of large volumes of algae (Ukeles 1980). With regard to the first, De Pauw et al. (1984) estimated that monospecific algal cultures produced indoors or in a greenhouse range in cost from US$120 - 200/kg dry weight. While costs may be lower for some operations, there is no question that commercial production costs for phytoplankton are high (Taub 1975; Ukeles 1976, 1980; Laing and Utting 1980; Persoone and Claus 1980; Horstmann 1985, De Pauw and Persoone 1988; James et al. 1988).

The economic woes of cultivators stem from the fact that most microalgal culture today is labor intensive and requires a great deal of space (inside and/or outside). Additionally, the cost of energy (for lighting, pumping, aeration/mixing and heating/cooling) and nutrients is high. De Pauw and Persoone (1988, also see Helm et al. 1979) reported the following cost breakdowns for culturing algae by the bloom induction technique: labor (50 – 85%), pumping (4 - 24%), nutrients (4 - 20%) and mixing (5 - 8%) of the total production costs. Better system designs could help reduce expenditures. For example, money could be saved through design features that reduce labor costs by improving the efficiency of algal transfer during scale-up and harvesting (feeding the primary consumers). Automation is another means of reducing labor requirements. For example, computer technology has recently been used to facilitate automation in microalgal cultures (Hill et al. 1985, Rusch 1989, Wangersky et al. 1989). Finally, systems should be designed to promote maximal growth with minimal expenditures for energy, nutrients, labor, materials and space (Table 2).

The problem of reliability has two components: maintaining a regular supply, and producing algae of consistently high quality. All live cultures are subject to occasional failure ("crashes"). We can consider two types of culture failure: one in which the organisms do not multiply as predicted, and another in which the entire culture simply dies.
Table 2. Some of the problems associated with microalgal culture and examples of potential design-related solutions.

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<thead>
<tr>
<th>Problems</th>
<th>Potential Design-related Solutions</th>
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<tr>
<td>COST</td>
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<tr>
<td>Labor intensive</td>
<td>■ Improvements in the efficiency of transfer/harvesting</td>
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<tr>
<td>High energy costs</td>
<td>■ Designing more cost-effective culture enclosures and techniques; e.g., optimizing culture depth, flow rate/residence time, mixing regime, etc.</td>
</tr>
<tr>
<td></td>
<td>■ Automation</td>
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<tr>
<td>High cost of nutrients /culture media</td>
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<tr>
<td>RELIABILITY</td>
<td></td>
</tr>
<tr>
<td>Regularity of supply</td>
<td>■ Designing systems over which culturists have the highest degree of control while balancing costs</td>
</tr>
<tr>
<td>Consistency of quality</td>
<td>■ Consider designing and implementing larger, more cost-effective continuous culture devices</td>
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Either would result in starvation of the target species for some period of time. Depending on the particulars of the operation, the consequences could be minor, or they could be devastating. Many facilities often keep back-up cultures, an expensive, but prudent precaution. The frequency of culture collapse may even prohibit the large-scale production of certain species of algae altogether: “a major handicap in the cultivation of algae is our inability to grow selected species with known food value in substantial volumes (hundreds of m³)” (De Pauw et al. 1984 p. 126). Similarly, De Pauw and Pruder (1986) state that “the consistent production of large quantities of desirable algae remains an elusive goal” (p. 95). Note that these authors previously defined a “modest” algae requirement as 5 x 10^{12} cells/day.

As one might imagine, the likelihood of crashes increases as the amount of control a culturist has over factors such as illumination, temperature, nutrient quantity, etc. decreases. Hence, crashes are particularly problematic in outdoor cultures, where one usually has little control over these important variables:

“Upscaling the cultures... to larger volumes (mostly in outdoor enclosures) and thus leaving the artificially protected environment of (semi) sterility, rapidly leads to collapse of the culture or take-over by other species better adapted to the prevailing outdoor conditions” (De Pauw et al. 1984 p. 126).

With regard to culture quality, the nutritional value of algae (when grown in non-continuous systems) changes with the state of the culture. Culture age/growth phase, light intensity, temperature, nutrient limitation and source, and cell density can all affect the chemical composition of algae (De Pauw and Persoone 1988, also see Volkman et al. 1989). The problem of consistent nutritional quality is also especially pronounced in outdoor cultures. So, we must design systems that, in addition to producing the required volume of feed in an economical fashion, can turn out a high quality product with a high degree of reliability (Table 2).
2.3 General Principles

2.3.1 Upscaling

Most commercial-scale production today is probably unialgal semi-continuous or batch culture. Algae from stock cultures are “grown up” in successively larger enclosures until harvest. The following description of The Oceanic Institute’s (OI) shrimp hatchery algae upscaling protocol demonstrates some important principles (also see Ukeles 1971 pp. 55-59, Guillard 1975 pp. 120-122, Fox 1983 pp. 22-37, Treese and Yates 1988 pp. 37-42 and Hoff and Snell 1989 pp. 28-33).

Stock cultures of the diatom Chaetoceros gracilis are maintained in screw-top test tubes in a 22°C room. Test tubes containing 10 ml of a mixture of nutrient medium and filtered seawater are inoculated with one drop of the stock culture and allowed to grow for three days; illumination is provided with fluorescent tubes at all stages. In this time, the cells will have multiplied, reaching a density of approximately two million cells/ml. The contents of one test tube are then used to inoculate a sterile 500-ml flask to which enriched seawater has been added. After two days, there may be as many as 3 - 5 million cells/ml in the flasks. One flask is enough to inoculate a 20-liter carboy. Carboy cultures are also grown for two days, to approximately two million cells/ml. Finally, 15 - 20 liters of a carboy culture are added to one 150-liter cylinder. Two more days are needed to bring the density back up to two million cells/ml (for a total of nine days from stock to final culture), at which time the cylinder can be harvested to feed Penaeus vannamei larvae.

While upscaling, it is crucial to inoculate a new container with a sufficiently large amount of algae. De Pauw and Persoone (1988) recommended starting with 5 - 10% of the total volume, or an initial concentration of $10^5$ to $10^6$ cells/ml. There are two reasons for this: to ensure rapid population growth; and for open cultures, to prevent unwanted species of algae, zooplankton, protozoa and/or bacteria from outcompeting, grazing, or otherwise harming the desired species of algae. Moreover, phytoplankton should be transferred while in the log phase of population growth to ensure rapid multiplication (see discussion below).

Another vital aspect of upscaling is the preparation of culture vessels. Although time consuming, this step is necessary to help prevent contamination in closed systems and to forestall serious contamination troubles in open systems. Hoff and Snell (1989) for example, recommend that small containers be 1) washed in detergent, 2) rinsed in hot water, 3) acid cleaned with 30% muriatic acid, 4) rinsed again in hot water, and 5) dried before use. Other culturists sterilize their test tubes, flasks, and even carboys in autoclaves or by other means. Because vessel preparation is so time consuming, disposable culture vessels such as sturdy polyethylene bags are gaining popularity (e.g., Baynes et al. 1979, Trottta 1981).

2.3.2 Population dynamics

Depending upon whom you ask, there are three to five generally recognized “phases” of population growth. For microalgae, these are thought to correspond to the nutritional state of the cells (Droop 1975). First is the lag phase in which cells in culture have just begun to absorb the nutrients present in the medium. Reproduction is slow, as is net population growth. Upon absorption of nutrients, the population enters the log phase (or exponential phase) in which reproduction is extremely fast (population growth is exponential). The
transitional phase or phase of declining growth comes next. Net population growth proceeds, but at a slower pace (some authors may refer to this as the late log phase). Finally, the stationary phase, in which there is no net population growth, follows the growth phases. If the culture is allowed to continue, cell death will follow (some authors consider this to be a separate phase).

Generally, it is best to harvest cells during the log phase, and to use these cells as inocula for other cultures. Log phase inocula will divide more rapidly than cells taken from other phases, thus they yield cultures that are, in general, more viable. Also, the biochemical composition, and hence the nutritional quality of algae varies with the stage of population growth. For example, Flaak and Epifanio (1978, cited in De Pauw et al. 1984) reported that log phase cells contain a relatively greater proportion of protein than cells in other growth stages, and that stationary phase cells have a higher proportion of carbohydrates.

2.3.3 Types of culture

Microalgal cultures may be coarsely divided into indoor and outdoor systems. Indoor cultures typically produce small volumes of algae under controlled conditions. There is some overlap. For example, the early stages of large, outdoor unialgal cultures are almost always grown indoors where it is relatively easy to prevent takeover by predators, competitors and disease. Furthermore, illumination, temperature, and nutrient levels can all be controlled within strict levels, allowing for very predictable growth. In general, then, the specifics of culturing microalgae indoors have been worked out. This is in sharp contrast to outdoor mass culturing:

"One of the major disappointments of algal mass culturing has been an inability to control algal speciation in outdoor cultures, except in unique chemical environments ... the large size and openness of outdoor algal systems makes it virtually impossible to inoculate with and maintain a desired species in culture for extended periods" (Goldman 1979a p. 14);

and,

"... virtually all attempts to grow specific algal species outdoors for sustained periods have failed ... due to the rapid generation periods of algae, certain species tend to dominate through natural selection regardless of which alga is used as an inoculum. Invariably, the weed species such as Chlorella, Scenedesmus and Micractinium in freshwater culture and Phaeodactylum and Skeletonema in marine systems tend to become dominant over time" (Goldman 1979b p. 134, also see Goldman and Mann 1980).

One can also distinguish between open and closed cultures (see Ukeles 1980). Closed cultures are maintained in tubes, flasks, carboys, bags, etc. This is in contrast to phytoplankton cultured in uncovered pools or ponds (indoors or outdoors). Open cultures are more readily contaminated:

"The possibility of unsuitable algae, predator populations, and the growth of disease-producing bacteria appearing in the algae is an ever-present danger with the consequence of mortality in the grazing population and/or loss of the food supply" (Ukeles 1980, p. 296).

However, for producing large quantities of algae, open cultures are the only practical systems at this time.

Some indoor, closed cultures are axenic (also referred to as "sterile"): that is, they are free of foreign organisms such as bacteria. Axenic cultures require that glassware, tubing, water, pipettes, nutrient media, etc. all be scrupulously sterilized — care is taken at every step to avoid contamination (see Ukeles 1980 for a detailed description of the maintenance of axenic algal cultures). There is, of
Table 3. The primary types of microalgal culture.

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<th>Culture type</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td>Indoors</td>
<td>A high degree of control (predictable) Cheaper</td>
<td>-Expensive, difficult -More prone to crashes</td>
</tr>
<tr>
<td>Outdoors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed</td>
<td>Contamination less likely Cheaper</td>
<td>-Expensive -Contamination more likely</td>
</tr>
<tr>
<td>Open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axenic</td>
<td>Predictable, less prone to crashes Cheaper, less difficult</td>
<td>-Expensive -More prone to crashes</td>
</tr>
<tr>
<td>Nonaxenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>Efficient, provides a consistent supply of high-quality cells, automation, highest rate of production over extended periods</td>
<td>-Difficult, usually only possible to culture small quantities, complex, equipment expenses may be high -Sporadic quality, less reliable -Least efficient, quality may be inconsistent</td>
</tr>
<tr>
<td>Semi-continuous Batch</td>
<td>Easier, somewhat efficient Easiest, most reliable</td>
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course, an increased expense associated with sterilization; however, axenic cultures may be less prone to failure and are essential for many types of algal research. Furthermore, some primary consumers may be successfully cultured only with axenic microalgae. However, it is impractical for commercial operations to produce large volumes of microalgae axenically.

Axenic and nonaxenic cultures notwithstanding, we shall consider the following three basic types of phytoplankton culture: continuous, semi-continuous and batch. Droop (1975) defines the two most common types of continuous culture (chemostats and turbidostats) as “steady-state continuous flow cultures in which the rate of growth is governed by the rate of supply of the limiting nutrient” (p. 71). These are delicately balanced systems, often axenic, in which the culture organisms are harvested continually and receive constant nutrient replenishment. It is very important to adjust the rate of “wash out” in continuous systems so that the rate of harvest is a bit slower than the maximum specific growth rate (Taub 1970, Gold 1973).

Turbidostats simultaneously harvest algae and add fresh medium when cell counts in the culture vessel exceed a certain level. Hence, the culturist sets the cell density at a certain value, and the wash out rate is continually adjusted automatically to keep the density from changing (see Sorgeloos et al. 1976, Laing and Jones 1983).

Chemosstats, by contrast, act on the principle of limiting a vital nutrient, such as nitrate. When the concentration of that nutrient drops below a certain level, a fixed quantity of medium containing algae is removed, and a fixed quantity of nutrient is added. Hence, it is the growth rate (regulated by the supply of a limiting nutrient), not the cell density, which remains constant in chemostats (see Droop 1975, James et al. 1988).

There are many advantages to continuous algal cultures, including a steady supply of high-quality, log-phase cells; a greater rate of production; and automation (Table 3) (also see Taub 1970, 1975, 1980; Droop 1975). Furthermore, continuous cultures, like axenic cultures, may be preferred for research purposes. From a commercial production standpoint,
however, illumination and temperature must be maintained within tightly defined limits, hence these systems are almost always housed indoors (but see Camacho et al. 1990). This is one reason continuous cultures are, for the most part, only feasible for the production of relatively small amounts of microalgae. Advances are being made, however, in the adaptation of continuous culture technology to the large-scale production of microalgae. James et al. (1988), for example, report on an indoor, vertical chemostat system, five 200-liter “translucent tubes”, with which they achieved extremely high yields. It is unclear, however, how long the authors were able to maintain those yields.

If a great deal of algal biomass is required, semi-continuous or batch culture is usually employed. In semi-continuous cultures, a given population is allowed to grow until it reaches a desired cell density. Then it is partially harvested, and fresh medium is added. The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable, especially outdoors. Competitors, predators and/or other contaminants and metabolites eventually build up, rendering the culture inviable. A further drawback is the variability in the nutritional quality of the cells produced.

The alternative is batch culture, complete harvest when the population reaches its maximum or near-maximum density. The entire volume may not be needed at once, so it may take several days to harvest a tank. Used both indoors and outdoors, this technique is considered by many to be the most reliable method of algal production. Once a working protocol has been established at a site, there is little uncertainty about how long a tank will last (even outdoors), and contamination is less troublesome than it is in the older stages of semi-continuous cultures. However, because a given tank is harvested completely, it will yield less algae than will a tank of the same size run semi-continuously. This is one reason batch culture requires more tanks than semi-continuous culture. Also, the quality of cells produced in batch cultures is not as predictable as those produced in continuous cultures (Taub 1970, 1980). One important variable may be the timing of harvest. For example, Liao et al. (1983) reported that Skeletonema costatum batch cultures harvested in the morning were better feed for shrimp larvae than cells that were harvested in the afternoon of the same day.

The decision to use semi-continuous or batch culture may depend on many factors, among them: experience, level of quality and consistency needed, available space and facilities, the target species, the algal species and site characteristics. Some environments are more stable than others with regard to temperature, illumination, water quality and other variables, hence they may be more suited to semi-continuous culture than less stable sites. The trade-off seems to be one of reliability. Semi-continuous systems can realize savings over batch systems in that fewer tanks and labor are needed to produce the same volume of algae. Finally, Taub (1970) contends that batch cultures are less efficient than continuous cultures because a given population must be maintained long after the maximum specific growth rate has been attained (and growth rate is declining). See Table 3 for a list of advantages and disadvantages of the major types of algal culture.

Less common culture types include single-species or multi-species systems (e.g. Hirata 1974, 1979; Pruder 1975; Pruder and Bolton 1978; Hirata et al. 1983) that employ
feedback culture. The waste products of primary and/or secondary consumers provide nutrients for the algae. These may or may not be closed recirculating systems that have the added benefit of conserving water (Epifanio et al. 1975 cited in Ukeles 1980). Finally, bloom induction may also be considered as a different culture type (see discussion below).

2.3.4 Species

*Chlorella, Chaetoceros, Isochrysis, Nannochloropsis, Dunaliella, and Tetraselmis* are the genera of algae most commonly cultured for aquacultural purposes (also see Persoon and Claus 1980, Ukeles 1980, Laing and Millican 1986, and Loosanoff and Davis 1963 for lists of species commonly used to feed bivalve larvae; see Liao et al. 1983 for a discussion of species choice for penaeids; and see Guillard 1975 and De Pauw and Persoon 1988 and Gladue, this volume for a listing of some commonly cultured species and the classes to which they belong). Species are usually chosen on the basis of size, nutritional value and ease of culture. A species must also be non-toxic. Naked flagellates like *Isochrysis galbana* seem to be particularly good feed for bivalves (Ukeles 1980), whereas taxa with thick cell walls (like *Chlorella*) apparently cannot be digested by bivalves. Moreover, mixed diets containing several species of microalgae have been reported to give better results for some organisms (Davis and Guillard 1958 cited in Brown et al. 1989, Hu 1990; but see Laing and Millican 1986).

Not all species lend themselves to large-scale culture, hence those that are both nutritious and relatively simple to culture tend to be used most frequently (see Witt et al. 1981). Also, some species or geographic strains within species are better suited to certain geographic regions. For example, for the nursery culture of bivalves, Christine Claus (1981) lists *Skeletonema costatum, Phaeodactylum tricornutum, Tetraselmis suecica* and *Pavlova lutheri* as species that have been successfully mass-cultured in temperate climates. *Bellerocchia spinifera, Chaetoceros simplex, Thalassiosira pseudonana* and *Isochrysis galbana* (Tahiti strain), on the other hand, have been grown in subtropical conditions.

Not only must a culturist decide between species of algae, there can also be significant differences between strains (genetically distinct groups within species), or even clones (genetically identical descendants of one asexually reproducing organism). For example, some algae have been selected to grow in the absence of so-called “essential” vitamins (Ukeles 1976) or under some other stress. Other strains have simply been found to exhibit good or poor growth under certain conditions. That intraspecific variation can be great is attested to by the fact that researchers often report the strain and/or clonal type of the species studied, along with the source of the stock culture.

2.3.5 *A history of the culture of phytoplankton as an aquacultural feed*

In 1910 Allen and Nelson cultured diatoms monospecifically to feed a variety of invertebrates (Ryther and Goldman 1975) whereas Bruce et al. (1939, cited by Ukeles 1980) are reported to have been the first to isolate and maintain unialgal cultures (in this case *Isochrysis galbana* and *Pyramimonas grossii*) to feed oyster larvae.

The first successful attempts at culturing microalgae for shrimp were carried out to feed *Peneaus japonicus* larvae. Dr. Fujinaga (also known as “Dr. Hudinaga”) of Japan pioneered two very different techniques (detailed in De Pauw and Prude 1986). With the first method,
a desirable species of algae was isolated from natural seawater and then, with the addition of nutrients, light and air, allowed to multiply. For shrimp, Fujinaga grew mainly the diatom Skeletonema costatum. This is probably how most microalgae are grown in aquaculture today. Under the correct conditions, this method can produce large, fairly dense, unialgal cultures.

The second technique has been termed bloom induction. Dr. Fujinaga adopted this method in 1946. Addition of inorganic fertilizer to coarsely filtered, illuminated, natural seawater was found to result in multispecific algal blooms. Similar techniques were also used by Loosanoff (1951) and Loosanoff and Davis (1963) for feeding bivalves. Originally, fertilizer was added directly to the shrimp rearing tanks. Not only did algae grow, but with time, populations of zooplankton such as rotifers and copepods would often become established. These organisms were ideal feed for the later omnivorous shrimp stages, the late zoeae and mysis (also see Yang 1975).

One variation on this theme is the "Wells-Glancy method" used early in the history of bivalve larviculture (Glancy 1965, cited in Ukeles 1980). Natural seawater was coarsely filtered either by centrifugation or by passing it through fine mesh cotton bags. It was then placed in a greenhouse-type enclosure, whereupon blooms of plankton eventually resulted (fertilizer was not added, see Figs. 2 and 3 in Ukeles 1980).

As one might imagine, the bloom induction and Wells-Glancy techniques are much easier and cheaper than monospecific culture. In general, though, the lack of control over the species composition of induced blooms makes this technique less reliable, hence the predominance of unialgal culture. Some research in Belgium, however, has produced promising results for the bloom induction technique. Growing feed for juvenile bivalves, researchers were able to control the dominant phytoplankton species by manipulation of culture conditions, especially nutrients, while at the same time avoiding serious infestation by zooplankton (De Pauw et al. 1983, De Pauw and de Leenheer 1985; cf. Dunstan and Tenore 1974, Srna 1976, Goldman and Mann 1980, Riva and Lelong 1981).

2.4 General Requirements and Considerations

The most important parameters regulating algal growth are nutrients, light, pH, and turbulence (Persoon and Claus 1980). In this section, we will discuss all of these factors, in addition to temperature, salinity, monitoring, equipment and supplies. In most cases, the "most optimal" parameters as well as the tolerated ranges for these variables are species specific, hence only broad generalizations can be made here. It should also be noted that the effects of factors such as nutrient quantity and quality, pH, salinity, temperature, light and turbulence are often interdependent. For example, a parameter that is "optimal" for one set of conditions is not necessarily optimal for another.

2.4.1 Culture medium/nutrients

Most of the microalgae grown as feed for commercially important aquatic animals are marine or brackishwater species. Hence, they require a culture medium with a chemical composition similar to that of seawater. Water containing toxins such as oils, pesticides, organics, or unchelated heavy metals should be avoided. For most outdoor mass cultures, natural seawater is the only economically feasible culture medium. Seawater used in
both indoor and outdoor cultures is usually filtered to rid it of algae, zooplankton, and protozoa. The degree of filtration may be site-specific (Fox 1983), however, water for outdoor cultures is usually not filtered as finely as that destined for indoor use. The former is commonly passed through a filter ranging in size from 2.0-0.4 μm, while the latter may be filtered down to 0.2 μm or less. Additionally, water for indoor cultures may also be sterilized via an autoclave, UV radiation, or some other means. This reduces the possibility of culture collapse due to bacterial contamination. For small operations, it may be preferable to use artificial seawater (see Ukeles 1976, 1980; Kaplan et al. 1986; Boussiba et al. 1988), depending on the need for uniformity, the quality of the natural seawater available and the amount of money available.

Besides carbon, the principle nutrients phytoplankton require are nitrogen and phosphorus, in an approximate ratio of 6:1 by weight, respectively (e.g. Valero et al. 1981). Additionally, diatoms require silicate. Trace minerals (iron, copper, zinc, cobalt, manganese, and molybdenum) and vitamins (especially B12 and thiamine, and sometimes biotin) can also be added, and are necessary in most axenic cultures. Other additives like chelating agents may also be used (see Ukeles 1976, Fabregas et al. 1987), but they are usually expensive. Whether or not these are used will likely depend upon the species of algae to be grown, the cost-effectiveness of using the additive, and the philosophy of the culturist. It should also be noted that there are now commercially available nutrient solutions that can reduce preparation labor.

Numerous published descriptions of algal nutrient media exist; however, one of the most recent and comprehensive is that compiled by Borowitzka (1988). Additionally, Guillard (1975) provides detailed instructions on making stock solutions for the major elements, trace elements, vitamins, etc. and for making soil extract (see also Ukeles 1976). Finally, see Fox (1983) for a detailed discussion of enrichment media used in shrimp hatcheries.

The OI shrimp hatchery uses F/2 Guillard’s medium (Guillard 1975) containing NaNO₃, NaH₂PO₄ x H₂O, and Na₂SiO₃ x H₂O as the source of major nutrients, along with a commercial vitamin mix, EDTA, and sodium metasilicate. However, for economic reasons, the complexity of the culture medium is usually inversely proportional to the size of the culture volume. Thus, large, outdoor cultures are usually supplied with only the barest essentials, and agricultural-grade rather than laboratory-grade fertilizer is often used (see Gonzalez-Rodriguez and Maestrini 1984).

2.4.2 Light

Light is the source of energy which drives photosynthesis. In order to maximize yield, one must maximize the efficiency with which available light is converted into algal biomass. Maximum culture depth and cell density are the key variables regulating light utilization efficiency (Roels et al. 1977, Goldman 1979b, Richmond et al. 1980).

With respect to the illumination regime to be used, intensity, spectral quality, and photoperiod are all important considerations. Indoor microalgal facilities usually have fluorescent tubes (80 watts is common) arranged to provide maximal illumination of the culture vessels (although the heat produced by the lights must also be taken into consideration). Hoff and Snell (1989) state that an intensity in the range of 2500 - 5000 lux is optimal, and Guillard (1975) recommends 3500 and 4500 lux for stock cultures of Thalas-
*siosira pseudonana* under continuous and 14 hours/day illumination, respectively (see Table 4). Plants are known to utilize only a portion of the spectrum of visible light (PAR). “Cool White” fluorescent bulbs are commonly used.

Cultures may be kept in continuous light, or timers may be used to maintain a fixed schedule of light and dark hours (photo-period). The amount of incident light a culture needs depends on its cell density and increases as populations increase (Guillard 1975). Of course, outdoors the natural sunlight and photoperiod is used. However, direct sun may not be tolerated (see Pruder and Bolton 1978), depending on the intensity of the sun (which, in turn, varies with season and weather conditions), the species or strain being cultured and the density of the culture (Guillard 1975).

When, during upscaling, an algal culture is transferred outdoors, the cells often suffer from photic shock. This is because they are not adapted to light of such a high intensity, and they require a period of time to adapt. On the other hand, in greenhouses, supplemental light may be needed (e.g. Loosanoff 1951, Loosanoff and Davis 1963). Also see Lorenzen (1980).

### 2.4.3 pH

A hydrogen ion concentration (pH level) that is too high or too low will slow algal growth by disrupting cellular processes. The optimum pH range for most of the species cultured falls between 7 and 9. The “most optimum” range, furthermore, is reported to be 8.2 - 8.7 (Ukeles 1971) (Table 4). However, Kaplan et al. (1986) found that as long as the concentration of Fe$^{3+}$ was high enough, *Isochrysis galbana* grew equally well within the range of 5.0 - 9.0 pH.

Complete culture collapse can result from a failure to properly monitor and maintain an acceptable pH. Fortunately, this is easily accomplished in moderately dense cultures through aeration, a process which serves other purposes as well (see below). The addition of carbon dioxide, naturally present in air, serves to increase the buffering capacity of the culture medium and prevent the pH from getting too high (becoming too alkaline, see below).

| Table 4. A generalized set of conditions for culturing microalgae. |
|--------------------|-------------------|-------------------|
| **Parameters**     | **Range**     | **Optima**        |
| --                 | 16 - 27$^1$    | 20 - 24, 18 - 22$^2$ |
| Temperature (°C)  | 12 - 40 (for neritic flagellates)$^3$ | 20 - 24$^3$ |
| Salinity (ppt)     | 1,000 - 10,000 (depends on vol., density)$^2$ | 2,500 - 5,000 |
| Light intensity (lux) | 7 - 9       | 16:8 (minimum)$^1$ |
| Photoperiod (light hours:dark) | 7 - 9     | continuous (maximum)$^1$ |
| pH                 |                 | 8.2 - 8.7$^4$     |

$^1$Hoff and Snell (1989).
$^3$Ukeles (1976).
$^4$Ukeles (1971).
2.4.4 Aeration/mixing (turbulence)

Aeration is beneficial for three reasons. First, air is a source of carbon in the form of CO₂, which is fixed during photosynthesis. Second, adding CO₂ provides essential pH stabilization. CO₂ addition must keep pace with assimilation, or else the pH of the medium will rise. This is because the balance maintained between bicarbonate, carbon dioxide, and hydroxide ions [HCO₃⁻ ↔ CO₂ + OH⁻] serves to buffer the water against pH changes. Air, which is approximately 0.03% CO₂ by volume, may or may not be enriched with additional CO₂ before it is added to a culture. The need for extra CO₂ will depend on the density of the culture, pH, light intensity and growth rate (Ukeles 1971), but because of the expense, CO₂ is usually supplemented only in extremely dense cultures.

The third benefit derives from the fact that for many cultures, aeration is the sole means of mixing. Agitation is essential for a host of reasons (see Goldman and Ryther 1977, Goldman 1979b, Persoone et al. 1980, Richmond et al. 1980, Terry and Raymond 1985, Oswald 1988). It keeps both nutrients and cells evenly distributed, promoting uniform nutrient and light absorption. The supply of light is often a limiting factor in dense cultures, and mixing helps decrease the loss of production due to self-shading and/or photo-inhibition (a decrease in photosynthesis due to an excess of light). For outdoor cultures, an adequate level of mixing can also prevent thermal stratification, and the resulting precipitation of heavy metals, as well as the settling and subsequent decay of organic matter. See Persoone et al. 1980 for descriptions and results of experiments with continuously mixed, discontinuously mixed, and non-agitated *Chlorella saccharophila* cultures. Mixed cultures yielded approximately 30% more algae than non-mixed cultures. Also see Molina et al. 1990.

Not all species can tolerate vigorous mixing, however. The intensity of agitation should be adequate but not so intense as to slow or prevent growth. Guillard (1975) recommends gentle aeration for a day or two after inoculation, followed by increasing amounts of mixing as the culture grows.

Bubbling air as a means of mixing may be more appropriate for small-scale cultures than for larger ones (Persoone et al. 1980). Note, however, that there can be a trade-off between the efficiency of mixing and the efficiency of aeration. Large bubbles achieve the highest degree of mixing, whereas small bubbles are best for diffusing gases to a liquid medium.

Common alternatives for large vessels include jet pumps, paddle wheels, continual circulation of the water mass and air-lift pumps. Vessels that are shaped differently will likely have different optimal means of ensuring adequate aeration and mixing:

"... for each type of culturing unit the most economic aeration regime for which the algal output is maximal, should be determined" (Persoone et al. 1980 p. 520).

2.4.5 Temperature

When nutrients are present in excess, temperature and illumination are the sole limiting factors in algal cultures (Goldman 1979b). However, Goldman (1979b) indicated that temperature is not as important as sunlight in controlling productivity. Furthermore, Payer et al. (1980) investigated the direct and indirect effects of temperature on a number of species and strains of algae in order to select those that would be suitable for production in Thailand. In addition to finding strain-specific results, they concluded that
Temperature, however, can be important in determining which species will predominate in open, outdoor cultures (Goldman and Ryther 1977, Goldman 1979a, Goldman and Mann 1980, De Pauw et al. 1980, and Witt et al. 1981).

With regard to the effect of temperature on growth, temperature tolerance may vary with the nutritional composition of the medium, the species and the strain cultured (Table 5) (Ukeles 1976). Each species is regarded as having minimum, maximum, and optimum temperature ranges for growth. In general, though, the most commonly cultured species of microalgae tolerate temperatures between 16 and 27°C. Twenty to 24°C may be considered “optimum” (Guillard 1975, Hoff and Snell 1989) (Table 4). Temperatures lower than 16°C will slow growth, but those higher than 35°C are lethal for a number of species (Hoff and Snell 1989). For outdoor cultures, it is very important to choose a species of algae that will tolerate the range of temperatures likely to prevail at the culture site. For nonaxenic, indoor systems like that at the OI shrimp hatchery, temperatures may be kept somewhat below the optimum level for algal growth in order to discourage the growth of bacteria. This may be accomplished by several means, for example, air conditioning the culture room and/or using water baths.

According to Ukeles (1971):

“The optimum temperature for growth will vary with species, to some extent, and 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” (p. 58).

Finally, Kaplan et al. (1986) determined that the optimal temperature for culturing a strain of *Isochrysis galbana* — that with which they achieved the highest yield — was 27°C, whereas temperatures above 32°C and below 19°C caused a marked reduction in yield.

### 2.4.6 Salinity

The tolerance of marine phytoplankton to changes in salinity is considered to be extremely broad. Most species grow best at a salinity that is a bit lower than that of their native habitat. Tolerated and optimum salinities have been investigated by a number of authors for

<table>
<thead>
<tr>
<th>Species</th>
<th>No Growth</th>
<th>Growth less than control</th>
<th>Growth equal to control at 20.5°C</th>
<th>Growth less than control</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Monochrysis lutheri</em></td>
<td>8 - 9</td>
<td>12</td>
<td>14 - 25</td>
<td>27</td>
<td>29 - 35</td>
</tr>
<tr>
<td><em>Isochrysis galbana</em></td>
<td>8 - 9</td>
<td>12</td>
<td>14 - 22</td>
<td>24 - 25</td>
<td>27 - 35</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>—</td>
<td>—</td>
<td>8 - 24</td>
<td>27</td>
<td>29 - 35</td>
</tr>
<tr>
<td><em>Dunaliella euchlora</em></td>
<td>8 - 9</td>
<td>—</td>
<td>12 - 35</td>
<td>—</td>
<td>39</td>
</tr>
<tr>
<td><em>Platymonas (= Tetraselmis sp.)</em></td>
<td>—</td>
<td>8 - 9</td>
<td>12 - 32</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td><em>Chlorella sp.</em> (isolate #580)</td>
<td>8 - 9</td>
<td>12</td>
<td>14 - 35</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Chlorella sp.</em> (UHMC isolate)</td>
<td>8 - 9</td>
<td>12</td>
<td>14 - 29</td>
<td>—</td>
<td>32 - 35</td>
</tr>
</tbody>
</table>

Table 5. Growth response of different microalgal species at various temperatures (°C) (from Ukeles 1961).
a variety of commercially important species (also see Table 4). For example:

Most neritic flagellates, (those inhabiting the relatively shallow waters over the continental shelf), are reported to grow in salinities ranging from 12 - 40 ppt., but 20 - 24 ppt. is optimal (Ukeles 1976; also see Duerr and Mitsui 1982). *Isochrysis galbana* grew well within the range of 5 - 60 g/liter NaCl (15% the salinity of seawater to twice the salinity of seawater; Kaplan et al. 1986). Additionally, Fabregas et al. (1984) concluded that good *Tetraselmis suecica* growth occurred between 25 and 35 ppt. (coupled with concentrations of 2 - 8 mM NaNO3). Finally, Laing and Utting (1980) found 15 - 25 ppt. and 25 - 30 ppt. to be optimal salinities for *I. galbana* and *T. suecica*, respectively.

### 2.4.7 Monitoring

"Culture crashes can happen overnight, so it is important to closely monitor cultures for early warnings of a crash like declining cell densities or changes in culture color. Algal cells from declining populations also have little nutritional value for zooplankton" (Hoff and Snell 1989 p. 23).

"High quality control in food cultures may be maintained by frequent observations of cultures, both macroscopically and microscopically, as well as density and pH measurements" (Ukeles 1971 p. 59).

"Color and pH are often guidelines to the condition of the algal culture. If the color appears a somewhat opaque gray and the pH is lower than about 7.5, it is likely that the bacterial population is too high to attempt a rescue of the algal culture" (Ukeles 1980 p. 296).

The aforementioned attest to the fact that monitoring (especially pH, nutrient levels, contaminant levels and density) is a vital component of any algal production system. The primary motivation, of course, is the prevention of culture collapse and the maintenance of high quality live feeds. Cell densities can be monitored in a variety of ways, but Secchi disks and hemacytometers are probably the most commonly used devices. Secchi disks are quick and easy to use, but hemacytometers provide more accurate measurements (also see Valero et al. 1981).

#### 2.4.8 Equipment and supplies

The equipment needed to culture micro-algae is highly dependent on the type and scale of culture. For details on the materials needed to grow axenic cultures see Stein (1973) and Guillard (1975). Guillard provides descriptions and evaluations of a variety of common culture vessels used for growing small, indoor cultures of algae as well as guidelines for the selection and arrangement of heating and cooling units. A list of "various materials," pasteur pipettes, cotton stoppers, etc., required by an indoor algal culture facility is also included.

Justice et al. (1972) tested the effects of different materials (e.g. pyrex vs. kimax glassware, surgical Tygon tubing vs. regular Tygon tubing, etc.) on algal growth. Additionally, the equipment and supplies needed for a small-scale, simple algal culture system are outlined in Hoff and Snell (1989), while Davis (1971) gives some guidelines for the selection of piping, pumps, and heat exchangers.

Regarding the types of large culture containers to be used, tanks, ponds and even commercial swimming pools have been tried successfully:

"Pools and ponds may be natural or man made; the bottom may be natural or lined with cement, asphalt or synthetic materials such as polyethylene or polyvinyl-chloride sheets. Tanks are usually rectangular, square or circular, and built on a solid base, either at ground level or excavated; the commonly used materials are reinforced concrete, fibre glass, plywood, bricks coated with cement,
resins and plastic sheets" (De Pauw and Persoon 1988 p. 206).

Culture depths in such large-scale systems are typically .25 - 1 m but may be greater than 1.5 m. Finally, De Pauw and Persoon recommend that algal culture systems have draining devices and be easy to clean.

2.5 Design Examples

Some examples of algae production system designs will be presented here. The list is not comprehensive, rather it is meant to introduce the reader to some of the many designs which have been used, either experimentally or on a larger scale. Section 2.5.1 describes relatively simple systems, modifications of which have been used since the early days of controlled microalgal culture. The examples in Sections 2.5.2 - 2.5.4, by contrast, have been proposed as improvements to the traditional techniques. In most cases, they were developed to increase production efficiency and/or decrease cost while maintaining reliability and high yields.

2.5.1 Commonly used systems

Indoor, closed algal culture systems are often used to produce relatively small amounts of monospecific microalgae. Depending on the particular system, cultures may be axenic or non-axenic, and they may be run as batch, semi-continuous or even continuous systems. One of the simplest types of indoor culture employs 10 to 20-liter glass or plastic carboys as primary culturing vessels (see Fig. 2). The resulting algae can be used as feed or as inocula for larger cultures. Lighting is provided by fluorescent tubes; the temperature is kept fairly constant; aeration is provided; and a high-grade nutrient medium is used. Several carboys may be kept on shelves, backlit with fluorescent bulbs (Fig. 3).

When more algae is needed, open, semi-continuous or batch cultures are the simplest and most common type of system employed. Tanks ranging in size from .5 m$^3$ to 200 m$^3$ or more can be kept in the open air or in covered sheds or greenhouses. They are inoculated with indoor cultures and provided with agricultural-grade nutrients, natural illumination and aeration. Contamination and fluctuations in sunlight and temperature are potential problems that necessitate keeping backup cultures. To facilitate transfer and harvest, the tanks may be connected to each other and/or to the target species' culture vessels by piping.
2.5.2 Semi-continuous and continuous culture in polyethylene bags

Examples of this type of system are described in Baynes et al. (1979) and Trotta (1981). Baynes et al. (1979) detail the design and operation of a system that employs vertical, 480-liter capacity plastic bags to grow *Pavlova lutheri*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Dunaliella tertiolecta* to feed rotifers. Their culture vessel design is based on that used by Seasalter Shellfish Co. Ltd. (depicted in Farrar 1975). Bags were created from tubing that was heat-sealed on both ends and suspended from a frame. Care is taken to avoid contamination at all steps; sterile medium and air + CO₂ are used.

Good results were obtained with both indoor and outdoor units. The outdoor cultures could be operated semi-continuously for six to eight weeks before contaminants reduced algal growth rates. Improvements over "traditional" culture methods included the fact that the closed cultures were easy to manage and the cost of materials, bags and frames, was low. Additionally, the vessels could be used indoors or outdoors and took up little horizontal space. One drawback was that the relatively large diameter of the bag, 600 mm, prevented the growth of dense cultures because self-shading occurred. Whereas densities of 16,000 *Pavlova lutheri* cells/μl could be cultured indoors in 20-liter (300-mm diameter) flasks, densities of 8,000 and 6,000 cells/μl were the maximum obtained from outdoor and indoor polyethylene bags, respectively.

A similar system was designed by Trotta (1981). This time, however, the bags were
only 50 liters in volume and the algal cultures were run continuously (indoors only) with an automatic supply of culture medium and air. These vessels were small enough to be suspended from hangers; no frame was needed. Benefits were the same as those mentioned above — cheap materials that needed no sterilization and took up little floor space, with the added bonus of automation.

2.5.3 *Internally illuminated vessels*

These containers were designed to provide maximal light exposure to closed, cylindrical cultures. For example, Helm et al. (1979) designed and tested a 200-liter vessel in which they grew *Tetraselmis suecica* semi-continuously. The primary aim was to reduce the labor input per unit volume of algae produced while maintaining reliability. Their container was constructed largely of “white pigmented glass-fibre.” The white surface reflected light that emanated from an inner, transparent acrylic tube. The latter held four 80-watt, 150-cm long, “daylight” fluorescent lamps. After some experimentation, the cultures could be run semi-continuously for more than 70 days, and the amount of labor needed was estimated to be only 3 hours per day for a ten-vessel system. The total cost of production was also estimated for a system having ten vessels, each of which produced 52 liters of *T. suecica* day (cell density: 1,000 cells/μl). This system would be large enough to supply the majority of feed to an oyster hatchery producing one million 5-mm juveniles per month. The cost per 520 liters was £15.64, or by today’s exchange rate, US$8.30.

A modification of this system is that described by Laing and Jones (1988) for the production of *T. suecica* and *Isochrysis aff. galbana* (also see Laing and Jones 1983). This time a 40-liter, internally illuminated, turbidostat vessel was devised in which the algae were contained in “polyethylene tubing supported around a core of six 80 W fluorescent lamps” (see Figs. 4 and 5). *Tetraselmis suecica* cultures could be run continuously for 24-69 days, whereas cultures of *I. galbana* lasted only 18-24 days. Average yields were .56 x 10¹¹ cells/day for 33 days and 5.21 x 10¹¹ cells/day for 21 days for *T. suecica* and *I. galbana*, respectively.

Finally, Knowles and Edwards (1971) described a 330-liter “water-jacketed vat” system in which they cultured a variety of algal species for bivalves. They could be operated either as batch cultures or semi-continuously (the latter was found to be more economical). The three internal lights (each 250 watts, 21.1 cm long and 8.9 cm in diameter) were enclosed in glass pockets attached to the lip of the vat. One container provided 780 liters of *T. suecica* (mean cell density: 800,000 cells/ml) over a two-week period. Prolonging the life of these semi-continuous cultures resulted in algae that was unsatisfactory in quality.

2.5.4 *Other continuous systems*

Canzonier and Brunetti (1975) reported on an unusual system in which they produced *Phaeodactylum* and *Chlorella* continuously for more than eight months. They utilized 35 1.5-m long sections of thin-walled, soft-glass tubing, 1-cm internal diameter, connected with Tygon plastic tubing. Illumination was supplied by a bank of fluorescent tubes. Best yields were obtained at a harvest rate of 3-4 liters/day; cell density averaged one million cells/ml. Advantages of this system included the high degree of automation and the ease with which it could be scaled up and adapted to fit a variety of spatial configurations.

Palmer et al. (1975) described a continuous culture apparatus in which they