Services du Premier Ministre
Programmation de la Politique scientifique
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ACTIONS DE RECHERCHE CONCERTEES

ACTION INTERUNIVERSITAIRE

OCEANOLOGIE

Rapport final

Volume 4

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Programmatie van het Wetenschapsbeleid
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GECONCERTEERDE ONDERZOEKSACTIES

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SELECTED TOPICS IN MARINE AQUACULTURE :

Microalgae
Mollusc nursery
Artemia

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TRENDS IN NURSERY REARING OF BIVALVE MOLLUSCS

Christine CLAUS

Abstract.

Nursery rearing of bivalve molluscs, as the intermediate step between the controlled production of larvae in commercial hatcheries and the growth-out in the wild, is a practice which is receiving more and more attention in mollusc farming. The purpose of nursery rearing of bivalves is to culture cultchless spat of a few millimeters in size, up to 1-2 cm, in a minimum of time, in densities as high as possible, and at minimal costs and risks.

In the last decade various technologies for nursery culturing, indoors as well as outdoors, have been developed at different places, ranging from suspended culture in the open sea to controlled onshore culture in upwelling cylinders. This paper reviews the different systems developed until now, with special emphasis on one of the key problems, namely the supply of the right amount of suitable food for the juvenile bivalves, in function of flow rates, stocking densities, and temperature.

The growth and mortality rates of *Ostrea edulis*, *Crassostrea gigas*, *Venerupis semidecussata*, *Mercenaria mercenaria*, *Argopecten irradians* and *Crassostrea virginica*, under nursery conditions is discussed. A few considerations are made with regard to the economic aspects of nursery operations.

1.– Introduction.

Nursery rearing of bivalve molluscs is an intermediate step between the controlled production of larvae in commercial hatcheries and the growth-out of the shellfish in the wild.

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Hatcheries for oyster and clam seed are in operation and have become an essential part of the shellfish farming. Each year millions of juvenile bivalves are produced artificially by known methods and techniques. A review is given by Loosanoff and Davis (1963). Without over-simplifying the matter, one can say that bivalve hatcheries have reached a level at which the developed techniques only need to be optimized.

As a matter of fact, the problems came from maybe an unexpected angle. Indeed, some hatcheries have failed due to the unexperience of growers in handling small cultchless seed and their unwillingness to develop new techniques and equipment. Generally, ongrowers prefer seed of 8-10 mm and larger, instead of the 3 mm spat sold by the hatcheries. This indeed simplifies the counting, the maintenance and reduces the mortality and tray losses. Rearing spat to this size puts, however, considerable pressure on the hatcheries. It is clear that for economic reasons, industrial mollusc hatcheries can barely scale up their very expensive indoor algal production to fulfil the increasing food demands of the ongrowing spat.

This gap between hatchery and on-growing can be bridged by the so called nurseries. In these nurseries, which may considerably vary in their approach to the problem, large quantities of bivalve postlarvae are stocked in protected conditions. These nursery conditions are less sophisticated than those in the hatchery. Therefore the spat can be held longer and at less cost, until it is sold to the ongrowers. The nursery furthermore ensures a more gradual transition from the hatchery to the natural environment, and thus improves the chances of survival. In this regard Bayes (1981) made the following statement : "It is the development of the nursery stage, that has made a sound business of the hatchery production of bivalves". While Le Borgne (1981) reported : "the nursery culture of postlarvae is the key for the further development of bivalve hatcheries".

Various authors consider that successful oyster culture should be based on five subsequent stages, and that it is particularly important not to push any one stage past its limits (Gerard, 1976; Bayes, 1979). This five stages are :

1. the hatchery operation in strictly controlled conditions and leading to the production of stock of maximum 5 mm;
2. a nursery stage with ongrowing in running sea-water under protected conditions to a size of approximately 10 mm;
3. ongrowing in the sea in trays. This stage is considered economically feasible up to a size limit of approximately 25 mm, above this size a less costly method of ongrowing to maturity is essential;
4. ongrowing to maturity using bottom-laying or alternative methods;
5. hardening before marketing by exposure between tides.

In order to illustrate the complexity of the nursery problem and the numerous ways in which scientists as well as commercial people are trying to find solutions to it, this paper briefly reviews the present state of the art of nursery rearing systems.

2.– Biotechnical aspects of nursery culturing and culture devices.

2.1.– *Offshore culturing.*

The biological requirements which must be met by any method for handling young spat, concern primarily the feeding. Bivalves feed by filtering sea-water and extracting mainly phytoplankton. To what extent other solid or soluble substances in the water contribute to the bivalves' diet as well, is not taken into consideration here. To sustain growth of the oysters, they need to be kept in a constant flow of plankton-rich sea-water. This requirement is normally fulfilled in the estuaries in the temperate climatic zone. Consequently to date, nursery culturing is mostly performed with the natural phytoplankton present in the sea-water being the sole source of food for the juvenile bivalves.

The most classic and the oldest technique for rearing post-larval bivalves is simply to place the cultchless seed in some kind of stocking device in a suitable environment. Since seed oysters of 2-3 mm may suffer high mortality, special techniques must be employed to ensure maximal growth and survival. Several methods can be used to achieve these aims :

- intertidal racks arranged on structures on the sea shore;
- trays suspended near the surface from rafts moored in open water;
- trays suspended in mid-water on long lines without costly floating structures;
- upwelling systems.

Shore-based systems can be reached only at low tide, while rafts are usually accessible at all times. Rafts, however, are costly to build and maintain, which must be considered in relation to the value of the crop. In the shore based systems, the position of the racks with regard to the tidal height is critical, since it is a compromise between continuous total immersion, when maximal growth is obtained, and sufficient periods of exposure to enable servicing. For the Pacific oyster *Crassostrea gigas* (Thunberg) it has been shown that exposure to air in the range of 10 to 30 % of the time, led to a marked reduction in growth rate (Spencer et al., 1978).

Plastic meshes are used to retain the oysters in trays which vary widely in design and size. Prefabricated trays which nest on one another have recently become available, and although they are more expensive than light mesh trays, their durability may make them attractive for long-term use.

The majority of the oyster growers, particularly those concerned with Pacific oysters, nevertheless uses rafts of one form or another. The waste of capital in building too large a raft, or cramping of stock on too small a raft, is to be avoided. These problems can be eliminated by using modular designs in which the raft consists of a number of small separate units (E. Hoet, personal communication). If the units are too small an auxiliary boat may be needed for servicing, because the raft does not represent a stable working platform.

The stocking device for oysters must be well designed, to allow an adequate water flow over and around every individual animal, and yet offer protection and support. A few examples of devices for the suspension technique are, the stacked wooden trays with mesh bottom (Spencer and Gough, 1978; Neudecker, 1981), the self stacking plastic trays (Davidson, 1974), the Japanese lantern-nets (Ley, 1978; Shaw, 1981), the "casiers-tiroirs" (Lucas and Gerard, 1981), the plastic mesh bags (Yelf, 1978), and the prefabricated plastic circular trays with a central suspending rope (George, 1975). Bunching of bivalves, particularly of the small spat may be avoided by keeping the support surfaces as flat and horizontal as possible.

Holding small spat in trays represents a particular problem, because the fine retaining meshes tend to become blocked very quickly. In silt-loaden water, fine mud gathers on the meshes, and where siltation is not the problem, biofouling usually is. Therefore, trays with fine mesh may require servicing every few days. This is usually done by periodic air drying of the trays, followed by vigorous flushing with a high volume and high pressure water stream. This method, although efficient, adds significant cost to the grow-out procedure.

Recently trays have been developed with a removable mesh curtain to surround a stack of circular trays. These curtains are easily replaced whenever they begin to suffer from fouling (George, 1975). Alternative solutions need further investigation. Research has been carried out on the biological fouling control in which natural enemies, such as crabs are used to eliminate the biofouling material – usually mussels – (Hidu et al., 1981). Reasonable success has been booked by replacing the plastic nettings with nettings in a Cu-Ni alloy, which greatly inhibits the biofouling on the trays (Huguenin and Ansuini, 1975). Bio-accumulation of these heavy metals in the juvenile shellfish is, in some cases, important, but has been proven to be reversible (Benijts, personal communication).

The classical suspended culture of bivalves in trays is a monolayer practice requiring more than a hundred times as much tray space as compared to the three-dimensional (3D) upflow systems which has been developed for the rearing of spat. The idea behind the upflow technique, is to increase the water flow-rates through the cultures and to meet the food requirements of the shellfish by circulating larger volumes of food-containing medium through the cultures.

The upflow system from which all modern spat-rearing systems have been derived (Bayes, 1979, 1981; Le Borgne, 1980, 1981; Guerrero et al., 1981; Lucas and Gerard, 1981; Williams, 1981) has originally been developed by W. Budge (1970, US Pat. n° 3526209). Originally it has been designed to operate with pumped sea-water. The water is forced to flow upwards through the container with a mesh bottom holding the oysters. The water leaves the container through an overflow at the top of the cylinder, and eventually can be recycled several times.

The advantage of such upflow systems is that they allow culturing of spat piled up in several layers. The young oysters do not stick to the trays, and the faeces and pseudofaeces are not accumulated upon or between the spat and thus do not clogg the meshes of the bottom. A few commercial operations in Scotland and at Guernsey, Channel Islands, apply the upwelling principle in their offshore nursery (Lintell, 1980, 1981; Williams, 1981).

Both systems consist of a raft with a series of upwelling units and air compressors supplying the air flow that operates the airlifts which suck water up through the oyster bed.

This system appears to have many advantages as compared to the ongrowing of seed in trays, especially as far as man-power for maintenance is concerned. An additional benefit can be made by working in sheltered conditions in a natural or man-made closed basin connected to the sea by means of a sluice allowing the controlled exchange of water with the sea at high tide. Both the temperature and the primary production of such enclosures are somewhat higher than in the open sea which results in a faster growth of the spat. Another new application of the upflow technique in the natural environment, is based on the tidal action (Spencer and Hepper, 1981).

2.2.– *Land-based operations using natural phytoplankton.*

Because of the rather difficult manipulation of rafts and floating devices, it is preferable to operate an onshore unit with a continuous flow of natural sea-water through the culturing devices.

If the nursery is located in an area where phytoplankton is abundant, the sea-water can be pumped directly through the nursery. From the technological point of view, two different nursery systems are interesting to cite.

First, the raceway system used in Milford (U.S.A.) and which Rhodes et al. (1981) describe as "an ideal compromise between costly controlled systems and uncertain field plants", and second, the gravity-fed system designed as an extension of the storm-surge barrier in the Netherlands, namely the project MARIOS developed by Drinkwaard (1981).

Persoone and Claus (1980) and De Pauw (1981) emphasized that in most cases the primary production in open sea, even in sheltered areas, is far from sufficient to fulfil the nutritional requirements of several millions of bivalve spat in an onshore nursery-plant with natural sea-water being the sole food source. Therefore, impounding the water and eventually adding fertilizers to the water to induce phytoplankton blooms, is a technique which has been adopted by several commercial firms in Europe (Le Borgne et al., 1978; Bayes, 1981; Lucas and Gerard, 1981; Rabeux, personal communication). The bivalve spat is kept in appropriate culturing units, mostly upwelling cylinders, through which the pond water is directly pumped. Placing the nursery unit proper in a greenhouse can be favorable for the bivalves' growth (Lucas, 1976). Recirculation of the pond water in various percentages is also becoming a more widely spread practice. Another attempt to take full advantage of the eutrophic sea-water was made by Hughes-Games (1977) who introduced oyster trays in the draining channels of a marine fish-culturing station in Israel.

In all cases, however, the operation remains entirely dependent upon incontrollable environmental conditions, and therefore suffers from important differences in growth rate of the bivalve spat, which can be related to the variation in the natural phytoplankton density. Uncontrollable blooms of unsuited or even toxic microalgal species or the excessive growth of macroscopic algae in the pond competing for the nutrients, may be disastrous for this kind of nursery operation.

2.3.– *Land-based operations using cultured phytoplankton.*

With all the shortcomings of the previous systems in mind, a fair number of people, mainly scientists, working in the field of nursery rearing of bivalves, have tried to develop more controlled culturing-systems in which supplemental feeding was given to the bivalves.

The emphasis has been on the use of live algae. Besides its close resemblance to the natural diet, the advantages of this food are that it remains in suspension, and that the cells which are not eaten do not decay and pollute the water. For commercial application, however, the disadvantage of an algal diet resides in the fact that the production of a sufficient quantity of algae is technically the most difficult part of the operation, and when used to feed juveniles it becomes relatively costly (De Pauw, 1981).

Since a long time many attempts have been made to induce blooms of natural phytoplankton in algal tanks of various dimensions, by adding agricultural fertilizers to the sea-water. This technique has been developed and improved worldwide by many mariculturists. Although it looks very easy to do, it has been proven that this method is only reasonably successful, if one keeps a large number of parameters under control. "Unmanaged water is frequently better than mismanaged water" (Bayes, 1979). Details on this subject were given by De Pauw (1981). Recent experiments on nursery rearing of bivalve spat by means of induced blooms were described by Lucas (1976), Riva and Lelong (1978, 1981), Guerrero et al. (1981), Mercer (1981a,b), reflecting the latest evolution in the matter.

A classic example of mass production of marine microscopic algae in outdoor ponds to feed commercially important bivalves, is the series of experiments carried out by the research team of Ryther in the Woods Hole Oceanographic Institution in Massachusetts, U.S.A. (Ryther et al., 1972, 1975; Mann and Ryther, 1977; Mann, 1979a,b; Mann and Taylor, 1981). In all these experiments the algae were cultured in six ponds of 15 m diameter. The cultures were enriched with a continuous addition of secondary treated sewage effluent. The harvested algae overflowed into concrete raceways containing the shellfish. The shellfish were held in plastic trays and the water was heated to 15 and 20°C.

More or less based on this model our laboratory very recently built an onshore nursery rearing-system at pilote scale at the border of the Sluice Dock in Ostend at the Institute for Marine Scientific Research (IZWO). It consists of four outdoor algal tanks of approximately 100 m^2 surface and an indoor nursery-unit. Blooms of natural phytoplankton are induced by adding agricultural fertilizers. The nursery proper consists of four tanks with rows of eight upflow cylinders (figs 1 and 2).

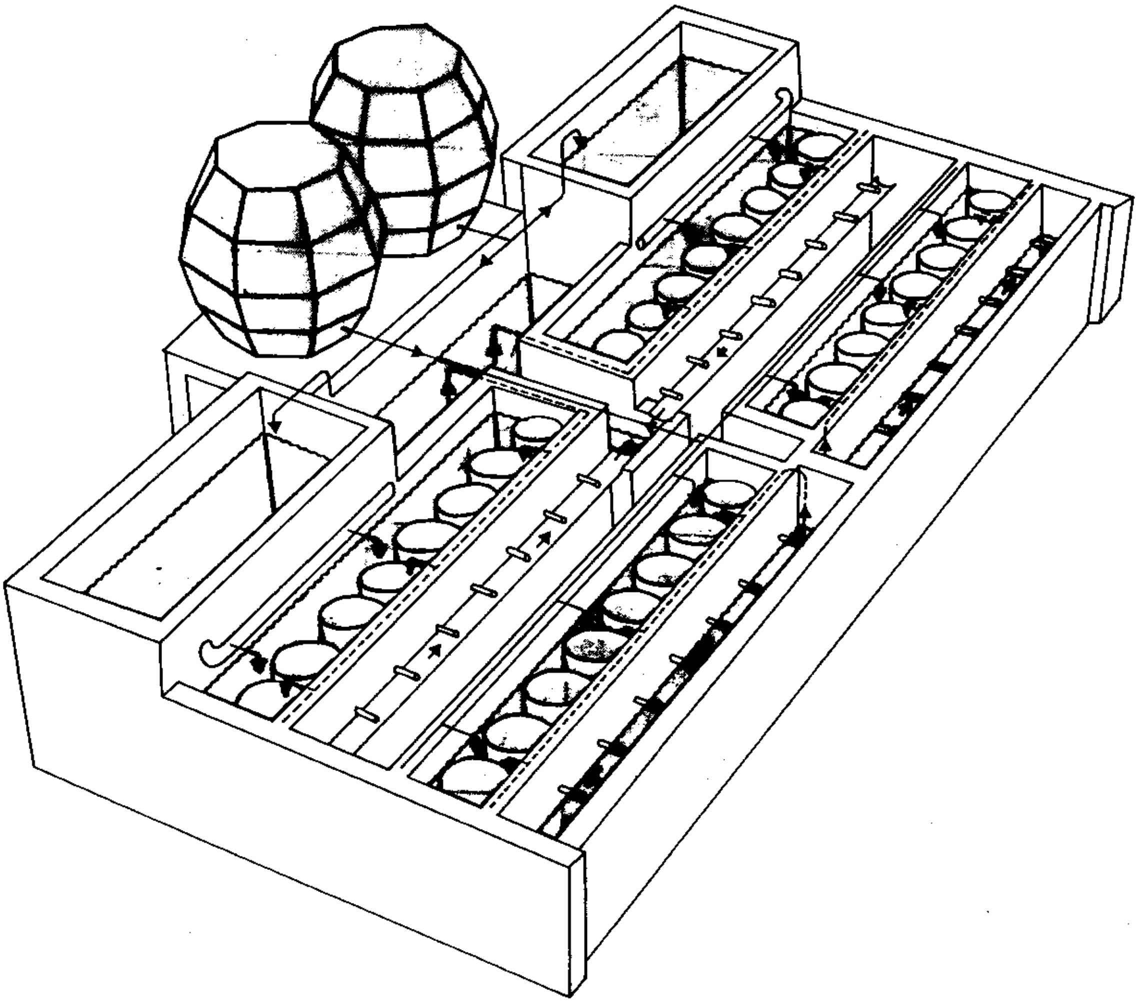

fig. 1.

General schematic view of the experimental indoor bivalve nursery at the Institute for Marine Scientific Research (IZWO), Ostend

Sea-water is pumped into two constant-head devices from which the water is distributed by gravity into the four rearing tanks. For two rearing tanks the sea-water flows through an intermediate heating tank. Heating occurs indirectly by means of an electric boiler and two radiators. The algal suspension is pumped into a separate storage tank from which it is distributed by gravity to the rearing tanks at a maniable constant flow-rate. The outflowing sea-water runs through a reservoir from which it can eventually be recycled through the cultures. The outflowing

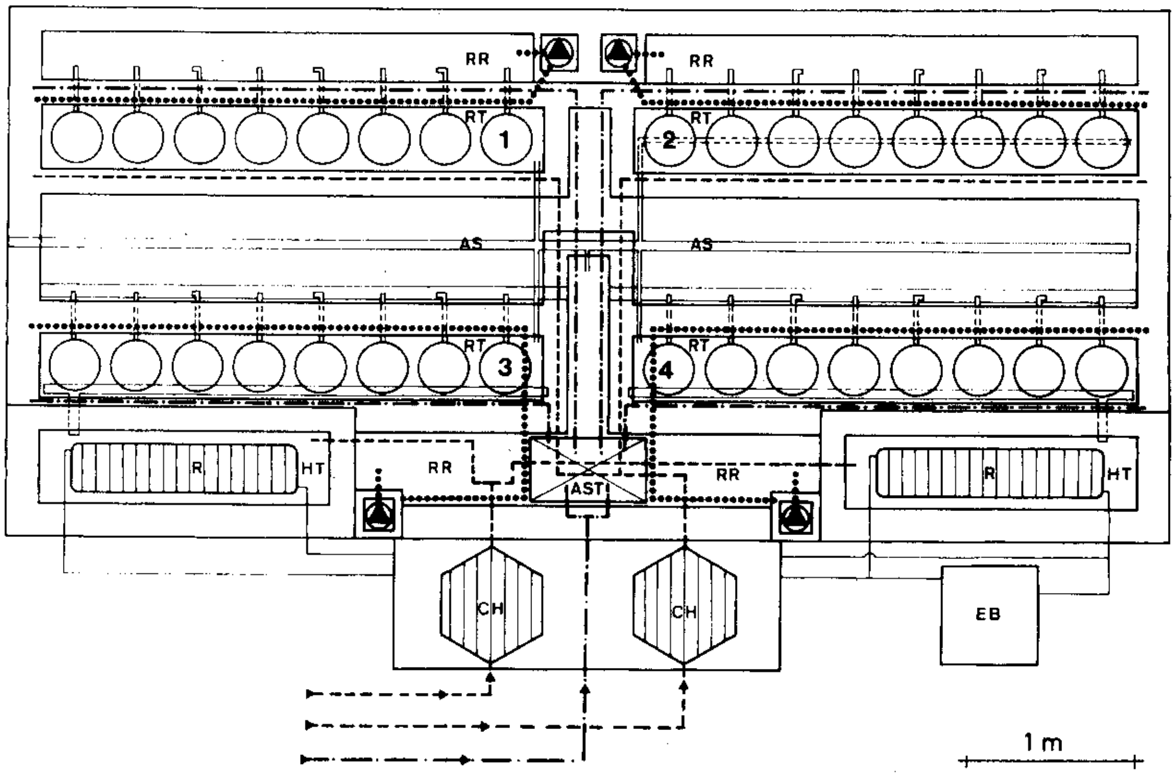

fig. 2.

Scheme of the indoor bivalve nursery at the Institute for Marine Scientific Research (IZWO), Ostend
CH : constant head; HT : heating tank; RT 1, 2, 3, 4 : rearing tanks; RR : recycling reservoir;
EB : electric boiler; R : radiator; AST : algal storage tank; AS : air supply; ▲ : recycling pump;
— — —: water supply; —·—·: algal supply; ··········: water recycling

heated sea-water is used to preheat the inflowing fresh sea-water. The spat is stocked in upflow cylinders with a mesh bottom.

The whole system is conceived in such a way that the influence of the variation of many parameters such as temperature, flow rate, feeding rate, stocking density, etc. can be assessed. The ultimate goal of this pilot plant is to make a cost benefit analysis of an industrial nursery utilizing a heated effluent.

A second trend in the production of microscopic algae as food source for nursery bivalves, relies on the completely controlled production of specific phytoplankton species. This implies keeping stocks of monospecific algae, progressive inoculation of cultures of increasing volumes, and controlled culturing conditions in either indoor or outdoor conditions.

From the many laboratory experiments with young bivalve spat fed different monospecific algal suspensions in various concentrations and mixtures, many interesting conclusions with regard to the nutritional value of these algal species could be drawn. These are directly applicable in the large-scale rearing systems. A review of the literature on this topic was given by De Pauw (1981).

Why the different algal species vary in their food value is not yet understood, but since there is such an important difference in food value, it is not surprising that the mass culturing of the most suitable species for bivalves has been tried out under indoor as well as outdoor conditions in various places (Walne, 1970a,b).

A few experimental and commercial installations are described because they brought some important innovations in the concept and the technology of the bivalve nursery. In the University of Delaware, U.S.A., an experimental closed-cycle controlled mariculture system has been developed, with the aim to raise bivalve molluscs from egg to market size in a recirculating system on a diet of monospecific algae *Thalassiosira pseudonana* and *Isochrysis galbana*. These algae are cultured in a large greenhouse in circular tanks of 9.5 m^3. The algal suspension is pumped continuously through the oyster growing-tanks. Oysters and algal cultures are grown at the same temperature of 18 to 20°C. A cost benefit can be made if the heated sea-water and the unassimilated algal cells can be conserved and recycled. There is also a potential value in recycling the nitrogen and phosphorus by recycling the overflow of the oyster tanks back to the algal cultures. The levels of the micronutrients for the algal culture and of calcium for the shellfish need to be monitored and adjusted (Pruder et al., 1978).

The water quality is one of the critical factors for a controlled bivalve rearing-system based on monospecific algal cultures. In most cases surface sea-water must be filtered or centrifuged to remove the undesirable organisms, and in most cases the water is sterilized. This kind of water treatments can be minimized if one utilizes salt well-water, free of pollutants, undesirable species, diseases, and predators harmful to the shellfish. This is how the Cultured Clam Corporation in Dennis, Massachusetts, U.S.A., operates. The hard clam *Mercenaria mercenaria* is grown on pure algal strains cultured in large outdoor tanks. Similarly, in two commercial plants in Hawaii *Crassostrea gigas* is reared up to commercial size on algae mass-cultured in open ponds of 2 500 m^2. From the engineering point of view, the design of the technology used in Aquatic Farms Ltd. in Hawaii is remarkable. The trenches receiving the oyster trays have been built as a cascade. Each trench is located slightly below the level of the previous one and slightly above the next one. The phytoplankton suspension flows over the oyster trays, is forced down by gravity through each stack of trays and then flows to the next trench where it is enriched again with new phytoplankton to bring the concentration back at the original level (Burzell, 1978; Pryor, 1978).

The idea of coupling a mollusc mariculture plant with an OTEC plant (Ocean Thermal Energy Conversion) has been studied in St Croix, Virgin Islands in the Caribbean Sea (Roels et al., 1976). Deep-sea water, very pure and rich in nutrients, is pumped continuously from 870 m depth into two concrete pools in which unialgal cultures of diatoms are grown. The algal cultures are pumped continuously into the shellfish tanks where several species of oysters and clams have been raised up from spat to market size in approximately one year (Sunderlin et al., 1975, 1976; Rodde et al., 1976).

Unfortunately, in none of these four large-scale operations, the upwelling system has been used to stock the bivalve spat. Undoubtedly this sytem would better reduce the problem of the large quantities of faecal material sticking to the trays than some of the sophisticated devices that were used (Langton et al., 1977).

3.– Mortality rate in the nursery.

It is clear that seasonal variations in the parameters of the natural environment are reflected in both the growth and the mortality curves of the bivalve populations.

Walne (1974) stated that when very young *Ostrea edulis* L. spat of less than 1 mm is moved outside, whatever the season, mortality is usually high, and mortality is always high, when spat is moved outside in the winter and early spring, whatever its size. These mortalities are attributed to the large temperature shocks. Spat grown in cooler water, has a relatively higher meat content and can better withstand the transition to the cooler outdoor conditions.

The seasonal mortality of newly planted spat in their first month on a raft, has been studied by Spencer and Gough (1978). These authors showed that for *Ostrea edulis* the mortality is maximal in the warmest months of the year, and for *Crassostrea gigas* in April and May.

In some of our own experiments similar phenomena were observed. Other factors than temperature must be taken into consideration, such as the density and the species composition of the phytoplankton. In our experiments the summer mortalities of both oyster species in the Sluice Dock in Ostend were attributed to the consecutive shocks of high levels of free ammonia at the end of the day as a result of the very important photosynthetic activity in this eutrophic water, and the nearly complete depletion of dissolved oxygen during the night caused by respiratory activity.

Blooms of toxic algal species such as toxic dinoflagellates and of *Phaeocystis* and to a certain extent *Chlorella* can cause severe mortality of the spat (Loosanoff and Davis, 1963; Walne, 1976). Summarizing a number of experience papers on the rearing of bivalve spat in the natural environment, one can say that mortality is unpredictable and thus hard to control. An average mortality of 50 % is not unusual. Mortality in a controlled onshore nursery installation is generally far less. From literature data, the averaging mortality rate during the nursery stage is expected to be 10-30 % for *Ostrea edulis*, 5-25 % for *Crassostrea gigas*, 20-60 % for *Mercenaria mercenaria*, 10-40 % for *Venerupis semidecussata* Adams and Reeve, 30-50 % for *Argopecten irradians* Lmk, and 5-20 % for *Crassostrea virginica* (Gmelin).

4.– Growth performances.

All the nursery practices described above result in different growth rates of the various bivalve species. In the systems used in the natural environment in the temperate climate zone, the growth of the shell and the soft tissues of the spat is limited to the warmer period of the year. The growing season usually starts in April and ends in October. As established by laboratory studies, in which the natural sea-water was heated during the winter, temperature is not the only growth-limiting factor during this time of the year (Malouf and Breese, 1978; Clause et al., 1981; Malouf, 1981). The amount of available food is at least as important. In nature these two environmental requirements for growth may often be mutually exclusive. On one hand tropical and subtropical waters are usually too poor in phytoplankton to give a substantial growth of the bivalves, and on the other hand the more eutrophic temperate waters have temperatures too low for bivalve growth for often as long as half a year.

An intensive nursery-system in a temperate climate, that aims at having the bulk of its production ready for spring sales, has to make the choice of either to grow the spat in autumn when phytoplankton is present in adequate densities in the natural sea-water, and then store the graded spat during the winter at low temperatures, or to grow the spat during the winter and to add an additional amount of food, usually consisting of cultured microscopic algae, to the culturing water.

It is clear that in the latter systems, the same increase in shell length and live weight of the spat must be achieved in a much shorter period than in the natural environment (i.e. three months versus seven-eight months) and this for economic reasons.

In order to estimate the growth performances that one can expect in such a controlled nursery system, an extensive literature survey was made regarding the growth rates of spat of different commercial bivalves in various culturing devices and at various temperatures.

Since no standard procedure has been used in the studies, the original data have been transformed in various ways to give comparable information for each locality. Data from 64 papers on six species were examined : i.e. *Ostrea edulis*, *Crassostrea gigas*, *Venerupis semidecussata*, *Mercenaria mercenaria*, *Crassostrea virginica* and *Argopecten irradians*.

The growth rate is expressed as the instantaneous growth rate for 30 days (Ricker, 1968; Spencer and Gough, 1978),

$$G_{30} = \frac{30}{t} \ln \frac{W_t}{W_0}$$

where G_{30} is the instantaneous growth rate for 30 days, W_0 the initial live weight, dry meat weight or shell length, W_t the final live weight, dry meat weight or shell length, $t = t - t_0$ the time.

For a sample the value for G_{30} is usually higher when considering the increase in live weight instead of the increase in shell length. The G_{30} values are strongly affected by temperature and for G_{30} (live weight) also by the initial weight of the spat. The duration of the experiment affects the G_{30} values as well, and since the scope of this article is the growth of the bivalves during the nursery phase, data regarding experimental periods longer than 150 days have been omitted, as well as data concerning the growth of bivalve spat smaller than 1.5 mm and juveniles larger than 20 mm .

4.1.– *Ostrea edulis.*

The G_{30} values for shell length and live weight were derived from nine papers, and are given in figs 3 and 4 (Walne, 1974; Barry, 1975; Sunderlin et al., 1976; Le Borgne et al., 1978; Gimazane and Medhioub, 1979a; Moerman, 1979; Drinkwaard, 1981; Guerrero et al., 1981).

The values for shell length are maximal (i.e. almost = 1.0) at the highest temperature taken into consideration, namely 25.5 °C (fig. 3). From the G_{30} values for live weight (fig. 4) it appears, however, that the instantaneous growth rate is maximal at 20°C. At 10°C the *Ostrea edulis* spat completely ceases to grow. The influence of the initial size of the spat on the G_{30} value is important especially at 20°C. For example the G_{30} value at 10 mg equals 2.90 , whereas at 100 mg it is only 1.30 .

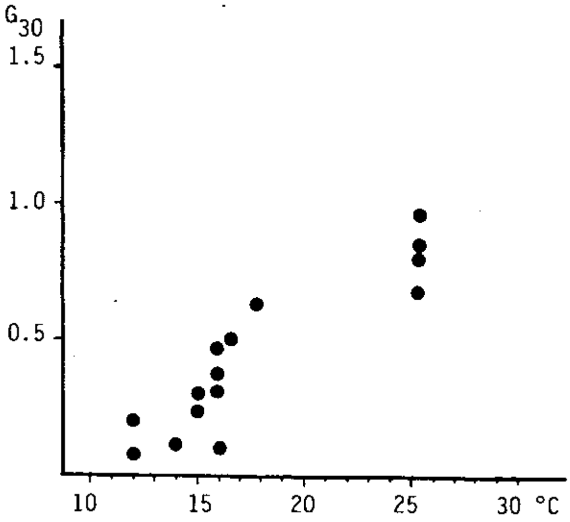

fig. 3.

Instantaneous growth rate for 30 days (G_{30}) for the shell length of *Ostrea edulis* in function of the temperature

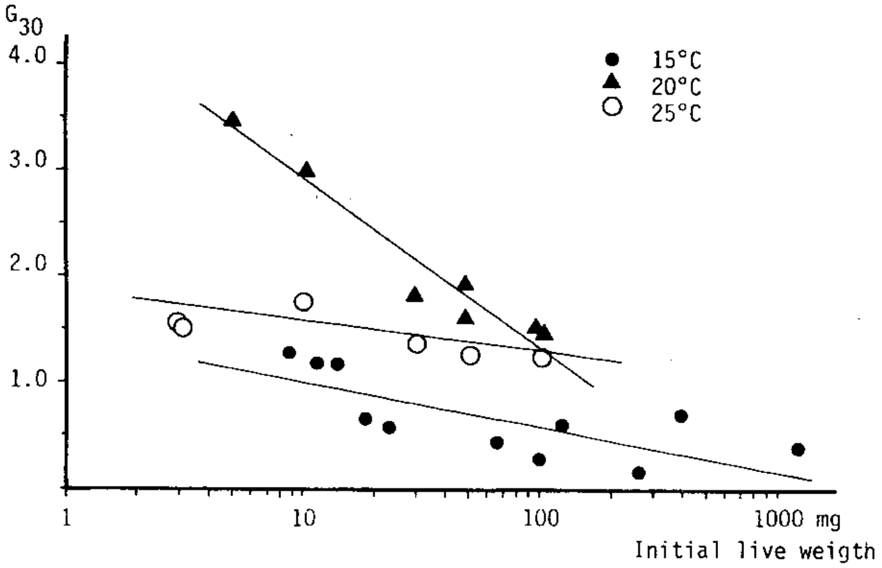

fig. 4.

Instantaneous growth rate for 30 days (G_{30}) for individual live weight of *Ostrea edulis* in function of the initial live weight

4.2.– *Crassostrea gigas.*

The G_{30} values for shell length and live weight were derived from 12 papers and are given in figs 5 and 6 (Cooke et al., 1975; Mann and Ryther, 1977; Askew, 1978; Le Borgne et al., 1978; Ley, 1978; Malouf and Breese, 1978; Spencer and Gough, 1978; Yelf, 1978; Gimazane and Medhioub, 1979b; O'brien, 1979; Breber, 1981; Spencer and Hepper, 1981).

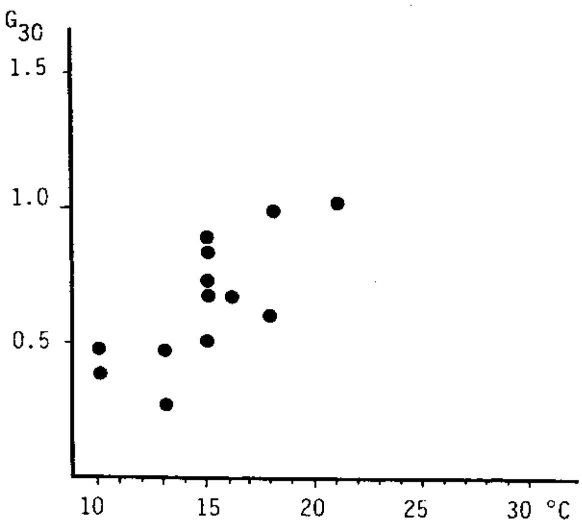

fig. 5.

Instantaneous growth rate for 30 days (G_{30}) for the shell length of *Crassostrea gigas* in function of the temperature

The G_{30} values for length are maximal (i.e. 1.0) at the highest temperature taken into consideration, namely 21°C (fig. 5). Since *Crassostrea gigas* is a subtropical species, it may be expected that even higher values may be encountered at higher temperatures. At a temperature of only 10°C there is still a substantial growth of the shell ($G_{30} > 0.30$). As for *Ostrea edulis* the influence of the initial live weight of the spat strongly affects the G_{30} values (fig. 6). At 20°C a specimen of 10 mg has an instantaneous growth rate of 4.00, whereas a specimen of 100 mg has a G_{30} value of only 2.00. Generally, *Crassostrea gigas* grows faster than *Ostrea edulis* under the same circumstances.

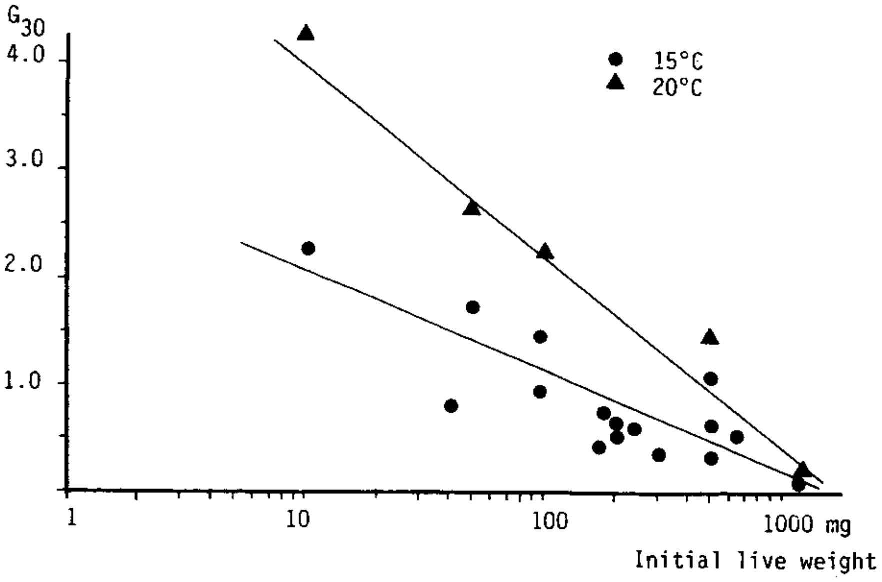

fig. 6.

Instantaneous growth rate for 30 days (G_{30}) for individual live weight of *Crassostrea gigas* in function of the initial live weight

4.3.– *Venerupis semidecussata.*

The G_{30} values for shell length and live weight were derived from 11 papers and are given in figs 7 and 8 (Lucas, 1976, 1977; Rodde et al., 1976; Le Borgne et al., 1978; Dreno, 1979; Gimazane and Medhioub, 1979c; Moerman, 1979; Claus et al., 1981; Guerrero et al., 1981; Riva and Lelong, 1981; Spencer and Hepper, 1981).

The G_{30} values for shell length (fig. 7) are more or less constant from 10 to 20°C (approximately 0.25). A maximal value of 0.75 is observed at a mean temperature of 25.5 °C. Contrary to the trend in the growth rate of both previous species, the G_{30} for live weight is apparently unaffected by the initial live weight of the spat (fig. 8). The G_{30} equals 0.45 for the range from 5 mg to 1 g. The Japanese little-neck clam is a slow grower as compared to the two oyster species.

4.4.– *Mercenaria mercenaria.*

The G_{30} values for shell length and live weight were derived from five papers and are given in fig. 9 (Walne, 1970a; Hartman et al., 1973; Sunderlin et al., 1975; Roels et al., 1976; Epifanio, 1979).

From this limited number of data it appears that the instantaneous growth rate of 3 mm spat is optimal at a temperature of 20°C. This corroborates the data given by Ansell (1968) for specimens of 4 cm (fig. 10). The hard clam grows faster than *Venerupis semidecussata* but more slowly than the oysters.

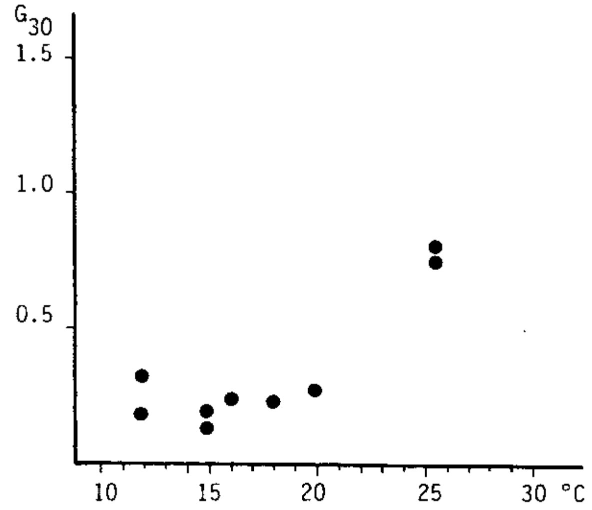

fig. 7.

Instantaneous growth rate for 30 days (G_{30}) for the shell length of *Venerupis semidecussata* in function of the temperature

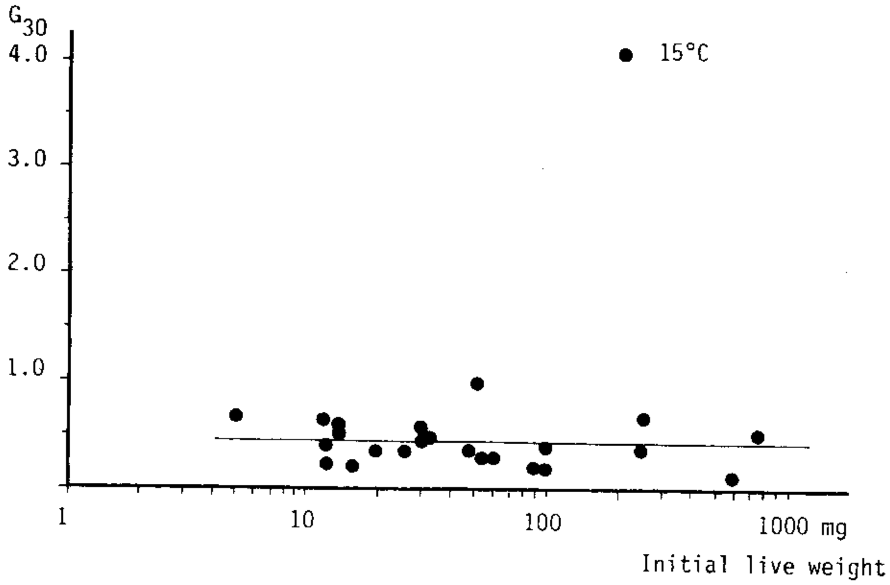

fig. 8.

Instantaneous growth rate for 30 days (G_{30}) for individual live weight of *Venerupis semidecussata* in function of the initial live weight

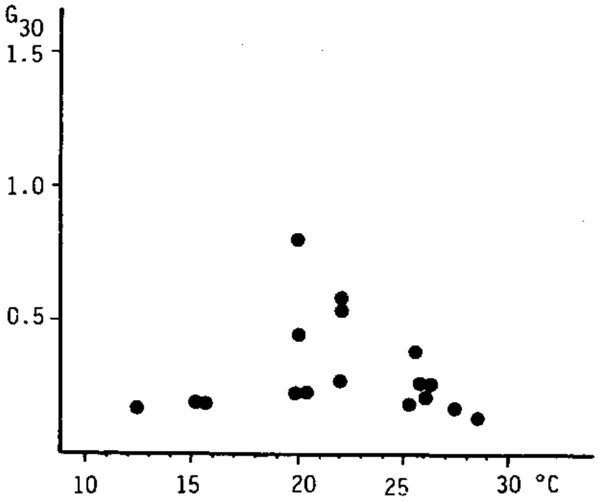

fig. 9.

Instantaneous growth rate for 30 days (G_{30}) for the shell length of *Mercenaria mercenaria* in function of the temperature

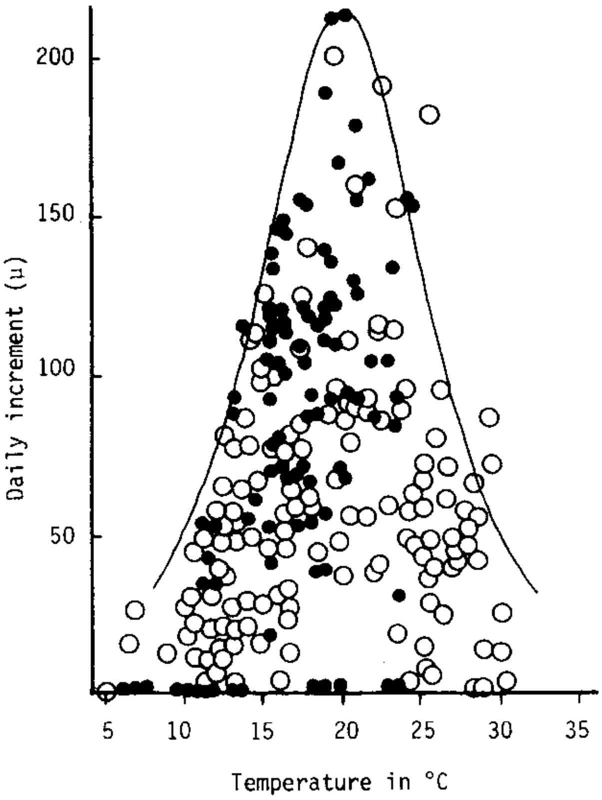

fig. 10.

The general relationship between the rate of shell growth (μ) of *Mercenaria mercenaria* based on field measurements and temperatures (●) Sites in England; (○) North American sites

4.5.– *Argopecten irradians.*

Data from only three papers were retained. At temperatures ranging from 22-29°C, a maximal G_{30} for shell length of 0.46 has been recorded for 2 mm spat (Roels et al., 1976). Rhodes et al. (1981), however, found G_{30} values of 1.97 to 1.54 at 20°C for specimens of 2 to 4 mm. Larger specimens of 4-5 g have G_{30} values for live weight at 12 and 25°C of respectively 0.21 and 0.81 (Mann and Taylor, 1981). Since the nursery culturing of scallops is a fairly new practice, additional data are needed to draw meaningful conclusions.

4.6.– *Crassostrea virginica.*

Growth rate data of *Crassostrea virginica* in nursery operations are very scarce. From the information reported by Pruder et al. (1976) a G_{30} for shell length of 0.61 was calculated for 4 mm spat at 24°C. Based upon the figures given by Singh and Zouros (1978) a G_{30} for live weight of 0.97 for spat of 870 mg at 18°C could be derived. These very few data, however, compare very well with the figures for *Crassostrea gigas*.

It is interesting to consider these results in relation to commercial practice.

From the data shown in figs 3 through 9, one can derive the average growth rate of spat in the nursery system under various temperatures, assuming an unlimited food supply. This average growth rate is expressed as the mean time necessary to achieve the size at which the spat is ready to leave the nursery. These values have been arbitrarily set at 15 mm and 250 mg for *Ostrea edulis* and *Crassostrea gigas*, at 10 mm and 250 mg for *Venerupis semidecussata*, and at 10 mm for *Mercenaria mercenaria*. The results are summarized in table 1.

In a commercial operation, the turnover of the spat in the culturing devices, is one of the major factors determining the yearly benefits. The duration of the nursery phase is different for each species and strongly influenced by the temperature of the culturing water (Table 1). The nursery periods are very similar when calculated on a length basis and on a live weight basis. At a temperature of 15°C it takes *Crassostrea gigas* nearly ten weeks to complete the nursery stage, *Ostrea edulis* four to five months, and both clams more than six months. At 20°C *Crassostrea gigas* needs only six weeks, *Ostrea edulis* and *Mercenaria mercenaria* two months, and *Venerupis semidecussata* four months. At 25°C *Venerupis semidecussata* can achieve the final nursery stage in two months. It should, however, be pointed out that the daily food rations of the bivalves at these high temperatures are substantial. In most cases the production of these large quantities of live food is not economically feasible (De Pauw, 1981).

5.– Temperature.

The effect of temperature on the growth of bivalve spat has been studied by several authors (Mann and Ryther, 1977; Malouf and Breese, 1978; Mann, 1979a,b; Claus et al., 1981). The optimal temperature for growth usually ranges from 20 to 25°C depending upon the species. It has, however, been shown by Lough (1975) that in a mariculture installation, sustained high temperatures at which a maximal growth response is obtained, may put an abnormal stress on the animals which can result in high mortality.

An organism probably operates most efficiently when it finds itself in a set of environmental conditions which maximizes all its biological responses and not only growth. In this regard it should be emphasized that increasing the temperature will hasten growth but may divert energy from somatic growth to gonadal growth (Walne, 1976).

A temperature regime optimal for growth has to be a compromise between the stimulation of feeding and meat production on one hand, and minimizing excessive shell growth, production of gonadal material and potential physiological stresses associated with high temperatures, on the other hand. The exploitation of the advantages of increased temperature results in a greater vulnerability for prolonged stress. The animal indeed depends on limited stored reserves to maintain a normal metabolism rate during periods when the collected food is unadequate to support this process (Ansell and Sivadas, 1973).

Walne and Spencer (1974) demonstrated that there is little difference between growth at 14°C and 24°C when *Ostrea edulis* spat were only fed five *Tetraselmis* cells/µℓ/day. If the ration was increased to 10 cells/µℓ/day some positive influence could be seen resulting from the higher temperature. The experiments carried out on a larger scale by Mann and Ryther (1977) with spat of *Crassostrea gigas*, *Crassostrea virginica*, *Ostrea edulis*, *Tapes japonica*, *Mercenaria mercenaria* and *Mytilus edulis* corroborate these findings. At 15°C consistant higher values for live weight, dry meat weight, and condition index were recorded throughout the study as compared to 20°C. In our experiments the minimal food level to sustain growth at 15°C and higher, is considered to be 10 µg/ℓ of chlorophyll α (Claus et al., 1981), which was also found by Rhodes (1978, personal communication) and Rhodes et al. (1981).

From an economic viewpoint the artificial heating of large volumes of sea-water needed for commercial mollusc production is theoretically prohibitively expensive. A solution to this problem can, however, be found in the use of the waste heat of a power plant.

Some scientists advocate the direct use of marine cooling water as a culturing medium for molluscs (Malouf and Breese, 1978; Malouf, 1981). Because of the various problems inherent to a direct utilization it seems, however, that indirect use of thermal effluents (of all kinds) in a shellfish nursery system might be far more interesting (Claus et al., 1981).

Table 1

Mean time (days) necessary to achieve the nursery cycle
for *Crassostrea gigas*, *Ostrea edulis*, *Venerupis semidecussata* and *Mercenaria mercenaria*
at different temperatures

Species	Temperature (°C)	Initial		Final		Time for length (days)	Time for live weight (days)
		Length (mm)	Live weight (mg)	Length (mm)	Live weight (mg)		
C. gigas	12	3	10	15	250	127	
	15					69	68
	18					55	
	20					48	36
O. edulis	12	3	10	15	250	402	
	15					172	136
	18					82	
	20					64	59
	25					58	70
V. semidecussata	12	3	15	10	250	201	
	15					190	188
	18					157	
	20					134	
	25					52	
M. mercenaria	12	3	15	10	250	212	
	15					201	
	18					144	
	20					52	
	25					103	

6.– Stocking density and water flow.

The growth of small cultchless spat in a nursery system is very sensitive to the stocking density and water flow. Whatever growing system is chosen, its mean feature must be a virtually unlimited supply of new water reaching every individual animal. In the monolayer practices such as trays, a rule of thumb for stocking density is that "the juveniles may touch but not overlap" (Maskell, 1973).

In most commercial operations working in the natural environment, however, much lower stocking densities are applied (0.2 g/cm^2). Although this may seem a waste of equipment space, one can consider it worthwhile in saving labor for thinning operations (Ley, 1978). Regular servicing remains, however, an absolute necessity. It is the experience of most growers that thorough cleaning of the stock and the meshes is essential if satisfactory survival and growth rates are to be achieved with small spat (George, 1975). In the natural environment it is also important to avoid the spat to bunch in the trays. This is usually accomplished by maintaining the support surfaces as flat and horizontally as possible.

In the onshore nurseries these problems are overcome by using trays and trenches, and subsequently increasing the stocking densities. Present work at Conwy, UK, indicates that good growth and survival of small oysters is obtained up to a final stocking density of 1 g live weight/cm^2 of tray (Spencer and Gough, 1978). This corresponds with 200 000 specimens of 50 mg/m^2. This is ten times more than the density recommended earlier by Walne and Spencer (1971) and Walne (1974) for their recirculating system; ten times more than the stocking densities applied in the subtropical nursery at St Croix (Rodde et al., 1976), and four times more than the density recommended by Lucas (1977) for the raceways with lantern nets.

The great breakthrough in technology that allowed to increase the stocking density substantially, is the upflow system as described in a previous chapter. In these upwelling systems with pumped sea-water, stocking densities of 20 g/cm^2 and more can be achieved (Le Borgne, 1981; Williams, 1981). The application of this technique making use of the tidal action (Spencer and Hepper, 1981) resulted in a compact system with a maximum capacity of 3 to 6 g/cm^2. For the gravity-fed nursery plant MARIOS in the Netherlands, the recommended stocking density, however, is only 0.5 g/cm^2 (Drinkwaard, 1981).

There is much diversity in the data on the flow rate of the water in nursery systems. A suitable water current is required to stimulate feeding and carry away faeces. From the very basic research of Walne (1972) on the influence of current speed, body size, and water temperature on the filtration rate of *Ostrea edulis*, *Crassostrea gigas*, *Mytilus edulis*, *Venus decussata* and *Mercenaria mercenaria*, it appeared that there was a significant correlation between the flow rate and the filtration rate. It was stated that the large volumes of water pumped by the bivalves are primarily for feeding rather than for respiration. At some point the animal reaches its maximal filtering rate, and increasing the water flow rate will have no further effect (Wilson, 1980). In an earlier study, Wilson and La Touche (1978), however, found that there is no evidence that current velocity alters ingestion rates of bivalves. Neither change in direction of flow, nor variation in magnitude caused a detectable variation in the pattern of intracellular digestion.

Very few studies have directly linked filtration and growth (Kirby-Smith, 1972; Walne, 1972). It was assumed by the first author that the limiting factor for growth was the concentration of suspended food present in the water, more than the flow rate itself.

Recently, a very interesting study has been made by Rodhouse and O'Kelly (1981) on the flow requirements of *Ostrea edulis* and *Crassostrea gigas* in an upwelling column. Since the stocking density of spat in this type of culturing device is very high, the flow rates become very critical. Flow rates below the optimum cause a reduction in growth rate, while flow rates above the optimum result in an unnecessary increase in the cost of pumping and heating of the water and of producing algal cultures necessary to feed the spat.

Flow requirements were assessed by determining the rate of clearance of the suspended algal cells by a population of oysters. This clearance rate is the equivalent of the filtration rate of an individual oyster as calculated by Bayne (1971) and Hildreth and Crisp (1976). A maximal clearance rate is obtained at a certain flow rate. At this maximal clearance rate no effect of food concentration on this rate was observed. Temperature and body size, on the contrary, definitely affect the clearance rate. The whole study resulted in two equations which can be used to predict the flow requirements for 90 % clearance between 10 and 20°C :

$$FR = (0.47 + 0.04\ T)\ LW^{-0.26} \qquad \text{for } \textit{Ostrea edulis}$$

$$FR = (-\ 0.92 + 0.17\ T)\ LW^{-0.32} \qquad \text{for } \textit{Crassostrea gigas}$$

where FR is the flow requirement for 90 % clearance expressed in mℓ/min/g, T the temperature in °C and LW the individual live weight in g.

Inversely these equations can be used to calculate the maximal stocking capacity of an upwelling tube at a given flow rate and temperature. In table 2, the maximal number of *Ostrea edulis* and *Crassostrea gigas* spat at various sizes are given for the nursery plant in Ostend. Maximum stocking density varies between 10 to 45 g/cm^2. It should, however, be pointed out that a clearance rate of 90 % is very favorable from the economical point of view, but the experience has shown that the growth rate of different species (especially *Venerupis semidecussata*) is decreased at such high clearance rates. Kirby-Smith (1972) suggested that the growth rate of the scallop *Argopecten irradians* begins to decline when the concentration of chlorophyll α drops below 60 % of the inflow and that growth ceases completely when the concentration drops below 30 % .

Table 2

Stocking capacity of nursery pilot plant at the Institute for Marine Scientific Research (IZWO)
Temperature : 15°C; flow rate of heated sea-water : 60 000 mℓ/min;
number of upflow cylinders : 16; diameter of one upflow cylinder : 33 cm;
surface of one upflow cylinder : 855 cm^2

Live weight (g)	Flow requirement (Rodhouse and O'Kelly, 1981) (mℓ/min/g)	Maximal total weight (g)	Maximal total number of individuals	Maximal number of individuals per cylinder	Maximal stocking density (g/cm^2)
Ostrea edulis					
0.010	3.54	16 934	1 693 432	105 840	19.80
0.050	2.33	25 751	515 000	32 000	30.11
0.100	1.95	30 769	308 000	19 230	35.97
0.250	1.53	39 000	156 000	9 800	45.60
Crassostrea gigas					
0.010	7.11	8 439	843 265	52 704	9.87
0.050	4.25	14 113	282 270	17 642	16.50
0.100	3.41	17 618	176 182	11 011	20.60
0.250	2.54	23 621	94 485	5 905	27.62

As a rule of thumb Bayes (1979) uses the ratio :

$1\ m^3$ water/min/ton of seed.

For a temperature of 6°C this corresponds to the flow rate proposed by Drinkwaard (1981) at a temperature x :

$x\ m^3/h/100\ kg$.

The latter formula corroborates the equations of Rodhouse and O'Kelly (1981).

7.– Feeding regime.

Knowledge of feeding and biodeposition rates at different food concentrations and under different circumstances is important to optimize the nursery.

Various investigations have been performed to establish the food value of different algal species for bivalve spat. Reviews are given by Walne (1970a), Epifanio (1976) and De Pauw (1981). These studies provide valuable information, but often the experimental conditions are so specialized that extrapolation of these results to a larger scale is very difficult if ever possible, and any comparison as to the effects of mixed populations in the natural environment are very difficult to make. Even the laboratory studies with mixed diets which usually result in higher growth rates and lower mortality rates of the bivalves than the single foods (Hartman et al., 1973; Epifanio and Mootz, 1976; Epifanio, 1979; Ewart and Epifanio, 1981), are hard to extrapolate to pilot- or commercial-scale operations mainly because some algal species are very difficult to keep in large-volume cultures.

On a large scale *Skeletonema costatum, Phaeodactylum tricornutum, Tetraselmis suecica* and *Monochrysis lutheri* have been used successfully in temperate climates (Lucas, 1977; Mann and Ryther, 1977; Mann, 1979a,b). In subtropical conditions *Bellerochia spinifera, Chaetoceros simplex, Thalassiosira pseudonana* and *Isochrysis galbana* Tahiti strain have been used with success (Sunderlin et al., 1975; Rodde et al., 1976).

Efficient production of bivalves in controlled environments requires the definition of an adequate daily ration. Although considerable information exists concerning the filtration rates of bivalves, there is little concensus regarding rates of ingestion and selection of particles (Epifanio and Ewart, 1977).

A very clear relationship exists between the food concentration, the size of the particles, and the filter-feeding behavior of the bivalves. Particles are retained effectively by most bivalve species down to 3 µm in diameter (Owen, 1974; Winter, 1978). Particles of 1 µm are retained less efficiently but when considered in terms of total volume rather than in terms of efficiency, similar quantities of these small particles are removed by the bivalves than of the larger fractions (Haven and

Morales-Alamo, 1970). Particles greater than 55 µm are ejected for 90 % by small *Mytilus edulis* of 1.5 cm (Gabbott et al., 1976).

Generally, it has been assumed that bivalves have the ability to select particles for ingestion, based on the shape, the size, and the weight of the particles (Ali, 1970; Wilson, 1980). With regard to a selection based on chemoreception there is no consensus among the authors. Dwivedy (1973) gave evidence for the presence of chemoreceptors in the labial palps and suggested a chemical selection of the ingested particles. The results of the studies by Winter (1972) and Foster-Smith (1975a), however, are in contradiction with the hypothesis.

Various authors have shown that bivalves control the rate of phytoplankton intake in relation to the algal cell-concentration in the culturing medium (Ali, 1970; Winter, 1970, 1973; Tenore and Dunstan, 1973a,b; Foster-Smith, 1975a,b; Schulte, 1975; Langton and McKay, 1976; Epifanio and Ewart, 1977; Epifanio and Roels, 1977).

The quantity of phytoplankton consumed increases in proportion to the algal cell-density, reaching a plateau where it is constant, independently of the particle-concentration, while on the other hand the filtering rate decreases. Finally, the ingestion rate decreases again with further increasing particle concentrations. This occurs when the production of pseudofaeces starts (Winter, 1978).

The concentration which is just below the threshold of pseudofaeces production, the "pseudofaeces-free cell density" is very close to the optimal food concentration since at that level the minimal energy must be put into filtration activity by the animal and yet all the filtered cells are ingested.

The regulation of the filtration rate is not only influenced by cell density but is also a function of algal size. The relation between the cell volume and the most favorable density of algal food for spat of *Ostrea edulis* and *Mercenaria mercenaria* has been established by Walne (1970). For example, the optimal cell concentration of a medium-sized algal species of 100 μm^3 (± 6 µm in diameter) is ± 20 cells/µℓ for *Ostrea edulis* and ± 25 cells/µℓ for *Mercenaria mercenaria*. Generally, the optimal cell densities given by Walne (1970a) are slightly lower than the "pseudofaeces-free cell density" given by different authors for the given algal species (Foster-Smith, 1975a; Schulte, 1975; Epifanio and Ewart, 1977).

An estimation of the optimal cell density can also be made based on the knowledge of the energy budget of the oyster spat. The daily ration of a juvenile oyster of 10 mg live weight is estimated at 20 % of its dry meat weight [200 µg] (Walne and Spencer, 1974; Epifanio and Ewart, 1977). These 40 µg of dry algal material correspond on the average with 2 million medium-sized phytoplankton cells per oyster of 10 mg, or 10^8 cells/g live weight. Starting from a calculated flow requirement of 4 mℓ/min/g live weight for an oyster of this size (Rodhouse and O'Kelly, 1981), the cell concentration of the flowing suspension is thus approximately 35 cells/µℓ which corresponds with the mean optimal concentration given by Walne (1970a).

Langton and McKay (1976) have shown that discontinuous feeding using a 6 h feeding – 6 h unfed periodicity, maximized the growth of *Crassostrea gigas* spat when compared to continuous feeding. A digestive rhythm that determined the assimilation efficiency was suggested. Analogous experiments by Winter and Langton (1976) with *Mytilus edulis*, however, dit not corroborate these findings. Wilson and La Touche (1978) gave evidence that the cyclic feeding-pattern of *Ostrea edulis* is not endogenous but depends on the food availability. With respect to aquaculture this would imply that the spat should be maintained at a constant optimal food level.

8.– Economics.

Presently it is impossible to draw firm conclusions with regard to the future of mollusc nurseries. All systems presented in this paper have proven to be biologically feasible but the economics should be evaluated. The type of technology which shall be selected depends largely on the geographical location of the nursery. Indeed, different places may be characterized by different climatic and ecological conditions and divergent costs for land, energy, labor, water treatment, food production, purchase and transport of seed, taxes, etc.

Regardless of the system used, the market price of the produced spat is more or less constant, contrary to the price of natural seed which varies as much as 1:4 according to the amount of seed captured and collected. Currently, 100 million artificially-bred *Crassostrea gigas* spat are sold annually in France and the UK combined (Lintell, 1980). These countries are indeed the two major producers in Europe.

Directive prices of nursery spat from French and British firms are given in table 3.

Table 3

Price per 1000 in £ and in FF (converted to US $) of nursery reared spat

Size (mm)	*C. gigas*			*O. edulis*			*V. semidecussata*		
	£	FF	$	£	FF	$	£	FF	$
3-4	5.70		9.73	6.00		10.24	5.70		9.73
6	7.40		12.63	7.70		13.15	7.40		12.63
8	8.50	66	14.51	8.80	75	15.02	8.50	70	14.51
10	9.70	78	16.56	11.00	85	18.78	9.70	88	16.56
12-15	12.50	95	21.34	14.50	95	24.76	12.50	100	21.34
15-20	14.80		25.27	17.80		30.39	14.80		25.27
20-25	18.00		30.73	22.50		38.41	18.00		30.73
25-30	21.50		36.71	27.50		46.95	21.50		36.71

An estimation of the cost structure of a European nursery operation working in the natural environment was given by George (1975) :

Purchase of seed	46.5 %
Equipment (written off over 5 years)	4.5 %
Labor	22.5 %
Overheads (100 % of labor)	22.5 %
Operating costs	2.0 %
Insurance	2.0 %

Another estimation for an extensive operation has been made by Walker and Gates (1981) :

Seed stock purchase	16.7 %
Total maintenance + repair and replacement of equipment	19.9 %
Labor (inclusive social security payments)	58.0 %
Miscellaneous	9.3 %
Taxes	2.2 %
Insurance	9.0 %

The major costs are labor expenses and seed-stock purchase. Since under natural conditions a 50 % mortality is not unusual, the costs to purchase the spat are relatively high.

In the more complicated onshore operations investment costs, energy costs, and eventually costs to produce phytoplankton may become substantial as well. The advantages of these systems must be derived principally from the lower mortality rates (5 - 25 %), the higher stocking capacity and the higher growth rates.

The increase in value of the spat after the nursery stage is very small :

O. edulis	3 → 15 mm	US $	14.50/1000 = 1.5 cents/piece
C. gigas	3 → 15 mm	US $	11.60/1000 = 1.2 cents/piece
V. semidecussata	3 → 10 mm	US $	6.83/1000 = 0.7 cents/piece

If one compares these figures with the pumping costs as estimated by Drinkwaard (1981), namely 0.37 cents/spat estimated by Spencer and Gough (1978) for an experimental period of four weeks, taking the costs for algal food, namely 2 cents/spat for monospecific cultures into account (Walne, 1970b; Malouf and Breese, 1978), as well as 0.2 cents/spat for induced blooms (De Pauw, 1981), it is clear that setting up a nursery aquaculture-system, independently from a hatchery or an ongrowers operation, is not always an attractive investment.

Without a better understanding of the nutritional requirements of bivalve spat associated with the technological development of an optimized system, and next to that the development of an inexpensive artificial food, the economic feasibility of intensive nursery culturing must remain in doubt. To set up a nursery of a particular type, a reasonable compromise should be worked out, between the costs cited above and the corresponding biological responses of the bivalves that may be expected and which determine the total production in the system. For example, seasonally operated systems with unheated water, might be more

Table 4

Synopsis of the replies to the inquiry made by Persoone and Claus (1979) in the framework of the COST 46 program

Location	Country (*)	Type (**)	Spat cultured in the natural environment			Onshore culturing		Controlled food production			Inert foods	
			Bottom culture	Off-bottom		Pumping from		Natural phyto-plankton blooming		Monospecific cultures	Supplement	Substi
				Trays trestle	Trays raft	Open sea	Enclosures	Outdoors	Indoors			
Ostrea edulis												
Ostend	B	E			+		+	+			+	+
Nykøbing Mors	DK	C	+			+		+				
Barfleur	F	C				+	+			+		
Caen	F	E	+			+				+		
Ile de Ré	F	C				+	+	+				
Sète	F	E			+	+						
Chioggia	I	E		+	+							
Taranto	I	E		+		+		+				
Abbotstown	IR	E	+	+	+							
Carna	IR	E	+	+	+	+	+	+		+		+
Carna	IR	C			+	+		+		+		
Coleraine	IR	E	+	+	+							
Connemara	IR	C	+	+								
Derryherbest	IR	C	+		+							
Rossmore	IR	C	+	+		+	+	+				
Fort San Lucian	M	E	+	+	+							
La Coruña	S	E			+							
Villegarcia de Arosa	S	E			+			+		+		
Fawley, Southampton	UK	C		+								
Herts	UK	C			+							
Isle of Anglesey	UK	C	+	+		+	+	+		+		
Isle of Lewis	UK	C			+	+						
Guernsey, Channel Isl.	UK	C	+	+	+	+		+				
Totnes, Devon	UK	C		+								
West Mersea, Essex	UK	C	+	+	+	+						
Whitstable	UK	C					+					
Portoroz	Y	E			+							
Rovinj	Y	E			+							
Crassostrea gigas												
Ostend	B	E			+		+	+			+	+
Barfleur	F	C				+	+			+		
Brest	F	E	+		+			+	+	+		
Caen	F	E	+		+							
Ile de Ré	F	C				+	+	+				
Sète	F	E				+						
Langballigau	G	E		+	+	+		+	+	+		
Trieste	I	E			+							
Carna	IR	C			+	+		+	+			
Carna	IR	E	+	+	+	+	+	+		+		
Coleraine	IR	E	+	+	+							

Goldhanger, Essex	UK	C		+							
Herts	UK	C			+						
Isle of Anglesey	UK	C	+	+		+	+	+		+	
Isle of Lewis	UK	C			+	+					
Guernsey, Channel Isl.	UK	C	+	+	+	+		+			
Kilmartin, Argyll	UK	C	+	+							
Whitstable, Kent	UK	C					+				
Totnes, Devon	UK	C		+							
Rovinj	Y	E			+						
Venerupis semidecussata											
Ostend	B	E			+		+	+			+
Barfleur	F	C				+	+			+	
Brest	F	E		+	+	+			+	+	+
Ile de Ré	F	C				+	+				
Sète	F	E				+					
Carna	IR	E	+	+	+	+	+	+		+	
La Coruña	S	E			+						
Guernsey, Channel Isl.	UK	C	+	+	+	+		+			
Tapes decussatus											
Caen	F	E	+		+						
Chioggia	I	E	+							+	+
Chioggia	I	E				+	+				
Carna	IR	C			+	+		+		+	
Carna	IR	E	+			+	+	+	+	+	
Coleraine	IR	E	+								
Derryherbest	IR	C	+		+						
Huelva	S	E	+							+	+
La Coruña	S	E		+							
Villegarcia de Arosa	S	E	+		+			+		+	
Fawley, Southampton	UK	C		+							
Isle of Anglesey	UK	C	+	+	+	+	+	+		+	
Guernsey, Channel Isl.	UK	C	+	+	+	+		+			
Totnes, Devon	UK	C		+							
Whitstable, Kent	UK	C					+				
Mercenaria mercenaria											
Brest	F	E		+		+			+	+	
Carna	IR	E	+	+	+	+	+	+		+	
Fawley, Southampton	UK	C		+		+		+		+	
Pecten maximus											
Abbotstown	IR	E		+							
Carna	IR	E	+	+	+	+	+	+		+	
Derryherbest	IR	C	+		+						
Chlamys varia											
Brest	F	E			+						
Carna	IR	E	+	+	+	+	+	+		+	

(*) B : Belgium; DK : Denmark; F : France; G : Germany; I : Italy; IR : Ireland; M : Malta; S : Spain; UK : United Kingdom; Y : Yougoslavia
(**) E : experimental system; C : commercial plant

economical than the more sophisticated systems. More cooperative research is needed to assess the costs and productions of the different systems (Lucas and Gerard, 1981).

At the European level the COST 46 program is a step in the right direction to exchange experience and planning concerted actions in the field of nursery culturing of bivalve molluscs. In table 4 the answers are summarized of the inquiry made by Persoone and Claus in 1979 within the frame of the COST-46 Mariculture Program on nursery culturing of bivalve molluscs – COST (Cooperation in Science and Technology) is an EEC sponsored Committee. In comparing the economics of nursery operations, it is interesting to pay attention to the particular culture system chosen by the commercial enterprises. From table 4 it appears that nursery rearing of bivalves has become a popular technique in Europe. Most of the people involved in nursery rearing are working in the natural environment using trays to store the spat. Onshore pumping of sea-water is used at different places in Europe in both experimental systems and in commercial plants. Controlled food production is practiced in almost half of the cases. Natural blooming is preferred to monospecific cultures. It is, the however, worthwhile to note that probably some commercial firms carry out the latter type of food production, because to them it is an extension of their hatchery operation.

9.– Conclusions.

Nursery culturing of bivalve molluscs, as the intermediate step between controlled production of larvae and the growth-out in the wild, is practiced in a variety of ways. The majority of systems rely on the productivity of the natural environment and are utilizing untreated natural sea-water as sole food source of the bivalve spat. In nurseries located directly in the natural environment, high mortality rates and losses of spat are often recorded. Onshore nurseries provide better protection for the spat and allow an easier servicing.

In all these systems, however, growth of the bivalves is limited to the warmer seasons of the year. Heating of the water, or moving the nursery plant to a subtropical or tropical climate, can only partially offer a solution for the insufficient production during the winter, since the major limiting factor for optimal nursery growth of the bivalves is the food supply, and primary production under these circumstances is usually low.

Live phytoplankton is still the only satisfactory food source for bivalves on a large scale. Therefore the production of algae receives increasing attention from the experimental and commercial nursery operators. Several integrated aquaculture systems for microalgae and bivalves have been developed and are under trial. Recycling of the water from the bivalve through the algal tanks and the use of sterile and nutrient-rich well-water or deep-sea water are the most important innovations in this type of operations.

The most important technical breakthrough has been the design of the upflow culturing cylinder for bivalves, in which the spat is piled up in several layers and the water flows upwards in order to avoid the accumulation of faecal material upon and between the spat and on the container's walls.

Water flow-rate, water temperature, stocking density and feeding regime are the major parameters affecting the total production of bivalves in a nursery system, separately and in combination.

The selection of a particular technology and a specific set of environmental conditions will largely depend upon the economic aspects of the operation. The margin of profit in the nursery operation is very narrow, and therefore slight variations in the variable operating costs on top of the fixed charges for e.g. investment, labor, etc., may be determining the feasibility of an independent nursery operation.

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USE AND PRODUCTION OF MICROALGAE AS FOOD FOR NURSERY BIVALVES

Niels DE PAUW

Abstract.

The nutritional value of various foods and diets for juvenile bivalves is reviewed and commented. From all foods tested specific microalgae seem to be the most suited for young bivalves. Presently, serious alternatives for replacing living algae by inert, artificial feeds are practically inexistent, though research efforts are made in this direction.

The various microalgae production-systems used for feeding juvenile bivalves are also reviewed.

A literature survey and the COST inquiry held in 1979 by our laboratory revealed that nearly all commercial enterprises involved in nursery rearing of bivalve molluscs in Europe utilize natural phytoplankton as food. Culturing of the postlarvae is performed either *in situ* (in the sea) or in onshore constructions with flow through pumping of sea-water.

In contrast to these technologies, small-scale as well as large-scale experiments carried out in many countries, have shown the potential and reliability of culturing suited microalgal species for nursery bivalves, in analogy to the well established algal culturing for hatchery molluscs.

Some hatcheries continue to feed the spat with the same specific species of microalgae which are used to rear the larvae. The increasing quantities of algae needed to satisfy the growing food demand of the postlarvae soon, however, becomes a limiting technological as well as economical factor. For these reasons scaling up of the highly controlled and often sophisticated systems used to produce monospecific algae seems to be prohibitive.

To date two major trends can be distinguished for large-scale production of algae to be used in mollusc nurseries.

The first one relies on the completely controlled production of specific algal species. This implies keeping stocks of monospecific algae, progressive inoculation of cultures of increasing volumes, advanced water-treatment and controlled culturing conditions indoors (or in greenhouses).

The second trend is based on the induction of natural phytoplankton blooms in outdoor systems. This technology strives at a certain control of the species composition of the algal bloom by manipulating different internal and ambient parameters, such as nutrients, pH, detention time and mixing.

The pros and cons of these two trends are considered in the light of economic considerations.

The biotechnological aspects of large-scale production of algae are discussed : yields and harvest regimes, modes of sea-water enrichment, inoculation, collapsing and contamination of cultures, and culture systems and dimensioning of an algal plant for a bivalve nursery. The factors determining the production price of algae are analyzed.

The present trends and possibilities of a more controlled way to produce food for nursery bivalves are examined.

1.– Introduction.

In modern shellfish-rearing management, there is a tendency to introduce an intermediate nursery stage for rearing spat, following the larvae culture in hatcheries and preceding the transplantation of the spat to the natural environment (COST, 1978; Persoone and Claus, 1980; Claus, 1981).

The reason for developing such an intermediate nursery stage, is that too early transplantation of the fragile spat entails considerable risks of mortality due to handling losses, predation, temperature shocks and shortage of food.

Therefore nursery rearing of bivalve molluscs constitutes one of the major practices to be developed in mariculture, for which research should be stimulated and coordinated on an international basis (*cf.* COST, Mariculture Action 46, 1979).

Within the frame of an International Workshop on Nursery Culturing of Bivalve Molluscs, organized by our Laboratory (Claus et al., 1981), the methods and practices presently used are reviewed (Claus, 1981).

One of the major factors determining success or failure of these nursery operations, is the availability of large quantities of suited food organisms (Persoone and Claus, 1980).

The crux of the problem is the management of an adequate food supply and this review aims at exploring and commenting the various food-production systems and the nutritional value of different foods and diets which can be used to feed juvenile bivalve molluscs.

Close attention has been paid to the biotechnological as well as the economical aspects involved in the large-scale production of marine microalgae with the emphasis on practical problems encountered, and future research needed. An attempt is made to present some guiding data on production scale and costs of algal cultures needed for a bivalve nursery.

2.– Food requirements of juvenile bivalves.

Though disagreement exists among researchers regarding food of marine bivalves, from the numerous studies cited and commented by Ukeles (1971), we may conclude that in nature the following substances are important in bivalve nutrition :

- dissolved substances of organic origin (amino acids, vitamines, carbohydrates, lipids);
- organic detritus;
- living organisms : plants, animals (microalgae, protozoans, seaweed fragments).

The role of each of these categories is still a matter of discussion. This is also true for the role of bacteria as useful or harmful agents in the nutrition of bivalves at different stages of development. Many disagreements arise from the fact that the criteria for food utilization are not always as rigorous as desirable. Ingestion for example is not digestion. The presence or absence of certain foods in the stomach is not always a good criterium because of the rapid disintegration of many food items.

The most widely accepted view is that shellfish are basically microphagous herbivores, feeding mainly on phytoplankton and nanoplankton (Yonge, 1954; Ukeles, 1971; Walne, 1974; Imai, 1977). The food supply, however, that yields the most consistent results in feeding experiments, that is the most reliable and possible to control, duplicate, and adapt for large-scale development, is living phytoplankton (Ukeles, 1971).

The structure and function of food collecting and digestive organs in the different stages of development impose certain requirements on foods to be suitable for bivalve nutrition (Winter, 1978). The most obvious one is the size of the mouth and the oesophagus, determining the size of the food which can be particularly important for larvae. Juveniles have similar requirements though being less selective and more versatile with respect to their choice and utilization of food (Ukeles, 1971; Walne, 1974; Ryther and Goldman, 1975).

According to Winter (1978) juveniles and adults of *Chlamys* sp., *Mytilus edulis*, *Crassostrea virginica* and *Cardium edule* effectively retain particles down to 7 µm in diameter. *Mytilus edulis* is even capable of retaining particles down to 2 µm. Particles smaller than 1 µm are retained by all species, but only to a limited degree.

From Walne's investigations published in 1970, we can deduce that particles between 5 and 1 000 μm^3 with a corresponding diameter of 2 to 30-40 µm are assimilated by juveniles of bivalves belonging to the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*.

The physiological progress that mainly affects nutrition of bivalves is digestion. Of importance here are phagocytes and digestive enzymes for intra- and extra-cellular digestion (Ukeles, 1971; Owen, 1975; Imai, 1977; Lubet, 1978). In comparison to larvae, juveniles have larger digestive capabilities and are less sensitive to toxic and adverse conditions in food supply. Important factors for the usability of photosynthetic microorganisms are also the thickness of the cell wall and the degree of toxicity of the metabolites produced.

3.– Food value of microalgae.

A number of studies have examined the nutritive value of a variety of phytoplankton species for molluscan larvae (Walne, 1956, 1963, 1965, 1966; Davis and Guillard, 1958; Loosanoff and Davis, 1963; Loosanoff, 1971; Dupuy et al., 1977; Imai, 1977; Ukeles, 1980) and for juveniles and adults (Walne, 1970, 1973; Hartman et al., 1974; Loosanoff and Murray, 1974; Epifanio and Mootz, 1976; Epifanio, 1979b; Epifanio et al., 1976, 1981).

From the several thousands of phytoplankton species existing in nature, circa 40 distributed over eight classes have been tested as food for juvenile bivalves. Table 1 summarizes the relative food value of these species for several commercial bivalve molluscs belonging to the genera *Ostrea*, *Crassostrea*, *Mercenaria*, *Mytilus* and *Venerupis*. Table 1 has been compiled a.o. after Dean (1957), Guillard (1958), Walne (1970, 1974, 1976), Loosanoff (1971), Ukeles (1971, 1980), Baab et al. (1973), Hartman et al. (1974), Brown et al. (1975), Epifanio (1975, 1979b), Sunderlin et al. (1975, 1976), Epifanio et al. (1976, 1981), Rodde et al. (1976), Imai (1977), Dupuy et al. (1977), Pruder and Greenhaugh (1978), Malouf and Breese (1978).

Fom Table 1 we may conclude that considerable differences in food value exist among different algal species as food for juvenile bivalves.

Only 16 species out of 40 showed excellent growth : *Dicrateria inornata*, *Isochrysis galbana*, *Monochrysis lutheri*, *Pseudoisochrysis paradoxa*, *Tetraselmis suecica*, *Tetraselmis chui*, *Tetraselmis maculata*, *Tetraselmis tetrahele*, *Tetraselmis inconspicua*, *Chaetoceros calcitrans*, *Chaetoceros curvisetus*, *Chaetoceros simplex*, *Bellerochea polymorpha*, *Bellerochea spinifera*, *Skeletonema costatum*, *Thalassiosira pseudonana*.

Eighteen had very poor nutritive value and some of them were even toxic. In general, representatives of the Chlorophyceae, Cyanophyceae, Dinophyceae and Xantophyceae are less or unsuited as food for bivalves because of a thick cell wall or the production of toxic metabolites (*Amphidinum carterii*, *Chlorella* sp., *Gymnidinium* sp., *Prymnesium parvum*, *Stichococcus* sp., *Synechococcus* sp.) [Davis and Guillard, 1958; Guillard, 1958; Ukeles, 1971, 1980].

Chrysomonads on the contrary, having no thick cell wall, being small in size, and producing little or no metabolites, are good food as are Cryptomonads, green flagellates and diatoms.

A general rule, however, cannot be advanced, even related species within the same class, family or genus, may be good or bad. Within the class of the Prasinophyceae *Micromonas pusilla* is bad, while *Tetraselmis suecica* is excellent. Of the Haptophyceae

Food value of monospecific algae as food
for some commercially important juvenile bivalve species,
evaluation after different authors (see text)

Algal species	Size (μm)	Volume (μm³)	Bivalve species					
			1	2	3	4	5	6
HAPTOPHYCEAE								
Dicrateria inornata	3-4	21	+			+		
Isochrysis galbana	5-6	57	+	+	+	+	+	
Prymnesium parvum			–		–	–		
Cricosphaera carterae	12	900	+			±		
Pseudoisochrysis paradoxa	5-6			+	+			+
CHRYSOPHYCEAE								
Monochrysis lutheri	5-6	32	+	+	+	+	+	
Pavlova gyrans	5-6	43	–	–				
PRASINOPHYCEAE								
Micromonas pusilla	2	4.5	±	–		±		
Micromonas squamata	4-5	36	±					
Pyramimonas grossii	5-8		+			+		
Tetraselmis suecica	9-11	335	+	+		+	+	+
Tetraselmis inconspicua	4-7	580	+					
Tetraselmis tetrahele	10-16	250	+					
Tetraselmis chui	10-12	520	+					
Tetraselmis maculata					+			
CHLOROPHYCEAE								
Dunaliella euchlora	7-12	208	–	–	±	+		
Dunaliella tertiolecta	9-11	300	–	+	+	–		
Chlamydomonas coccoides	4-5	150	–	–		–		
Chlamydomonas sp.	10		±		+	–		
Chlorella autotrophica	2-3	5	–	–	–	–	–	
Chlorella stigmatophora	3-4	27	–	–		–	–	
Carteria chuii			+	+	+	+	+	
Chlorella sp.			–			±		
Nannochloris atomus	1-3		–			±		
Brachiomonas submarina	15-40	260	–			–		
XANTHOPHYCEAE								
Olisthodiscus sp.	14	1500	–			+		
CRYPTOPHYCEAE								
Chroomonas salina	9-16		+	+	+	+		
Cryptomonas sp.	4-5	46	±		+	+		
Cryptophyte S1			+	+				
Rhodomonas sp.					+			
CYANOPHYCEAE								
Synechococcus elongatus			–					
DINOPHYCEAE								
Amphidinium carterii					–	–		
Gymnodinium sp.					–	–		
BACILLARIOPHYCEAE								
Actinocyclus sp.					+	+		
Bellerochea polymorpha			+	+				+
Bellerochea spinifera			+					+
Chaetoceros curvisetus	10-30		+	+				+
Chaetoceros simplex	4-30		+	+				+
Chaetoceros calcitrans	3-5		+	+				
Phaeodactylum tricornutum	12-32	60	±	±	±	±	+	+
Skeletonema costatum	3-20		+	+	+	+	+	
Thalassiosira pseudonana	5-6		+	+	+	+	+	+
Thalassiosira fluviatilis	15-23		–					

+ : good; ± : moderate; – : poor.
1 : *Ostrea edulis*; 2 : *Crassostrea gigas*; 3 : *Crassostrea virginica*;
4 : *Mercenaria mercenaria*; 5 : *Mytilus edulis*; 6 : *Venerupis semidecussata*.

Prymnesium parvum is toxic, while *Isochrysis galbana* is excellent. In the genus of *Thalassiosira, Thalassiosira fluviatilis* is bad while *Thalassiosira pseudonana* is one of the best foods ever tested. *Chlorella* and *Chlamydomonas* having a rigid cell wall are unsuitable. *Dunaliella* on the contrary has no rigid cell wall but is nevertheless bad.

Most *Chlorella* species are not good food, but a small thin walled *Chlorella* sp. (Va52) isolated by Dupuis et al. proved excellent for feeding larvae of *Crassostrea virginica*.

The value of *Phaeodactylum tricornutum* on the contrary has been found to be very variable (Davis and Guillard, 1958; Walne, 1963, 1970; Wilson, 1978; Epifanio et al. 1981). Wilson (1978) recently demonstrated *Phaeodactylum tricornutum* to give the same growth results with larvae of *Ostrea edulis* and *Crassostrea gigas* as did *Isochrysis galbana* which is generally considered the best food. According to Walne (1963) and Wilson (1979) the factor causing variable growth in this case may be associated with the composition of the algal cells rather than their secretions or bacterial flora.

Also important to emphasize is that not all the bivalves considered are reacting in the same way towards given algal species. E.g. *Dunaliella tertiolecta* is bad for *Ostrea edulis* and *Mercenaria mercenaria* but good for *Crassostrea gigas*. *Monochrysis lutheri* and *Tetraselmis suecica*, both of excellent food value for most bivalve species, gave poor growth of *Ostrea lutaria*.

3.1.– *Food value of monospecific algal mixtures.*

During the last decade, considerable efforts have been made to determine the value of mixed diets of monospecific algae versus that of single algal diets as food for juvenile bivalves. The results show that with suitable species, far better growth is obtained with mixtures of two or more species than with either species alone (Matthiessen and Toner, 1966; Walne, 1973, 1974; Hartman et al., 1974; Sunderlin et al., 1976; Epifanio, 1975, 1979b). This is true not only for juveniles, but also for larvae and adults (Dean, 1957; Davis and Guillard, 1958; Calabrese and Davis, 1979; Helm et al., 1973; Dupuy et al., 1977; Helm, 1977; Shiraishi and Imai, 1977).

The algae mixtures resulting in better growth, consisted either of flagellates or diatoms, or flagellates and diatoms.

It should, however, be emphasized that the use of mixtures of algae species does not lead to an improvement of the value of poor species (Walne, 1974). According to Epifanio (1979b), the differences in food value between mixed and single algal diets may be explained by two types of interactions among combinations of algae.

Synergistic nutritional interactions could be explained by deficiencies in micronutrients or fatty acids (e.g. mixtures of *Thalassiosira pseudonana* or *Tetraselmis suecica* with *Isochrysis galbana*, resulted in better bivalve growth than with *Thalassiosira, Tetraselmis* or *Isochrysis*.

Non-additive interactions on the other hand could be explained by the ration of the algae versus the rate of extracellular digestion (e.g. *Carteria chui* or *Tetraselmis suecica* alone, resulted in less growth than did mixtures of these species with *Isochrysis galbana* or *Thalassiosira pseudonana*). These mixtures, however, resulted in equal growth in comparison with the latter species alone.

In connection with these observations, it is important to note the great differences observed in most cases between growth of bivalves fed monospecific algae cultured in filtered water and the same algae reared in unfiltered sea-water. Even with species such as *Isochrysis galbana* or *Tetraselmis suecica*, known to have an excellent food value, growth increased a 5- to 10-fold when mixed roughly filtered sea-water (Walne, 1970).

Moreover, species such as *Phaeodactylum tricornutum* and *Chlamydomonas coccoides* considered poor foods when cultured in filtered sea-water, gave no growth at all of *Mercenaria mercenaria* and *Ostrea edulis*, while growth was enhanced considerably by mixing *Phaeodactylum* of *Chlamydomonas* with unfiltered sea-water. The same is true for *Chlorella stigmatophora* as a food for *Ostrea edulis*.

It is clear that these observations may have serious economical implications for spat growing. More detailed studies are, however, needed to determine the value of raw sea-water supplemented with only small quantities of monospecific algae.

3.2.– *Explanations for differences in food value of microalgae.*

In conclusion of all the above mentioned findings regarding the food value of microalgae, we may quote Walne (1974) : "that the exact reason why one algae species is good food and another not, has not yet been found out".

The nutritionally inadequacy of algae may be due to :

- the lack of some trace constituents such as vitamines and minerals;
- the inability to digest cytoplasmatic bounderies of some algae;
- the rate of extracellular digestion related to the breakdown of the cell wall.

A remaining enigma is the relationship between the food value of an alga and its chemical composition (Epifanio, 1979a).

The growth of neither hard or soft tissue of a bivalve seems to be correlated with the gross chemical (protein, lipid, carbohydrate, ash) or the amino acid composition of the diet but seems to depend upon the presence or absence of particular algal species in the diet (Walne, 1970; Epifanio, 1975, 1979a,b).

This corroborates the findings of Parsons et al. (1961) that marine phytoplankton has a similar overall organic composition, when grown under similar physical and chemical conditions, regardless the size or the class it belongs to.

Walne (1974) noted that certain algal species are good foods even though the gross composition of algal cells may vary considerably as a function of culture conditions. The differences in gross composition of four algal species of vastly different nutritional values were no larger than the differences caused by changes in culture conditions within any one species. This, however, does not mean that the growth of bivalves is unaffected by the overall composition of algae. Flaak and Epifanio (1978) demonstrated that algae in the stationary phase containing more carbohydrates, resulted in oysters with more glycogen; algae in the exponential phase containing more protein gave less growth. The differences observed were, however, small.

According to Epifanio (1979a,b) the lipid quality rather than the quantity might be more important. However, to date, results are conclusive (*cf*. Watanabe and Ackman, 1974).

Differing food values can also be explained by the variation in the digestibility of proteins in different algal species (Walne, 1973). Temperature could also play a role in the digestibility of microalgae. According to Loosanoff and Davis (1963), *Chlorella* is better digested by oyster larvae at higher temperatures; a finding contradicted by Walne (1976).

The toxicity of some algae such as *Chlorella*, *Chlamydomonas* and *Stichococcus* could be due to the liberation of unsaturated fatty acids (Spoehr et al., 1949; Proctor, 1957, in Ukeles, 1971). Even good foods are occasionally toxic, because accumulation of algal and bacterial metabolites (Davis and Guillard, 1958; Walne, 1963). In this respect the age at which the algae cultures are fed may be of importance; stimulating as well as inhibiting reactions on bivalve growth have been observed (Wilson, 1979).

Finally, when considering the food value of microalgae, we must keep in mind that besides the quality of the food, the combined effect of flow rate and algal size and density are equally important (Walne and Spencer, 1974; Winter, 1976, 1978; Winter and Langton, 1976). The influence of these parameters are, however, beyond the scope of this review, but were researched by Claus (1981).

3.3.– *Alternative feeds instead of living algae.*

Finding an alternative inert feed to replace living microalgae would represent one of the major steps forward in the field of shellfish rearing. However, in spite of considerable efforts only limited success has been obtained, in most cases results were not consistent and often disappointing (Hidu and Ukeles, 1962; Haven, 1965; Chanley and Normandin, 1967; Dunathan et al., 1969; Claus and Adler, 1970; Walne, 1970; Lubet and De Longchamp, 1971, in Lubet, 1978; Winter, 1974; Castell and Trider, 1974; Flassch et al., 1975; Gabbott et al., 1976; Flassch, 1978; Epifanio, 1979a; Claus et al., 1981).

According to Walne (1974), the use of dead foods leads to difficult presentation problems. Small particles are indeed required, which are readily attacked by bacteria thus fouling the water as well as uneaten food.

Reviewing the various inert feeds tested, two major categories can be distinguished. One consists of preserved micro- or macroalgae, the other of non-algal feeds, including yeast, cereal or potato starch, cereal brans.

Freezedried *Isochrysis galbana*, vacuum dried *Monochrysis lutheri* and spray-dried *Chlorella* proved to be poor food for juvenile *Ostrea edulis* (Walne, 1970). Freezedried *Isochrysis galbana* and *Dunaliella euchlora*, heat-dried *Chlorella* and *Scenedesmus* and powdered macroalgae (*Fucus* and *Laminaria*), on the contrary, proved to be good food for larvae of *Mercenaria mercenaria* (Hidu and Ukeles, 1962; Chanley and Normandin, 1967; Walne, 1970; Lubet and De Longchamp, 1971, in Lubet, 1978).

Mixtures of yeast with algae (*Thalassiosira pseudonana*) gave less growth in juvenile *Crassostrea gigas* than algae alone (Epifanio, 1979a). Moderate growth of juvenile *Venerupis semidecussata* was obtained with rice bran, though it was inferior than with live algae (Claus et al., 1981). As a dietary supplement, starch might eventually be useful (Haven, 1965; Dupuy et al., 1977; Pruder and Greenhaugh, 1978).

With regard to the potential use of preserved algae, we should emphasize that provided suitable techniques can be developed, harvesting and preservation will add substantially to the cost of these algae, already expensive to produce.

4.– Algal production-systems used in nursery bivalve rearing.

The various possibilities and combinations to feed the different developmental stages of bivalve molluscs i.e. larvae, postlarvae, juveniles and adults, are schematically presented in figure 1.

Two major categories of microalgae utilized may be distinguished : phytoplankton naturally occuring in the sea, and cultured phytoplankton, either by induced blooming of natural phytoplankton, or in monospecific cultures. Both categories may be used alone or in combination depending upon the process developed.

Naturally occuring phytoplankton is traditionally used in shellfish rearing (Loosanoff and Davis, 1963; Walne, 1974; Korringa, 1976a,b,c; Imai, 1977) while cultured algae are used in more controlled enterprises, e.g. to rear larvae in the hatcheries (Loosanoff and Davis, 1963; Ukeles, 1965, 1971, 1976, 1980; Walne, 1966, 1974; Dupuy et al., 1977; Shiraishi and Nishikawa, in Imai, 1977), though the economical feasibility still remains a delicate question.

For the nursery rearing of spat, some hatcheries continue to feed the spat with the same specific algae already produced to rear larvae (Le Borgne and Vergonzane, 1975; Pruder et al., 1976; Loring, 1981, personal communication).

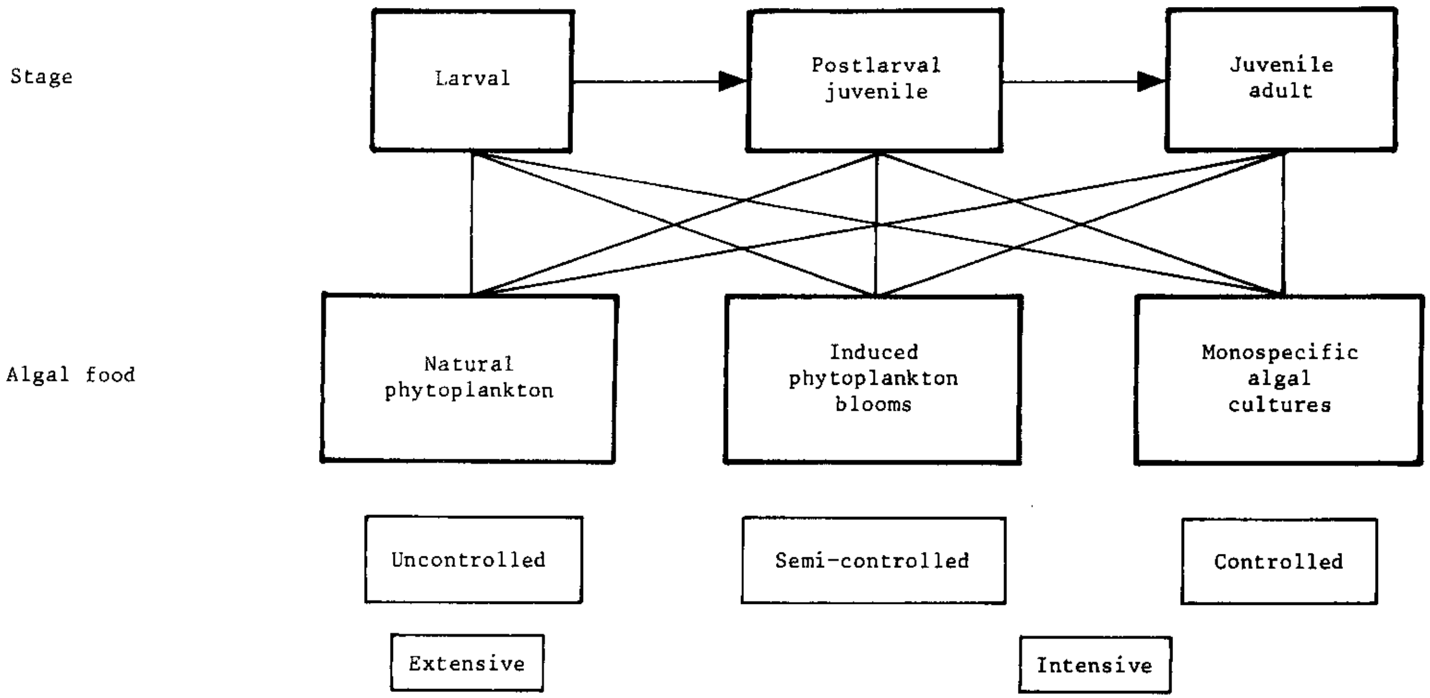

fig. 1.

Use of microalgae in bivalve mollusc rearing

The increasing quantities of algae needed to satisfy the growing food demand of the postlarvae soon become, however, a limiting technological as well as economical factor for the operation (see hereafter "Economical aspects of algal production"). For this reason most enterprises involved in nursery rearing of bivalve molluscs, even the modern ones, see themselves obliged to change to natural phytoplankton.

Table 2

Cost-inquiry 1979 on the use of microalgae
for the nursery rearing of bivalve molluscs in Europe

Country, place	Aim	Natural phytoplankton		Cultured phytoplankton	
		In situ	Onshore pumping	Induced blooms	Monospecific
Belgium, Ostend	E		+	+	
Denmark, Nykøbing, Mors	C	+	+	+	
France, Arradon	C	+	+	+	
France, Brest	E	+	+	+	+
France, Barfleur	C		+		+
France, Caen	E	+	+	+	+
France, St. Clément	C		+	+	
France, Sète	E	+	+		
Italy, Chioggia	C	+	+		
Italy, Lessina	E	+			
Italy, Taranto	C	+	+	+	
Italy, Trieste	C	+			
Ireland, Carna	C	+	+	+	+
Ireland, Carna	E	+	+	+	+
Ireland, Corelaine	C	+			
Ireland, Rossmore	C	+	+	+	
Malta, Ft San Luc	E	+	+	+	+
Spain, La Coruña	C	+			
Spain, Pemares	E				+
Spain, Villagarcia	E	+		+	+
Sweden, Lysekil	E	+			
The Netherlands, Yerseke	E	+			
UK, Argyll	C	+			
UK, Conway	E	+	+	+	+
UK, Essex	C	+			
UK, Lewis	C	+	+		
UK, Herts	C	+			
UK, Southampton	C	+			
UK, Whitstable	C		+		
UK, W-Mersey	C	+	+		
West-Germany, Langballigau	E	+	+		
Yugoslavia, Portoroz	E	+		+	
Yugoslavia, Rovinj	E	+			
Experimental	15	13	8	8	7
Commercial	18	15	11	6	2
Total	33	28	19	14	9

This trend is clearly illustrated by the COST-inquiry held by our laboratory among several commercial as well as experimental enterprises dealing with the nursery rearing of bivalve spat of oysters, clams, mussels and scallops (Table 2).

In most cases, the growing of larger spat is carried out, either *in situ* in the sea, or in onshore constructions with flow-through pumping of sea-water (Claus, 1981).

It should, however, be stressed that in temperate climates, consumption of natural phytoplankton is confined to the warmer period of the year because a minimal water temperature is required below which the animals stop filtering. In this regard, the use of heated effluents from power plants might have some future (Malouf and Breese, 1978; Malouf, 1981). In tropical areas, on the contrary, where temperatures are elevated year-round, growth is often limited due to the lack of phytoplankton (Persoone and Claus, 1980).

Therefore site selection plays an important role in determining the success or failure to be expected in shellfish rearing, as demonstrated by the many case studies described in detail by Korringa (1976a,b,c). Sheltered areas such as estuaries, bays, lagoons and inlets which are more productive thanks to eutrophication or natural upwelling have been favored over less productive oceanic sites (Oizumi, in Imai, 1977).

The major problem with the use of natural phytoplankton is, that one has no control on the production and composition of these populations which are subjected to predation of numerous consumers and influenced by many factors, among which the availability of nutrients. Moreover, often problems arise from the blooming of unwanted and even toxic dinoflagellates such as *Gonyaulax*, or Haptophyceae such as *Phaeocystis*, sometimes leading to mortality of the bivalves (Loosanoff and Davis, 1963; Sato, 1967, in Fujiya, 1970; Walne, 1976; Mercer, 1981; Rodhouse et al., 1981) and results inconsistent (Loosanoff, 1971; Walne, 1976).

On the contrary, as has been demonstrated in several pilot-scale as well as commercial operations, cultured phytoplankton, has a great potentiality and reliability as food for bivalves.

In table 3, some examples of the various algal production systems developed in different parts of the world are given.

To date, two major trends can be distinguished in the large-scale production of microalgae as food for bivalves. The first one completely relies upon the controlled production of specific algae. This implies keeping stocks of algae, axenically or non-axenically (with a low bacterial population), progressive inoculation of cultures of increasing volumes, advanced water-treatment, and controlled culturing conditions indoors or in greenhouses (Lucas, 1976; Loring, 1981, personal communication). A completely closed system has been devised to rear oysters from egg to market size on cultured food (Pruder et al., 1976; Pruder and Greenhaugh, 1978); its economic feasibility, however, remains to be proven.

The second trend in the large-scale production of microalgae is based on the induction of natural phytoplankton blooms (Ryther et al., 1972; Goldman and Ryther, 1976; Pryor and Lee-Watson,

Table 3

Examples and characteristics of land-based algal production-systems used for the nursery rearing of bivalve molluscs

Location	Culture device	Volume unit (m)	Enrichment type	Inoculum type	Algal type	Sea-water source	Aim	References
Ostend, Belgium	Tank/O	50-100	F	N	IB	Lagoon	E	De Pauw and De Leenheer, 1980 De Pauw et al., 1981
Le Brusc, France	Tank/O	33	F	N	IB	Lagoon	E	Riva and Vicente, 1978 Riva and Lelong, 1981
Tinduff, France	Tank/I	1.5	F	N	IB	Bay	E	Lucas, 1976
Carna, Ireland	Tank/O	20	F	N/A	M/IB	Sea	E	Rodhouse et al., 1981 Mercer, 1981
Oahu, Kaneohe	Pond/O	2000	F	A	M	Well	C	Burzell, 1978 Scura et al., 1979
Oahu, Kahuku	Tank/O	3000	F	A	IB	Well	C	Pryor, 1978 Pryor and Lee-Watson, 1978
Villagarcia, Spain	Tank/O	50	F	N	IB	Sea	E	Guerrero et al., 1981 Persoone et al, 1980
Milford, Ct, USA	Tank/I	12-40	F	N	IB	Bay	E	Loosanoff and Engle, 1942
Dennis, MA, USA	Tank/OI	23	F	A	M	Well	C	Zoto, 1978 (pers. comm.) Loring, 1981 (pers. comm.)
Woods Hole, MA, USA	Pool/O	120	W	N	IB	Sea	E	Ryther et al., 1972 Goldman and Ryther, 1976
Lewes, D, USA	Tank/I	10	F	A	M		E	Pruder et al., 1976
St Croix, VI, USA	Pool/O	50	AU	N/A	M/IB	AU	E	Roels et al., 1976a,b

O : outdoor; I : indoor.
F : fertilizer; W : Wastewater; AU : artificial upwelling.
N : natural; A : artificial.
IB : induced blooms; M : monospecific cultures.
E : experimental; C : commercial.

1978; Guerrero et al., 1981; Mercer, 1981; Riva and Lelong, 1981; Rodhouse et al., 1981) in outdoor systems. This technology strives at a certain control of the species composition of the algal bloom by manipulating different internal and external factors (De Pauw and De Leenher, 1980; De Pauw et al., 1980, 1981). Irrespective of the cost price, large-scale production of monospecific algae is feasible only in a few selected sites where large quantities of pure, practically sterile sea-water from wells or artificial upwelling are available (Roels et al., 1976a,b; Burzell, 1978; Zoto, 1978, personal communication; Scura et al., 1979). In all other cases, induced blooming of natural phytoplankton is necessary. In natural sea-water, it is impossible to maintain cultures of inoculated specific algae in large volumes over a prolonged period.

A major problem in outdoor mass-cultures of natural phytoplankton is to obtain control over the species composition of the induced blooms. In spite of the negative remarks formulated by several authors on the poor value of induced natural phytoplankton blooms there are indications that inducing blooms not necessarily results in the production of so called bad species such as *Phaeodactylum* (Ryther and Goldman, 1975) or *Chlorella* of suitable algae such as *Skeletonema costatum, Chaetoceros* spp., *Thalassiosira pseudonana, Nitzschia longissima,* and several flagellates (*Tetraselmis* spp., *Cryptomonas* spp.) [Dunstan and Menzel (1971), Tenore and Dunstan (1973a), Goldman and Ryther (1976), Laurence and Roels (1977), De Pauw and De Leenheer (1980), De Pauw et al. (1980), Mercer (1981), Riva and Lelong, (1981)].

Dunstan and Tenore (1974), Srna (1976), Graneli (1978), Harrison and Davis (1979), De Pauw and De Leenheer (1980), De Pauw et al. (1980), Mercer (1981) and Rodhouse et al. (1981) indicated that it is feasible to exert a certain control over the species composition of natural phytoplankton populations by manipulating different ecological parameters. Factors influencing the species composition are nutrient ratio's (N : Si : P), temperature, salinity, detention time and mixing. Besides these, the CO_2-O_2 balance in the medium, the light intensity and pH are also of great importance for the stability of the developing population (Pruder and Bolton, 1979, 1980; Pruder, 1981; Pruder et al., 1981a).

Mercer (1981) stated that *Phaeocystis* did not develop in cultures enriched with silicates. Diatoms, contrary to Chlorococcalean algae, are stimulated by silicate addition, low phosphorous levels and short detention times (De Pauw and De Leenheer, 1980). Depending upon the temperature, silica levels, detention time, different types of small diatoms will dominate the spectrum.

Over a period of more than three years, more than ten species were dominating at different periods of the year in our induced cultures, within a temperature range of 0°C to 25°C. A major task for the future will be to determine the reason why these species are blooming. Once this is accomplished we will be able to replace some of these species by more suitable ones.

Finally it must be emphasized that presently little or no precise information is available on the exact value of induced blooms of natural phytoplankton to be used as food for juvenile bivalves. To our knowledge, no growth comparisons have been made between bivalves fed pure monospecific algal cultures and induced blooms. Our own experience indicates that even mixtures of poor species such as *Phaeodactylum tricornutum* and *Chlorella* sp. result in good growth of several juvenile bivalves including *Ostrea edulis*, *Crassostrea gigas* and *Venerupis semidecussata* (Claus et al., 1981).

5.– Biotechnological aspects of large-scale microalgae production.

5.1.– *Culture devices and maintenance.*

Depending upon the needs and possibilities, ponds or pools as well as tanks of a few square meters up to several hectares are used for the large-scale production of algae (Table 3).

Pools and ponds may be natural or man made with a natural bottom or lined with cement, asphalt or synthetic materials such as polyethylene or PVC.

Tanks are usually rectangular, square or circular and built on a solid base, above or in the ground and may be temporary in design to allow flexibility of the system (Mercer, 1981; Rodhouse et al., 1981). The walls may be of reinforced concrete, wooden panels, bricks coated with cement, resines or plastic sheets. Commercial swimming pools may also be useful.

The culture depth in the various systems usually varies between 0.5 and 1 m, in some cases even 1.8 m (Persoone et al., 1980) and 1.85 m (Riva and Lelong, 1981). In particular cases vertical transparent culture tubes more than 2 m high are used as an intermediate step between carboys and tanks (Persoone and Sorgeloos, 1975; Flassch, 1978; Pruder et al., 1978; Zoto, 1978, personal communication; Trotta, 1980).

For a given detention time, shallow cultures will result in higher densities than deep ones. The latter ones require more pumping because larger volumes have to be moved, but they have the advantage to be more temperature stable, resulting in a greater stability in the plankton population composition (Bayes, 1981).

The culture system should be equipped with a draining device allowing complete, fast emptying and is important to allow cleaning. Cleaning of small vessels for growing monospecific algae require special and frequent care involving sterilization by heat or by chemicals (Guillard, 1973, 1974; Walne, 1974; Ukeles, 1976; Dupuy et al., 1977). Lucas (1976) preconized a weekly cleaning of small tanks used for monospecific algal cultures. According to Raymont and Adams (1958), chlorine sterilization of larger tanks was not effective to rid their cultures from phytoplankton species which had not been inoculated.

Usually the cleaning of larger systems can be restricted to the elimination of built-up sludge and periphyton. Systems with an artificial bottom may be hosed to eliminate periphyton whereas natural bottoms can only be cleaned by emptying and drying the ponds. This will at least eliminate the larger predators and unwanted consumers in the system. The dried organic material remaining in the system may serve as an extra enrichment. Particulate organic matter will, however, not originate immediately in phytoplankton but in bacteria and Protozoa, which break it down and mineralize it.

The experience of Pryor and Lee-Watson (1978) with 0.4 ha tanks in Hawaii indicated that maintaining cultures beyond thirty days allowed excessive growth of benthic sea-weeds on the sides and bottom of the reservoirs which competed with the desired cultures for nutrients, space and light. To get rid of benthic algae and *Enteromorpha* during summer, Rodhouse et al. (1981) suggest cleaning the tanks about every two weeks.

5.2.– *Inoculation of cultures and treatment of sea-water.*

Depending upon the purpose, algal cultures may be inoculated naturally or artificially. Mass cultures of selected monospecific algae require artificial inoculation with cultures grown in smaller volumes, first axenically later non-axenically (Walne, 1966, 1974; Guillard, 1973, 1974; Ukeles, 1973, 1976; Dupuy et al., 1977; Flassch, 1978). Cultures of monospecific algae also require advanced treatment of the water used including filtration down to less than 1 µm for the smaller inoculata, and heat- or chemical-sterilization to avoid contamination and fast take-over by other microorganisms. The use of artificial sea-water for smaller culture volumes has therefore been preconized by Ukeles (1965).

In case well water or artificial upwelling is used, selected algae species may be grown in volumes up to several thousands of m^3 (Table 3 – Roels et al., 1976a,b; Burzell, 1978; Pryor, 1978; Pryor and Lee-Watson, 1978; Zoto, 1978, personal communication; Scura et al., 1979). Because of contamination by other algae, regular restarting of these cultures is, however, necessary.

This type of cultures may last from a few days to two or three weeks depending upon the site. Unfortunately, in most cases no precise information on the management of such culture types is available.

Generally induced blooms of natural phytoplankton require no artificial inoculation. Introduction of non-sterile natural sea-water only roughly filtered is, however, a prerequisite to allow a variety of species to bloom. If well water or artificial upwelling is used, mixing with small quantities of surface water or trapping windborne algae in small tanks, may serve as an inoculum (Roels et al., 1976b; Pryor and Lee-Watson, 1978).

According to our experience, blooms of natural phytoplankton will occur within up to ten days depending upon light and temperature (De Pauw et al., 1981). Blooming can also be accelerated by inoculating natural sea-water or well water with blooms already developed in smaller preblooming tanks, thus making the system more efficient.

5.3.– *Enrichment of sea-water.*

In coastal and oceanic water, nutrients are present in only small quantities, often limiting the production of microalgae. Spärck (1927) and others (Edmonson and Edmonson, 1947; Pratt, 1949; Raymont and Adams, 1958; Ansell et al., 1963; Ukeles, 1965; Dunstan and Menzel, 1971) demonstrated that artificial enrichment of sea-water with organic or inorganic fertilizers is beneficial to the production of food for bivalves.

Two major categories of fertilizers can be distinguished. One consists of complex media with macro- and micronutrients, which are expensive and mainly used in the indoor small-scale production of monospecific algae (*cf.* a.o. Provasoli et al., 1957; Walne, 1966, 1974; Guillard,1974; Ukeles, 1976; Dupuy et al., 1977). The other comprises simple media with macronutrients composed of commercially available chemicals and is used in the large-scale production of algae, including induced blooms of natural phytoplankton (Loosanoff and Engle, 1942; Mann and Ryther, 1977; De Pauw and De Leenheer, 1980; Riva and Lelong, 1981; Rodhouse et al., 1981). The last category includes products such as ammoniumsulphate, ammonium chloride, triple superphosphate, calcium nitrate, ammonium nitrate and sodium silicate.

The necessity of various ingredients of the expensive complex substances has not been ascertained (Loosanoff and Davis, 1963; Guillard, personal communication, in Pruder et al., 1976). Indeed complete commercial N-P-K fertilizers as used in the tobacco industry, gave better results than standard chemical formulae prescribed for the cultivation of phytoplankton (Loosanoff and Engle, 1942).

Mineralized organic wastes such as sewage and manure, may also have a future (Ryther et al., 1972; Walrath and Natter, 1976; Faveris and Lubet, 1978; De Pauw and De Leenheer, 1980; De Pauw et al., 1980; Witt et al., 1981). Algae grown on artificial fertilizers and on secondary treated sewage resulted in the same growth of bivalves (Tenore and Dunstan, 1973a). Juvenile bivalves fed with algae grown in swine manure, grew better than those fed with algae cultured with inorganic nutrients (Claus et al., 1981).

To provide micronutrients, sea-water enriched with inorganic fertilizers may also be supplemented with small quantities of manures (Pryor and Lee-Watson, 1978; De Pauw and De Leenheer, 1980). When organic wastes are used care must be taken to avoid the eventual accumulation of viruses and heavy metals (Vaughn and Ryther, 1974; De Pauw and De Leenheer, 1979; Mann and Ryther, 1979).

A particular case of enrichment is the use of deep oceanic water, artificially upwelled for ocean thermal energy conversion (Roels et al., 1976a,b). Though richer in nutrients than oceanic surface water, the upwelled water is still poor in nitrogen (Roels et al., 1976b).

In the majority of cases, only nitrogen and phosphorus are added to the sea-water in large-scale systems because these elements are relatively cheap and easy to use. However, these types of enrichment often lead to the development of unsuited species such as *Chlorella* (Loosanoff and Engle, 1942).

Exceptionally no fertilization of the sea-water is needed as in the case with the N- and P-rich well water used in *Aquatic Farms* on Oahu, Hawaii for the large-scale production of monospecific algae (Scura et al., 1979).

In contrast, addition of silicates might favor the development of more suited diatoms (Dunstan and Tenore, 1974; Riva and Vicente, 1978; Scura et al., 1979; De Pauw and De Leenheer, 1980; Goldman and Mann, 1980; De Pauw et al., 1981; Riva and Lelong, 1981; Rodhouse et al., 1981). Silicates are indeed often limiting in sea-water (Officer and Ryther, 1979). According to our personal experience, inexpensive technical liquid sodium silicate is suitable for large-scale application provided it is acidified (De Pauw et al., in preparation).

Different growth responses are to be expected depending upon the nitrogen sources employed (Hayward, 1965; Ukeles, 1976). Nitrogen can be added as ammonium, nitrite, nitrate or ureum. Nitrite may be toxic in high concentrations (De Pauw and De Leenheer, 1979), while ammonium is often consumed with less energy expenditure from the cell than is nitrate (Syreth and Morris, 1963). Ammonium has, however, the disadvantage to be easily converted into free ammonium at high pH values due to photosynthesis and thus is lost to the atmosphere. According to the literature (Redfield et al., 1963; Riva and Vicente, 1978; Roden, in Mercer, 1981; Rodhouse et al., 1981) and our own experience (De Pauw and De Leenheer, 1980; De Pauw et al., 1981), N : P ratios by weight between 5 and 10, and N : Si ratios between 0.5 and 2 gave suitable blooms of diatoms. More studies are, however, necessary to determine the exact influence of nutrient ratios on the species composition of induced blooms of natural phytoplankton.

In practice, the difficulty consists in establishing that nutrient ration in the culture medium which is satisfactory for the nutrient uptake of the algae wanted, and the nutrient consumption of the system other than the uptake of algae.

In spite of the existing theoretical knowledge, we can conclude that the enrichment of sea-water is often done by a rule of thumb, leading to disappointing results due to a lack of control if the quantities applied have any effect. In this respect, fertilizer applications in very large natural open systems (bays, lagoons) are much more difficult to control than in more closed and smaller systems. The problem with natural systems is that the fertilizers may be taken up by a variety of organisms not directly useful to man and difficult to control (Korringa, 1976a). Moreover, part of the fertilizer may be captured by the sediment as demonstrated by Boyd and Musig (1981). In a large-scale algal system, nutrients are also removed by bacteria and periphyton developing on the walls and the bottom of the culture system, making regular cleaning a necessity.

With regard to sea-water enrichment, the elaboration of simple guide-lines for an efficient application of fertilizers will also be of utmost importance for the future. Hereby, the nature of the fertilizer must be taken into consideration. Some go immediately into solution but others become only slowly available. Boyd et al. (1981) demonstrated that liquid phosphorus fertilizers are more effective than granular phosphates. Bringing the granular phosphates into solution before application will, however, increase the effectiveness of the fertilizer. In our experiments good results were obtained with phosphoric acid of technical grade, which seemed to be better than triple superphosphate (De Pauw et al., 1979). Riva and Vicente (1978) reported that certain types of superphosphate are not practical because of the presence of inert parts in the fertilizer which hinder the culture.

According to Pruder et al. (1976) nutrients should be added at levels which each day can almost entirely be utilized by the phytoplankton. Too much fertilizer may lead to unsatisfactory buildups and growth of macrophytic algae at the surface and walls, leading to a reduction of microalgal production (Trévaillon et al., 1973). Frequent applications of small quantities of fertilizer seem to be more effective than less frequent applications of larger quantities. In our 100 m^2 tanks, nutrients are administrated daily or continuously in relation to harvest and algal growth. Analogue regimes have been worked out by Riva and Vicente (1978), Pruder et al. (1978) and Rodhouse et al. (1981).

5.4.– *Yield and harvest.*

In nutrient-saturated and light-limited algal cultures, yield is mainly determined by irradiation (Shelef et al., 1973; Stengel and Soeder, 1975; Goldman, 1979b). Estimated minimum and maximum yields for different latitudes and different conversion efficiencies were given by Oswald (1977). At our latitude 51°N, depending on the season, yields ranged from 0.1 to about 10 g algal dry weight/m^2/day (De Pauw and De Leenheer, 1979). These values correspond with an average realistic 1 % conversion efficiency of total incident radiation (Goldman, 1980). Below irradiation values of 200 J/cm^2/day, no algal growth was observed in our experiments, even with heating of the cultures (Goldman, 1972; De Pauw et al., 1980). At our latitude, such low irradiation only occurs during December.

To maximize the light energy conversion-efficiency it is necessary to adapt the concentration of the algae to the changing light irradiation. This implies that for a given depth the detention time of the harvest percentage is the variable factor (Shelef et al., 1973). At our latitude this means that between 5 and 80 % of the cultures could be harvested per day depending on the season (De Pauw et al., 1980, 1981). According to Mercer (1981) harvest regimes in Ireland during the summer could amount to 80 %/day. In tropical areas such as the Virgin Islands as much as 100 % of the culture volume can be harvested per day (Roels et al., 1976a,b). In that case, the detention time of the culture is only one day.

With respect to the harvesting, cultures can be run in batch or on a continuous basis. Batch cultures are usually associated with small-scale cultures of monospecific algae which have to be renewed regularly. Continuous or semi-continuous cultures are usually carried out on a large-scale, starting from induced blooms and lasting for longer periods (Table 3).

In some cases, (Loosanoff, 1951; Lucas, 1976; Pruder et al., 1978), cultivation of microalgae has been tested in greenhouses, supplemented or not with artificial illumination. In large-scale production, the use of artificial light has to be excluded for economic reasons. Our personal experience with greenhouses showed that up to 50 % of the light is lost due to the structure of the greenhouse itself, dust and precipitation of water vapor on the walls (De Pauw and De Leenheer, 1979). The profit obtained during winter time thanks to the extra heating was lost due to the decrease of the light intensity which became limiting. Covering algal outdoor tanks with different transparent materials may exclude 15 % of the light (Ansell et al., 1963). In areas where incident radiation is high and production is limited because of too low temperatures, greenhouses may, however, be profitable. This is certainly the case in countries where freezing of the water during winter prevents light penetration (Loring, 1981, personal communication).

5.5.– *Mixing of algal cultures.*

The major advantages of mixing algal cultures are to prevent sedimentation, thermal stratification, anaerobiotic conditions on the bottom, to keep the nutrients in active contact with the algal cell-surface and to increase the algal yield by a more effective utilization of the incident light (Stengel, 1970; Ukeles, 1971; Oswald, 1977; Märkl, 1980; Persoone et al., 1980).

Small-scale as well as large-scale experiments show that up to 30 % more yield can be obtained in a mixed versus an unmixed system (Persoone et al., 1980).To a certain extent, mixing will also prevent freezing of the water surface during winter. However, the economic repercussions of the extra energy input for mixing will have to be balanced against the increase in algal yield output. In this regard, the use of windmills for mixing should be studied.

Although mixing has advantages, it should be stressed that not all algal species grow better in mixed systems and that some prefer stagnant water as indicated by Walne (1970). The division rate of *Skeletonema costatum* is slower in turbulent water than in quiet water (Karsten, in Hustedt, 1930). Too vigorous mixing may also lead to hydrodynamical stress, influencing the development and the morphology of the algae (Bronnenmeier and Märkl, 1980). Ansell et al. (1963) observed the formation of non-motile cells of *Tetraselmis* in aerated high-density cultures.

Various mixing technologies including paddle wheels, air-bubbling from the bottom, air-lift pumps, jet-pumps have been developed (Stengel, 1970; Oswald et al., 1977; Goldman, 1979a). Circulation by simple pumping and air-bubbling from the bottom are most frequently used in aquaculture. These systems have also the advantage of easy introduction of CO_2 for pH stabilization and permit mixing of relatively deep cultures in contrast to paddle-wheels which are only suited for shallow systems.

Scarce precise information is available on the effect of different mixing devices, intensity, and duration on algal development. To evaluate the effects we presently run comparative experiments in 100 m^2 tanks equipped with different mixing devices including paddle-wheels, air-lift pumps and air-bubbling lines installed at the bottom. According to Bartels (1978) and Schroeder (1978) far better growth of *Nannochloris* was obtained with air-bubbling from the bottom than with air-lift pumps. Air-bubbling from the bottom, however, requires more energy. Introduction of air through air stones proved to be ineffective if too high pressures have to be overcome; rapid clogging of the stones' pores decreases their efficiency.

Our preliminary results indicate that mixing might influence the species composition and consequently the food value of induced phytoplankton blooms. During winter slow mixing in a 100 m^2 tank resulted in a pure culture of *Skeletonema costatum*, known to have an excellent food value for bivalves (Walne, 1970), while an unmixed parallel culture ended in a mixture dominated by *Skeletonema costatum* but accompanied by *Chlorella* an unsuitable food. Finally an important argument in favor of mixing algal cultures is the fact that mixing also reduces the development of zooplankton predators as recently demonstrated in small ecosystems by Oviatt (1981).

5.6.– *pH and CO_2 addition.*

In actively growing intensive algal cultures, rises in pH above 9 or 10 through photosynthesis will lead to severe limitations of nutrients due to precipitation and stripping (Soeder and Stengel, 1974; Ukeles, 1976).

The introduction of CO_2 is therefore of utmost importance, not only to stabilize the pH below a critical value and making nutrients available, but also as a carbon source avoiding unbalanced growth (Pruder and Bolton, 1979; Pruder, 1981; Pruder et al., 1981a,b). Addition of CO_2 in large-scale systems will, however, usually be economically prohibitive, exception made for a few cases in which cheap sources of carbon such as stack and flue gasses are available (Oswald et al., 1977). In this regard, the suggestion of Pruder (1981) that organic wastes might serve as cheap carbon sources deserves attention. The major problem with CO_2, is its efficient addition in large-scale open cultures, which till now remains unsolved (Märkl, 1977; Oswald et al., 1977; Märkl and Mather, 1980). A partial solution might be to work with deeper cultures with a less concentrated algal biomass per volume, which automatically could put a lower burden on the bicarbonate buffer. Examples are known, where CO_2 is added discontinuously when the pH is rising too high (Ansell et al., 1963; Zoto, 1978, personal communication). The lowering of the pH by CO_2 is only effective for a short time, soon after stopping the addition, the pH increases quickly.

A more effective but costly addition of CO_2 can be realized with pH transmitters only introducing CO_2 when needed (De Pauw et al., 1980). The pH cannot only be lowered by addition of free CO_2, but also with solid CO_2 (Ansell et al., 1963) or by the addition of acids such as chloric acid (Le Borgne and Vergonzane, 1975). Positive effects of CO_2 addition on the maximal cell density and prolonged growth of *Phaeodactylum tricornutum* were reported by Raymont and Adams (1958).

In practice, however, it is important to note that no cessation of *Phaeodactylum tricornutum* growth was observed due to extreme pH values. Le Borgne and Vergonzane (1975), on the other hand, reported that while CO_2 addition was positive for most species, this was not the case for *Skeletonema costatum* and *Phaeodactylum tricornutum*. This would confirm our own findings of excellent growth of *Skeletonema costatum* for several months even at pH values exceeding 9. Different species of algae have indeed different carbon requirements (Zelitch, 1971; Ukeles, 1976; Pruder and Bolton, 1979; Pruder, 1981). For the future it is of utmost importance to determine the conditions for balanced algal growth in order to decrease the risk of collapsing cultures and to allow the stimulated development of selected species in natural phytoplankton populations (Pruder and Bolton, 1980; Pruder et al., 1981a,b).

5.7.– *Predation and collapsing of algal cultures.*

Induced as well as natural phytoplankton species are subject to predation by numerous consumers. In practice, a distinction can be made between macropredators such as copepods, rotifers, meroplanktonic larvae of benthic invertebrates, and micropredators such as ciliates, zooflagellates and rhizopods. Macropredators may be effectively avoided by filtration of the incoming water. In our 100 m^2 tanks, good results were obtained with cheap 50 µm industrial cotton bag-filters (Ukeles, 1980). After four or five months of continuous operation, only small populations of copepods developed.

The water can also be filtered through combined gravel- and sand-filters, commonly used for swimming pools (Goldman and Ryther, 1976; Riva and Vicente, 1978; Witt et al., 1981). In actively growing algal cultures, developments of copepods and rotifers will also be counteracted by reducing the algal detention time below the generation time of the predators, as well as by the high pH values during the day (photosynthesis) and the low oxygen values during the night (respiration).

An alternative to eliminate predators is a selective chemical treatment of the cultures, as suggested by Loosanoff et al. (1957). Positive results were obtained with several insecticides in concentrations of 1 ppm and less without adverse effects to the shellfish. From a modern environmental protection standpoint such treatments should, however, not be encouraged.

Besides predation of algae by larger animals, protozoan predators are a problem. Protozoa have a short doubling time and are able to destroy a culture within 24 hours. Usually monospecific algae cultures, first grown axenically in a protected environment, are soon invaded by micropredators or other phytoplanktonic species better adapted to the given conditions, when transferred to open large-scale systems. Induced blooms of natural phytoplankton species seem to be less sensitive to this type of predation and may resist for months, provided that the culture conditions remain relatively the same (Pryor and Lee-Watson, 1978; De Pauw et al., 1981).

Though difficult to prove, we personally are convinced that severe predation of this nature is more an indication of poor culture conditions for a particular algal species.

Unfortunately little information is available on the morphology and ecology of these protozoan predators. The best described predation phenomena are those on *Phaeodactylum tricornutum* by *Monas* and *Oxyrhis* (Raymont and Adams, 1958; Ansell et al., 1963; Walne, 1976; Haas, 1979). It is remarkable that these predators do not seem to attack actively growing cells. Raymont and Adams (1958) demonstrated that *Monas* is only occurring after *Phaeodactylum tricornutum* has passed its exponential phase.

During the exponential phase, excretion of extracellular metabolites are apparently inhibiting the growth of these predators. Inversely, the growth of *Phaeodactylum tricornutum* was inhibited by extracellular products of *Monas* (Raymont and Adams, 1958). In some cases, cultures were reported to collapse without any apparent reason such as a shortage in nutrients or the occurrence of predators (Roels et al., 1976b; De Pauw, unpublished). Suggestions have been made that substances in the water, inimical to growth, might be responsible.

Presently no efficient treatment method to suppress monocellular predation exists. With small-scale cultures of *Dunaliella* reasonable success has been achieved by adding small quantities of formaldehyde, methylene blue and malachite green (De Pauw et al., 1979). Large-scale application of such treatments remain to be evaluated biologically and economically.

In conclusion we may say that because the collapsing of open large-scale algal cultures is unpredictable, the maintenance of parallel cultures seems to be an absolute necessity (Scura et al., 1979; Rodhouse et al., 1981).

6.– Economical aspects of algal production.

Few studies are devoted to the economics of large-scale algal production as food in aquaculture. According to Soeder (1978), this scarcity of information is readily explained by the relatively small number of projects devoted to the technology of microalgal production systems and by the hesitance of the experts to explore this field. A thorough economical approach would comprehend the analyses of investments, capital costs, maintenance and repair and operational costs.

Since no precise information is available on the costs of algal production as food for bivalves, the following is mainly derived from our personal experience with an experimental pilot plant for the nursery rearing of bivalve molluscs located at the *Institute of Marine Scientific Research*, in Ostend, Belgium. This plant includes four outdoor 100 m^2 tanks for algal production based on the principle of inducing blooms of natural phytoplankton. At a latitude of 51°N such a system could yield circa 160 kg dry weight algae/100 m^2/year, assuming a realistic light conversion-efficiency of 1 %. On the average this would correspond to a yield of 4.5 g dry wt/m^2/day. The operational costs of the algal production mainly include labor wages, electricity for pumping and mixing, fertilizers and eventually CO_2.

Labor costs are difficult to estimate and will largely depend upon the system's design, cleaning, addition of fertilizers and steering of the cultures. Depending upon the scale of the operation, the production of microalgae will be a part-time or a full-time job for a technician. An estimated half hour labor per day per 100 m^2 tank expenses, would already amount to US $ 2000 per year. Annual pumping costs may be estimated at US $ 125 and continuous mixing with a 1.5 kW motor at US $ 1000. Enrichment of sea-water with nitrogen, phosphorus and silicates would amount to US $ 50, 20 and 100 respectively. Costs for CO_2 estimated after Soeder (1978) who advanced at least US $ 2000 per ton of product, would represent US $ 320 per year. With continuous mixing of the algae and addition of CO_2, this would bring the annual operational costs at about US $ 3600 per year for a 100 m^2 tank.

Assuming an annual production of 160 kg dry weight/100 m^2, the price of 1 kg dry weight algae would be US $ 23. In comparison, Soeder (1978) estimated the price of 1 kg freshwater chloroccocalean microalgae from large-scale cultures to be US $ 1.50. This figure was obtained from 11 ha units under tropical conditions where yields are five times higher than in our climate.

Our data indicate that the price of the algae is primarily determined by the expenses for wages, mixing and CO_2. Reducing mixing and omitting CO_2, could lower the price to about US $ 14/kg, but this figure must be carefully interpreted because the impact of CO_2 addition and mixing on the algal yield has to be taken into consideration.

Prices (3 US cents/ℓ to 5 US cents/ℓ) given by Walne (1976) to produce monospecific algae such as *Isochrysis galbana*, containing 0.25 g dry weight would correspond with US $ 120 to 200/kg dry weight. Within the same order of magnitude, *Cultured Clam Corporation* (Loring, 1981, personal communication), forwards an estimated price of 0.5 US cents/ℓ for *Thalassiosira pseudonana* cultures containing 0.0308 g dry weight/ℓ

$$(2 \times 10^6 \text{ cells/m}\ell \times 1.54 \times 10^{-11} \text{ g/cell}),$$

which corresponds to US $ 162/kg.

In a cost-benefit analysis, the expenditures for algal production have to be weighed against the commercial value of the end product, namely the bivalve spat grown. For example growing spat of *Ostrea edulis* from 3 mm up to 15 mm, corresponding with an increase in meat dry weight of 10 mg, would require 100 mg dry weight of algae, assuming a food conversion-efficiency of 10 %. Based upon 5 US cents/ℓ of monospecific algae (Walne, 1976), 100 mg dry weight algae would be equivalent to 0.4 ℓ of algae or 2 US cents. The same 100 mg of algae, on the contrary, grown as induced blooms of natural phytoplankton would cost ten times less (0.2 US cents). Taking into account a commercial gain of 2 US cents between spat of 15 and 3 mm, costing respectively 3.4 and 1.4 US cents, we should conclude that the nursery rearing of large spat, exclusively fed with monospecific algae is not economically feasible.

The production price of induced blooms of natural phytoplankton, on the contrary, corresponding to less than 10 % of the commercial gain, could be economically rewarding assuming the algae grown are suitable for the bivalves, which is not always the case. Moreover, it should be emphasized that algal production in winter, will be more expensive than in summer while yields are lower and more surface is needed.

Dimensioning of an algal plant.

Essential for an efficient functioning of a controlled shellfish nursery, will be to determine and to provide sufficient quantities of suitable microalgae. Depending upon the production technology, algae grown indoors with artificial light, or outdoors with natural light, will be related respectively to culture volumes or surfaces and the approach to calculate the algal quantities needed, will differ.

According to Walne (1974), one million 5 mm spat of *Ostrea edulis* requires 840 ℓ of *Isochrysis galbana* culture of a concentration of 10×10^6 cells/mℓ/day whereas 15 mm spat needs 6000 ℓ/day of the same algal suspension. Similar data have been presented by Le Borgne and Vergonzane (1975). Pruder et al. (1976) calculated that from metamorphosis till marketable size, *Crassostrea virginica* consumed approximately 13×10^{11} cells of *Thalassiosira pseudonana*. Assuming a concentration of 2×10^6 cells/mℓ, a quantity of 650 ℓ of algal culture would be needed per marketable specimen. For 10×10^6 oysters this would correspond with 6.5×10^9 ℓ of algal suspension to be produced in twelve months. At a cost price of 0.5 US cents/ℓ (Loring, 1981, personal communication) this represents circa 30 million US $, which is above the commercial value of the marketable oysters.

Matthiessen and Toner (1966) determined the daily food requirement of juvenile oysters to be in the order of 10^6 cells of *Isochrysis galbana, Monochrysis lutheri, Thalassiosira pseudonana* or *Skeletonema costatum*.

For outdoor cultures in Woods Hole, Ryther and Goldman (1975) calculated that an 120 m^2 algal pond with concentrations of 10^5 to 10^6 cells/m , would be sufficient to grow some 10^7 juvenile shellfish. Such growth has, however, never been achieved because of the poor nutritional value of the algae, incorrect flow and feeding rates, and other undetermined causes.

It must be emphasized that the algal pond-surface required to grow a certain quantity of algae is related to latitude and season and thus to irradiation.

Guiding algal pond-surface estimates to rear ten million oyster spat from 2-3 mm to 15 mm, corresponding with an increase in dry weight meat of 10 mg over a three-month period, are presented in table 4.

Table 4

Estimated algal pond surface needed at latitude 51°N, during the four seasons, to rear ten million oyster spat from 2-3 mm up to 15 mm, corresponding with an increase of 10 mg dry weight over a growth period of three months. Calculations based on total incident radiation, algal yield (light conversion efficiency 1 %, 23 kJ/g dry weight of algae) and oyster yield (food conversion efficiency 10 %).

	Winter	Spring	Summer	Autumn
Total incident radiation (J/cm^2/3 months)	29433	121996	160681	65988
Corresponding algal yield (kg dry wt/100 m^2/3 months)	12.8	53.1	69.8	28.7
Corresponding oyster yield (meat/kg dry wt/100 m^2)	1.28	5.31	6.98	2.87
Algal pond surface (m^2) needed for 10×10^6 spat	7800	1900	1400	3500

These calculations were based on a number of theoretical assumptions with regard to algal yields and conversion efficiencies. Algal yields were computed for different seasons starting from a total incident radiation (10 year average) given for Ostend, Belgium (latitude 51°N), a 1 % light conversion-efficiency, and a caloric content of 23 kJ/g algal dry weight (Goldman, 1979b). Yields of bivalve meat were based on a 10 % conversion efficiency which has been set advanced by various authors (Tenore and Dunstan, 1973; Walne and Spencer, 1974).

According to these data and at our latitude, an algal pond-surface of circa 8000 m^2 in winter and 1500 m^2 in summer would be needed to provide sufficient quantities of algae as food for ten million spat. Starting from other assumptions, Malouf and Breese (1978) and Malouf (1981) estimated a surface of circa 2 ha to be necessary to grow sufficient quantities of monospecific algae to supply in food for ten million juvenile oysters and this at a latitude of 41°N (Woods Hole).

To date, few operators have, however, managed intensive marine algal cultures of this size (Burzell, 1978; Pryor, 1978; Scura et al., 1979). More experience with large-scale systems will be necessary if serious progress is to be achieved.

7.– Conclusions.

Feeding of juvenile bivalves may be achieved by using either natural phytoplankton, or induced blooms of selected algae or mixed natural phytoplankton. Presently satisfactory alternatives for live algae are unavailable.

The technology selected to produce algae will largely depend upon the site characteristics and objectives of the nursery plant, including availability of natural phytoplankton, quality of sea-water, possibility of land- or sea-based operations.

In temperate climates, the use of natural phytoplankton *in situ*, is restricted to the warmer period of the year. Though satisfactory in some cases, results are often inconsistent due to food scarcity or development of unsuited species. In this regard, site selection may be the determining factor for the success of the operation. Increasing the flow rate of the sea-water loaded with phytoplankton by natural (tidal action) or artificial (pumping) means may be beneficial to bivalve growth.

In tropical and subtropical climates, and in spite of the ideal temperatures for bivalve filtration, natural food is in most cases lacking due to nutrient limitation. Artificial upwelling of deep nutrient-rich water might be a solution in areas where this could be coupled to ocean thermal energy plants.

Algal cultures of selected species grown indoors or in greenhouses, may have excellent food quality. Their application is, however, limited to the rearing of larvae and early spat in hatcheries, the cost price for production being prohibitive for later nursery stages. Moreover, large-scale production of monospecific algae is only possible in some unique situations where very pure sea-water from wells or the deep sea is available.

Inducing blooms of natural phytoplankton, on the contrary, is technically and economically feasible. More research is, however, needed on the nutritive value of the algae in relation to the species composition in induced blooms of natural phytoplankton will be one of the major tasks for the future.

Extensive research on the bio-engineering of large-scale marine micro-algal production-systems is also an absolute necessity if serious progress is to be expected in the future. These projects should be accompanied by applied fundamental research to determine the conditions for balanced growth of suitable algal species as food for bivalves. Simultaneously continued efforts should be made to develop alternative inert feeds to replace living algae as food for bivalve molluscs.

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LIVE ANIMAL FOOD FOR LARVAL REARING IN AQUACULTURE : THE BRINE SHRIMP *ARTEMIA*

Patrick SORGELOOS*

1.– Introduction.

Intensive hatchery production of most juvenile fishes and crustaceans is still handicaped by the essential requirement for live foods (Jones and Houde, 1981). In the future this problem will most probably get solved through the use of suitable inert larval feeds (Meyers, 1979). In the meantime, however, hatchery activities, both at experimental and industrial level, still have to rely on live foods; i.e. several species of algae and yeasts (De Pauw and Pruder, 1981); the rotifer *Brachionus*; the cladocerans *Moina, Daphnia* and *Diaphanosoma*; the copepods *Eurytemora, Tigriopus* and *Tisbe* (Nellen, 1981) and last but not least the anostracan brine shrimp *Artemia*.

Brine shrimp nauplii are indeed most widely used, both in terms of cultured species, e.g. crab, shrimp, prawn, sole, sturgeon, sea bass, sea bream, etc. (Kinne, 1977) and in terms of quantities, i.e. at present more than 350 metric tons live weight annualy.

For the predator larvae that can handle this 0.5 mm prey, *Artemia* constitutes the most practical food (Sorgeloos, 1980a). It might not be the best among live foods – copepods appear to be a better diet for marine fish larvae (Watanabe, 1979) –, *Artemia* has the unique property that it can be produced from commercially available inert powder, namely its dry cysts (Sorgeloos, 1980b).

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This means that, except for a 24 hours hatching incubation of the cysts, one is entirely independent of live stock culture maintenance and its inherent production problems which constitute the big draw-backs with all other live foods.

Problem number one with *Artemia* is thus availability and price of the cysts. Until about five years ago commercial cyst supplies were from two natural sources in the United States and Canada. The increasing demands for cysts by aquarium hobbyists and aquaculture hatcheries soon exceeded by far the commercial provisions of approximately 30 to 50 metric tons per year. As a consequence cyst prices skyrocketed and the quality of the product delivered became less and less reliable.

We argued at the 1976 FAO Technical Conference on Aquaculture in Kyoto (Japan) that the *Artemia* problem of that day was artificial and could be solved (Sorgeloos, 1979); i.e. more *Artemia* biotopes could be considered for exploitation, either natural sources or environments where *Artemia* can be artificially introduced; better techniques could be adopted for cysts processing and hatching; one could also save on the quantities of cysts needed by feeding the predator, at least from a certain larval stage onwards, with cultured *Artemia*.

It took a while before significant changes were noticed. However an *Artemia* inquiry held in preparation of this review among more than seventy aquaculture hatcheries that use *Artemia*, clearly reveals that five years after Kyoto the *Artemia* situation greatly has improved. The main reason for this has been the steadily growing aquaculture research interest in *Artemia*. Nationally and/or internationally financed projects are now in progress in Belgium, Brazil, Cuba, Ecuador, India, Indonesia, Mexico, New Zealand, the Philippines, Thailand, the United Kingdom, the United States and for sure more countries that I am not aware off. At the 1979 first international symposium on the brine shrimp more than forty papers were presented in relation to the use of *Artemia* in aquaculture (Persoone et al., 1980). The purpose of this review is to present a brief overview of the various achievements and prospects with regard to the use of *Artemia* in aquaculture.

2.– The production of *Artemia* cysts.

In comparison with 1976 there is now not only a larger choice of commercial cyst-products, provision is also more reliable and hatching qualities have generally improved. Referring only to those exploitations that produce more than one metric ton of cysts per year there are now two sources in the United States and one each in Argentina, Australia, Brazil, Canada, Colombia and the People's Republic of China (Table 1).

Table 1

Updated list of major *Artemia* cyst dealers

AQUAFAUNA BIO-MARINE Inc.
P.O. Box 5, Hawthorne, CA 90250, USA
(distributor of Great Salt Lake, Utah - USA)

AQUARIUM PRODUCTS
180L, Penrod Court, Glen Burnie, MD 21061, USA
(harvestor-distributor of sources from Argentina and Colombia)

ARTEMIA Inc.
P.O. Box 2891, Castro Valley, CA 94546, USA
(harvestor-distributor of Shark Bay, Australia;
distributor of Macau, Brazil)

CHINA NATIONAL CEREALS, OILS & FOODSTUFFS
Import & Export Corp. – Tientsin Branch
N° 134 Chih Feng Road, Tientsin, PR China
(harvestor-distributor of Tientsin, PR China)

HL MARINOCULTURA Ltda.
Caixa Postal 25, Macau, RN 59500 Brazil
(harvestor-distributor of Macau, Brazil)

JUNGLE LABORATORIES CORPORATION
P.O. Box 66, Comfort, TX 78013, USA
(harvestor-distributor of Chaplin Lake, Canada)

SANDERS BRINE SHRIMP Co.
1255 West 4600 South, Ogden, UT 84403, USA
(harvestor-distributor of Great Salt Lake, Utah - USA)

SAN FRANCISCO BAY BRAND Inc.
8339 Enterprise Drive, Newark, CA 94560, USA
(harvestor-distributor of San Francisco Bay, CA - USA)

Cyst consumption by aquaculture hatcheries is estimated at about sixty metric tons in 1981 (Department of Fisheries Thailand; UNDP/FAO, Programme for the Expansion of Freshwater Prawn Farming in Thailand; our own data).

Although many people complain not to know where to buy cysts, we may say that right now there is no more shortage of cysts on the market. However, this will probably not last for long. The industrial expansion in commercial farming of *Macrobrachium* and several *Penaeus* species will provoke a very significant increase in *Artemia* cyst demands (Sandifer, 1981).

According to data gathered with the *Artemia* inquiry, less than ten major corporations will need more than fifty metric tons of cysts for their hatcheries in the next three to five years. This means that a new period of cyst shortage only can be avoided by much faster developments in *Artemia* cysts provisions than those achieved during recent years. We believe that this is possible, the more that since Kyoto 1976 several theoretical assumptions with regard to *Artemia* matters have been demonstrated in practice.

First of all the exploitation of natural resources : although over 250 findspots of *Artemia* have been reported in scientific literature (Persoone and Sorgeloos, 1980), only less than twenty are tapped for aquaculture purposes. Most of the others have not been seriously surveyed for potential exploitation.

The potential should however not be overestimated; indeed many of these sources will be either too remote, too small or unproductive to justify commercial exploitation; on top of that many other business factors such as property rights, export regulations and currency problems might further discourage cysts dealers to invest in such explorations.

Transplantation of brine shrimp in a suitable environment, for example a solar salt work, into which *Artemia* has not been dispersed yet can lead to the development of a very successful *Artemia* business. Indeed the unique culturing properties of brine shrimp assure that in an operational salt work, only minimal investments are needed to develop an *Artemia* by-product operation : in Brazil, for example, the more than forty metric tons of Macau cysts that have been marketed since early 1978 all originated from a 250 g cyst introduction in 1977 (Persoone and Sorgeloos, 1980; Van Tilburg, personal communication).

Although the basic biological principles of an *Artemia* transplantation are clear now, problems with regard to reduced cyst production (i.e. dominant ovoviviparous reproduction mode) in transplanted *Artemia* in Australia (Geddes, 1981) and Brazil (Insulata and Lai, personal communication) remain unsolved. Better scientific information should be generated with regard to the selection of appropriate strains of *Artemia* for transplantation in specific areas.

Temporal salt works that are found in monsoon-climates also can be valorized for an integrated *Artemia*-production. The technical feasibility of this type of man-managed *Artemia*-production during the dry season has been proven first in the Philippines in 1978 (De los Santos et al., 1980; Primavera et al., 1980) and later in India (Royan, 1981), Thailand (Vos and Tansutapanit, 1979; Tunsutapanick, 1980), Costa Rica (Naegel, 1980, 1981) and Indonesia (Djajadiredja, personal communication).

Commercial feasability of such *Artemia* inoculation projects is however much less obvious than with the earlier cited *Artemia* transplantations. Since waterdepths have to be increased to assure acceptable water-temperatures for the brine shrimp (Vos and de la Rosa, 1980), extra investments are needed for pond modifications which on the other hand will reduce the salt returns per unit of surface area. In a country like Thailand, where solar salt making is a marginal venture and *Artemia* cysts are in high demand for the expanding *Macrobrachium* hatcheries, integrated salt *cum Artemia* production was very well received and apparently is a profitable industry (Department of Fisheries Thailand, UNDP/FAO, Programme for the Expansion of Freshwater Prawn Farming in Thailand, 1980).

In other countries like the Philippines salt making is very profitable (Vos, 1980). Local salt bed operators only will go into integrated *Artemia* production when the return with brine shrimp can be maximized, which means through intensified production and harvest of cysts + biomass from fertilized ponds (see further on the potential valorisation of adult biomass in fish and crustacean hatcheries and nurseries).

It should be clear thus that one cannot generalize on both technical and economical feasibility of *Artemia* transplantation and inoculation projects. Site selection work is of primary importance. The feasibility of new projects should also be verified at pilot scale prior to take off on large budgets for fabulous production forecats. In this regard the Christmas Islands *Artemia* project (Helfrich, 1973; Environmental Consultants, 1979) will make history as production site of golden *Artemia* cysts. Brine shrimp production on this remote island in the Pacific is not justified (Sorgeloos, 1980c) : i.e. salinity levels in the so-called "hypersaline" lagoons are too low for *Artemia*, cheap nutrients are not available and transportation costs can never be justified.

We are convinced however that there exist many other sites especially on continents like Africa and South America that have unique potential for large scale *Artemia* production.

It has recently be found that several 100,000 ha of alluvial land areas in Peru migt be suited for *Artemia* pond development. Vast amounts of salt water, infiltrated from irrigation fields are just at 30 to 50 cm depth. Chicken manure, known to be a good fertilizer for *Artemia* ponds (Vos and de la Rosa, 1980) is available by metric tons from nearby industrial farms. However, since one will have to start from scratch, it is clear that the development of these and similar *Artemia* projects will take a long time.

Much quicker developments can be expected from the integration of *Artemia* production in operational solar salt works. Salt farmers have indeed realized that their vast areas of evaporation ponds can be valorized for more than just salt production. For a long time these chemists were afraid that "... *Artemia* might eat the salt", better evidence is now available that the presence of *Artemia* in salinas assures not only more salt production, but even a better quality salt (Davis, 1980; Jones et al., 1981; Sorgeloos, 1979). Knowing that in the tropical-subtropical belt several 100,000 ha of solar salt works are in operation, these areas might be very well suited for vertically integrated aquaculture projects.

3.– The practical use of *Arremia* cysts in aquaculture hatcheries.

The hatching quality of commercial cyst products has greatly improved since Kyoto'76 (Smith et al., 1978; Sorgeloos et al., 1978; Vanhaecke and Sorgeloos, 1981a). Cyst processors have indeed adopted improved techniques for harvesting and cleaning of their cysts from sand, cracked shells and other debris.

Hatching techniques have also been improved (Sorgeloos, 1980a) as a confirmed by about 80 % of the aquaculture hatcheries that were recently questioned with regard to *Artemia* uses. Better standardisation, illumination of the cysts, change of equipment, incubation at lower salinity and decapsulation of the cysts appear to be the most important innovations.

In addition, better criteria have been developed for evaluating the hatching quality. One now considers hatching rate and synchrony, hatching efficiency and hatching output. Incubated at 25°C in 30 ppt sea-water, the first nauplii should appear after 15 to 20 hours cyst incubation and the last nauplii should have hatched out within a time-laps of less than ten hours (Vanhaecke and Sorgeloos, 1981a). The hatching efficiency criterium (Sorgeloos et al., 1978) is widely used (Dye, 1980; Prescot, 1980). However, since it has been shown that the nauplii from different geographical sources greatly differ in size and weight (Vanhaecke and Sorgeloos, 1980) it became obvious that the hatching efficiency criterium underestimates the quality of a batch of large cysts. This lead to the introduction of the concept hatching output (Vanhaecke and Sorgeloos, 1981a), which precises the total weight of nauplii that can be produced from one gram cysts.

Table 2

Hatching characteristics of different commercial batches of *Artemia* cysts (from Vanhaecke and Sorgeloos, 1981a)

Source of cysts	Hatching efficiency* (nauplii/g)	Nauplius dry weight (in µg)	Hatching output* (mg/g cysts)
San Francisco Bay (USA)	267,200	1.63	435.5
San Pablo Bay (USA)	259,200	1.92	497.7
Macau (Brasil)	304,000	1.74	529.0
Barotac Nuevo (Philippines)	214,000	1.68	359.5
Great Salt Lake (USA)	106,000	2.42	256.5
Shark Bay (Australia)	217,600	2.47	537.5
Chaplin Lake (Canada)	65,600	2.04	133.8
Buenos Aires (Argentina)	193,600	1.72	333.0
Lavalduc (France)	182,400	3.08	561.8
Tientsin (PR China)	129,600	3.09	400.5
Margherita di Savoia (Italy)	137,600	3.33	458.2

* Hatching efficiency and hatching output data are not source but batch specific !

As appears from an at random testing of commercial cyst batches (Table 2) there is still much room for improvement. Most cyst-customers that completed the *Artemia* questionnaire considered that an improved service by commercial cyst-dealers is a high priority need for future *Artemia*-developments. Cyst distributors could certainly avoid a lot of frustrations and arguing by a more explicit reference to the guaranteed hatching quality of their cyst products and to its keeping qualities.

The use of *Artemia* cysts in aquaculture hatcheries can further be improved by application of the cysts decapsulation technique, i.e. during a short exposure to a hypochlorite solution, the hard cyst shell is dissolved without affecting the viability of the embryo (Sorgeloos et al., 1979; Tunsutapanich, 1979; Bruggeman et al., 1980). This technique not only eliminates all problems with regard to the separation of the freshly hatched nauplii from the empty cyst shells, it has many other advantages not the least the fact that for many fish and crustacean larvae decapsulated cysts appear to be as good a food source as the freshly hatched nauplii (Bruggeman et al., 1980; Mock et al., 1980; Royan, 1980a). The handicap however is that decapsulated cysts do not stay in the water column but sink.

The main rational for the present using of decapsulated cysts in aquaculture hatcheries is the increased hatching output (Vanhaecke and Sorgeloos, 1981a). The *Artemia* inquiry furthermore revealed that the present technique for cyst decapsulation is still too complex. In this regard we are wondering if commercial availability of decapsulated cysts might not be a better solution for this technical problem at the hatchery level.

In view of the observed differences in hatching quality from one cyst source to another, it is logic to suspect differences in nutritional value of the nauplii for specific cultured species. A detailed characterization study of *Artemia* strains was initiated at an interdisciplinary level in 1978 (Sorgeloos et al., 1979) and is actually in progress with five laboratories from the United States, the United Kingdom, Spain and Belgium. The more than fifteen that have been published so far all reveal very significant differences for most characteristics studied. Of interest for this review here are the feeding tests with three crustacean and four fish species as well as the detailed biochemical analyses that were performed with ten commercial strains :

- Naupliar size variation (Vanhaecke and Sorgeloos, 1980) appears to be the first criterion that at least with some predator species determines the ingestibility of specific *Artemia* products (Beck et al., 1980; Beck and Bengtson, 1981).
- San Pablo Bay *Artemia* is very low in the essential fatty acid $20:5\omega3$ (Schauer et al., 1980; Léger et al., 1981) and provokes high larval mortalities in all marine fishes and crustaceans tested so far (Beck et al., 1980; Johns et al., 1980 and 1981b; Klein-MacPhee et al., 1980; Léger et al., 1981; see also Goy and Costlow, 1980). A similar correlation between low levels of $20:5\omega3$ in different *Artemia* strains and poor performances with cultured marine fish was reported by Fujita et al. (1980). Although San Pablo Bay *Artemia* was also found to be contamined with chlorinated hydrocarbons (Olney et al., 1980), it is an acceptable diet for freshwater fishes (Usher and Bengtson, 1981; Vanhaecke and Sorgeloos, 1981b).
- High mortalities are noticed with a diet of *Artemia* from Great Salt Lake origin for shrimp (review in Sorgeloos, 1980a), crabs (Sorgeloos, 1980a; Johns et al., 1980, 1981a) and flatfishes (Sorgeloos, 1980a; Klein-MacPhee et al., 1980); but mysid (Johns et al., 1981b), carp (Vanhaecke and Sorgeloos, 1981b) and atlantic silverside (Beck et al., 1980) do very well on the

same *Artemia*. The exceptional report of successful culturing of crabs with Great Salt Lake *Artemia* (Goy and Costlow, 1980) has recently been clarified : i.e. the batch of cysts received by Goy and Costlow had been harvested from a distinct source in the Great Salt Lake (Sanders, personal communication) and was significantly lower in copper content as compared to the levels found in commercial batches (Blust, 1981), which so far always have been harvested at the same spot (Sanders, personal communication).

- Except with Chaplin Lake Canadian *Artemia* which give intermediate results, the other *Artemia* strains tested (Lavalduc, France; Tientsin, PR China; Macau, Brazil; San Francisco Bay, USA; Margherita di Savoia, Italy and Shark Bay, Australia) assure acceptable survival and growth for the marine and freshwater test species (Beck and Bengtson, 1981; Vanhaecke and Sorgeloos, 1981b).

Since over two years now these differences in nutritional value of *Artemia* from various geographical sources have been the subject of several publications and were repeatedly discussed at international meeting. It nonetheless appears from the *Artemia* inquiry that many of the participants are unaware of these results. It is high time that hatchery people realize that there is *Artemia* and *Artemia*; that aside from hatching quality one should also consider nutritional quality; in other words that one should select the most appropriate *Artemia* sources for the specific predator one is working with. We know of at least six major hatcheries in the United Kingdom, the United States and the Philippines were *post factum* mass mortalities of marine fishes and crabs could be related to the source of the *Artemia* used ! As long as specific literature data are lacking on the nutritional value of different *Artemia* strains for commercially important fish and crustacean species, one should be very careful in deciding about an *Artemia* product to be used, i.e. a comparative bioassay should be run with an intercalibration product such as Reference *Artemia* Cysts (Sorgeloos, 1981).

4.– The use of ongrow *Artemia* as food source in aquaculture.

It is known from scientific literature that in fact (pre)-adult *Artemia* have a higher nutritive value than freshly hatched nauplii (Sorgeloos, 1980a); i.e. adults contain 60 % protein, are rich in all essential amino acids, high in poly-insaturated fatty acids and have an ash-content of 10 %. However aside from a few experimental tests (see also Palmegiano and Trotta, 1981) feeding with adult *Artemia* has never been verified for large scale application. This might soon change as a result of following recent findings and developments :

- a fish farmer in the Philippines has demonstrated that his milkfish production per surface area and per year can be significantly increased as a result of supplementary feeding of the

Chanos fry during their three week stay in the nursery ponds with adult *Artemia*; survival at the end of the nursery stage has not only increased, the milkfish has also grown faster and are thus more resistant which assures better results during consequent ongrowth (De los Santos, personal communication);

- a commercial shrimp farm in Brazil reports significant improvement in their *Penaeus* hatchery-nursery output since extra-feeding with adult *Artemia* has been applied in routine fashion (Guimaraes, personal communication).

Of course the basic question in this regard is where to obtain cheap *Artemia*.

As is done at the Brazilian farm (Sorgeloos, 1979), live *Artemia* can be collected from an operational solar salt work where it is mostly available on a year-round basis and by the metric tons. If not available from a nearby salt work cheap *Artemia* can be produced in either extensive or intensive culture systems.

As is applied by the Philippino fish farmer extensive *Artemia* culturing can be done in small ponds adjacent to the nursery ponds. Lime and crude salt are added to the sea-water to create an acceptable medium for monoculturing of brine shrimp. Phytoplankton development is enhanced by weekly additions of chicken manure and commercial inorganic fertilizer (see also Dwivedi et al., 1980; Royan, 1980b; Tunsutapanich, 1980; Vos and de la Rosa, 1980).

In tropical climates like in the Philippines, production yields of more than 10 g brine shrimp per square meter and per day can easily be maintained. This means that, in the Philippines for example, large scale practice of this type of extensive *Artemia* production could lead to a very substantial increase in milkfish productions. Such *Artemia* could also be used as a suitable food for other cultured species, even for freshwater organisms like *Macrobrachium*. Upon transfer in freshwater adult *Artemia* stay alive for several more hours, enough time thus to be captured and eaten by the predator.

Extensive *Artemia* productions, however, are limited to warm climated and dry seasons. In this regard intensive culturing of brine shrimp in controlled conditions is much more versatile in applicability.

Since Kyoto 1976 techniques for intensive *Artemia* culturing have grown from lab scale testing into pilot scale and, in some situations already into industrial application.

The major breakthrough in intensive culturing of brine shrimp has been the finding that cheap agricultural byproducts (James et al., 1981; Dobbeleir et al., 1980; Sorgeloos et al., 1981) such as e.g. ricebran and whey-powder, can be successfully used as food source. Ingestibility of the food is a critical factor; therefore particle sizes mostly have to be reduced below the 50 mikron limit (Dobbeleir et al., 1980).

The most widely applicable culture techniques is the batch culturing from nauplius to adult stage without any water renewal in air-water-lift operated raceways (Bossuyt and Sorgeloos, 1980) :

- air lifts assure optimal aeration and circulation without harming the animals;
- faecal pellets are selectively settled out in a plate separator which is connected to the raceway;
- food distribution is semi-automatic, the only adjustment needed being the frequency and rate of distribution as a function of the turbidity changes within the culture tank.

Present production figures that are obtained in routine operations amount to 5 kg brine shrimp per cubic meter after two weeks culturing on ricebran at 25°C (Bossuyt and Sorgeloos, 1981). The pilot plant of the *Artemia* Reference Center at the Belgian coast, which consist of four raceways of 5 m^3 each, can be operated by one technician only and can produce an average of 50 kg pre-adult *Artemia* per week. Variations on this culture technique in batch conditions have been reported by James (1980a,b) and Rallo et al. (1981).

More intensive *Artemia* culturing can be achieved with flow-through culturing. The basic requirement of course is the availability of large volumes of brine effluents (e.g. thermal effluents from desalination plants) or warm sea-water.

An interchangeable and self-cleaning screen system that retains the animals in the culture tank but allows drainage of water and faecal pellets is the key item that makes high density flow through culturing of brine shrimp technically feasible (Tobias et al., 1979). This culture system is very useful for converting algal biomass into animal protein in artificial upwelling projects (Roels et al., 1979) or, more realistically, in tertiary treatment systems (Milligan et al., 1980).

Using the effluent of a geothermal well, it has recently been demonstrated that with inert diets such as e.g. grinded ricebran, brine shrimp can be cultured in densities up to more than 10,000 animals per liter (Brisset et al., 1981). Production figures are indeed substantially higher than with raceways operated under batch conditions. Both with algae as with rice bran one can easily produce more than 20 kg *Artemia* in a 1 m^3 tank over a culturing period of two weeks only (Sharfstein et al., 1979; respectively Brisset et al., 1981).

It is to be expected that in the near future, the use of adult *Artemia* in aquaculture hatcheries and nurseries will contribute to improved production results. There is urgent need however for further research on the nutritional value of different products of adult brine shrimp; in this regard it is highly likely that for intensive culturing on inert feeds one might have to consider diet formulations for the *Artemia* as to assure an optimal nutritional value for specific predators (Dobbeleir et al., 1980; Metailler et al., 1981; Sorgeloos et al., 1981).

As production techniques both at the extensive and the intensive level further will develop it will become more and more realistic to consider mass scale production and use of *Artemia* meal as a protein-ingredient for artificial feed formulations (Aquaculture development for Hawaii, 1978; Sorgeloos, 1980a; Webber and Sorgeloos, 1980).

5.– Conclusion.

Five years after the FAO Technical Conference on Aquaculture in Kyoto it can be said that the overall *Artemia* situation has improved (Pillay, 1981) and that there is enough evidence now for an optimistic outlook into the future. This does not mean however that no action has to take place, i.e.

- extra cyst provisions have to be found in the near future;
- better cooperations between cyst dealers and customers should be finalized;
- nutritional differences between *Artemia* sources should be taken into account;
- nursery feeding with adult *Artemia* should be better explored.

The potential with *Artemia* has by far not been realized yet. It takes time however, not the least to learn the a,b,c of its culturing biology. The availability of inert cysts is one thing; one should not give up there, *Artemia* has many other unique characteristics that should be exploited for the benefit of aquaculture production.

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