Algae Biomass
G. Shelef and C.J. Soeder, Editors

MASS CULTURE OF ALGAE: A BOTTLENECK IN THE NURSERY CULTURING OF MOLLUSCS

G. PERSOONE AND C. CLAUS

Laboratory for Mariculture, State University of Ghent,
J. Plateaustraat 22, B-9000 Ghent (Belgium)

ABSTRACT

The controlled culture of larvae of molluscs of commercial importance (oysters, clams, etc.) till spatfall implies the daily production of substantial volumes of suitable species of microscopic algae.

For economic reasons, industrial mollusc hatcheries cannot, however, upscale the very expensive indoor algal production to fulfill the increasing food demands of the growing spat.

As too early transplantation of the postlarvae to the natural environment results in various technological and biological problems, the intermediate semi-controlled "nursery" culturing of the spat up to a few centimeters in length is presently more and more preconized.

The different technologies utilized for nursery culturing are reviewed with special emphasis on the major problem: the mass production of live algal food under outdoor or greenhouse conditions.

INTRODUCTION

The culturing of edible molluscs, a practice which treatises on history report to be as old as 20 centuries B. C. (in Japan) has grown out, especially during this century, to the second most important type of aquaculture. According to FAO statistics (Pillay 1976) the annual harvest of cultured molluscs, mainly bivalves, averages about 1 million tons i.e., roughly a quarter of the total amount of fishes (freshwater, brackish water and marine) produced by husbandry techniques.

Glude (1976) even projects the total aggregate consumption of oysters to increase to more than 2.3 million tons by the year 2000; this, however, precludes "a substantial annual production increase as well as the inclusion of several species not yet utilized in large-scale aquaculture operations" (Kinne and Rosenthal 1977).

The major advantage of bivalves over other groups of species, namely their sedentary character, has led to a type of maricultural industry which, contrary to crustacean and fish culture, can be practiced almost without any restriction in suitable open shallow natural environments.

Being primary consumers, and more specifically herbivorous filter feeders, bivalves are among the most efficient converters of plant protein to animal protein.

The natural reproduction, the settling of the meroplanktonic larvae on different types of suitable collectors and the further ongrowth in the wild of the spat have always been and still are the backbone of the traditional mussel and oyster culture practiced in many countries of the world.

The human reluctancy, however, to see the yearly crop depend entirely on non-controllable environmental conditions, with the risk of shortage of spat, poor spatfall, poor growth, diseases, etc. . . . has led, in analogy to the husbandry of terrestrial animals to intensive research on the controlled culturing and in the first place on the controlled reproduction of edible molluscs.

From the economic point of view, it is quite understandable that most of the research effort has been put in the first place on those species with the highest commercial value, namely oysters and clams.

PRODUCTION OF ALGAE FOR MOLLUSC HATCHERIES

In the USA the pioneering work of Wells and Prytherch, between 1920 and 1930, further developed by Engle and Davis, in the forties and fifties, led to the well-known Wells-Glancy and Milford oyster hatchery methods which opened the door to commercial intensive controlled oyster culture. An excellent review on the "Development of shellfish culture techniques" was published by Loosanoff in 1971.

On the other side of the North Atlantic Ocean, the endeavours in Great Britain of the team of Walne paralleled and confirmed some of the major findings of the Americans Davis and Guillard (1958):

1) bacteria can develop to fairly high numbers in mixed blooms of natural phytoplankton as utilized f.ex. in the Wells-Glancy technique and can be a serious threat for bivalve larvae, even leading to complete mortality of the cultures,
2) bivalve larvae have a definite preference for and thrive best on specific types of minute naked flagellates which are not always present in large numbers in mixed blooms of natural phytoplankton.

The result was that for a number of years intensive research has been focusing, on both sides of the Atlantic Ocean and also in Japan, on developing suitable techniques to grow bacteria-free algal cultures, and on the determination of the nutritional value for bivalve larvae of a whole array of tiny microscopic algae.

Table I, from Imai (1977) lists some of the algae species which have been tried out as food organisms for shellfish larvae during the fifties and early sixties.

TABLE I

LIST OF THE MOST IMPORTANT SPECIES OF MICRO-ALGAE USED AS FOOD FOR THE REARING OF SHELLFISH LARVAE (IMAI 1977)

Food organisms	Species of shellfish	References
Carteria sp.	*Mercenaria mercenaria*	Loosanoff and Davis 1963
Chaetoceros simplex	*Haliotis discus*	Sagara, Iino and Ari 1961
Chlamydomonas sp. (D)	*Mercenaria mercenaria*	Davis and Guillard 1958
Chlamydomonas sp.	*Mytilus edulis*	Hirano and Oshima 1962
Chlorella sp. (580)	*Mercenaria mercenaria*	Davis and Guillard 1958
Chlorella sp. (UHMC)	*Crassostrea virginica*	Davis and Guillard 1958
Chlorococcum sp.	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
Chromulina pleiades	*Crassostrea virginica*	Walne 1956
Cryptomonas sp.	*Crassostrea virginica*	Loosanoff and Davis 1963
Cyclotella sp. (0-3A)	*Mercenaria mercenaria* *Crassostrea virginica*	Loosanoff and Davis 1963
Dicrateria inornata	*Crassostrea virginica*	Loosanoff and Davis 1963
Dicrateria sp. (BII)	*Mercenaria mercenaria*	Loosanoff and Davis 1963
Dunaliella euchlora	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
Dunaliella sp.	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
Hemiselmis refescens	*Crassostrea virginica*	Loosanoff and Davis 1963
Isochrysis galbana	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
	Ostrea edulis	Walne 1956
Monas sp.	*Crassostrea gigas*	Imai and Hatanaka 1949
	Pinctada martensii	Kobayashi and Yuki 1952
	Haliotis gigantea	Iino 1952
	Mactra sachalinensis	Imai, Hatanaka, Sato and Sakai 1953
	Ostrea edulis	Imai, Sakai and Okada 1953
	Ostrea lurida	Imai, Sakai, Okada and Yoshida 1954
	Pteria penguin	Kagoshima Fisheries Experiment Station 1959
Monochrysis lutheri	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
	Ostrea edulis *Ostrea lurida* *Mactra sachalinensis* *Pecten yessoensis*	Imai and others
Olisthodiscus sp.	*Mercenaria mercenaria*	Loosanoff and Davis, 1963
Platymonas sp. (No. 5) *(= Tetraselmis maculata)*	*Haliotis discus hannai*	Imai and others
Platymonas sp. (No. 1)	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
Phaeodactylum tricornutum	*Mercenaria mercenaria* *Crassostrea virginica*	Davis and Guillard 1958
Pyramimonas grossi	*Crassostrea virginica*	Loosanoff and Davis 1963
Rhodomonas sp.	*Mercenaria mercenaria*	Loosanoff and Davis 1963
Skeletonema costatum	*Mercenaria mercenaria*	Loosanoff and Davis 1963
Stichococcus sp. (0—18)	*Mercenaria mercenaria*	Loosanoff and Davis 1963

In a recent circular sent to 13 institutions active in controlled shellfish rearing in Europe and North America, Walne asked to tell confidentially the species of algae which were found best suited for culturing bivalve larvae. With the answers of the 10 institutions which replied (report to COST-Mariculture Working Group, April 1978), Walne put up the following preference list indicating the frequency with which each algal species had been mentioned (Table II).

TABLE II

SPECIES OF ALGAE USED FOR THE CULTURE OF BIVALVE LARVAE (WALNE, COST, 1978)

Species	Frequency of mention (out of 10)
1. *Isochrysis galbana*	8
2. *Pavlova (Monochrysis) lutheri*	7
3. *Tetraselmis suecica*	6
4. *Phaeodactylum tricornutum*	5
5. *Pseudoisochrysis paradoxa*	5
6. *Thalassiosira pseudonana*	4
7. *Chaetoceros calcitrans*	4
8. *Skeletonema costatum*	2
9. *Isochrysis Tahiti*	2
10. *Chlamydomonas* sp.	1
11. *Pyramimonas obovata*	1
12. *Playtmonas chui*	1
13. *Rhodomonas* sp.	1

In the review paper of Ukeles (1971) on the "Nutritional requirements in shellfish culture", the successive steps in the scaling up of the volumes of algal cultures needed to feed bivalve larvae are: 1) axenic algal colonies on solid media (seawater agar slants necessary for long-term storage of the stocks), 2) small axenic Erlen-Meyer cultures, 3) larger Pyrex carboys (10 to 40 liter), 4) open cultures of several hundreds of liters. Steps 3 and 4 can be either batch- or semi-continuous and some commercial hatcheries have turned to continuous culturing of pure strains under almost axenic conditions, in large vertical plastic bags.

We cannot enter here in the details of the technological aspects of the daily culturing of the substantial volumes (several thousands of liters in some hatcheries) of micro-algae, necessary for the rearing of the mollusc larvae to

spatfall. It is clear, however, that the operational costs of this algal production constitute a serious financial burden on the cost-benefit of commercial mollusc hatcheries, since they include items such as:

— the filtration and sterilization of large volumes of seawater,
— the price of chemicals and vitamins for the complex media,
— the heating and illumination of the indoor cultures,
— the hardware and man power.

Let us quote Ukeles (1976) in this regard: "In questioning individuals who are building and maintaining commercial aquaculture plants . . . similar problems are identified time and time again as being the most significant. Leading the list is the one of providing an optimal nutritional support that is *efficient* and *economical* for culturing animals under controlled laboratory conditions."

PRODUCTION OF ALGAE FOR MOLLUSC NURSERIES

If oyster larvae already consume substantial quantities of algae during their pelagic life, their food demand increases a manyfold as soon as they metamorphose into sedentary spat (Fig. 1, from Walne 1974).

Most commercial mollusc hatcheries are not able to cope with the feeding of spat for more than a few weeks, because the scaling up of the algal production to meet the needs of millions of growing postlarvae is economically prohibitive.

Transplantation of the tiny spat to outdoor conditions has been and still is the technique most currently applied, but with variable success (mortality and/or poor growth) as a result of the many environmental variables which can affect the seed. Indeed, as emphasized by Walne (1974): "although spat are better at withstanding unfavourable conditions than are larvae, they are more sensitive than the adult stages and their successful culture requires care".

In the present state of the art it can be said without exaggeration that one of the major problems of commercial mollusc hatcheries is the ongrowth of the postlarvae till they reach the size or weight (which is species dependent) where they are hard enough to withstand further ongrowth in the wild.

The intermediate stage of controlled postlarval rearing of molluscs, the so-called nursery culturing, is now receiving more and more attention in many countries (Cost 1978) and data from experiments with different technologies are appearing at an increasing rate in the scientific literature.

Nursery culturing of bivalves includes two major aspects:

a) the qualitative and quantitative problems of the supply or production of food for the mollusc seed,
b) the technological problems of the specific culturing of the young bivalves.

In order to illustrate the complexity of the nursery problem and the numerous ways in which scientists as well as commercial hatcheries are

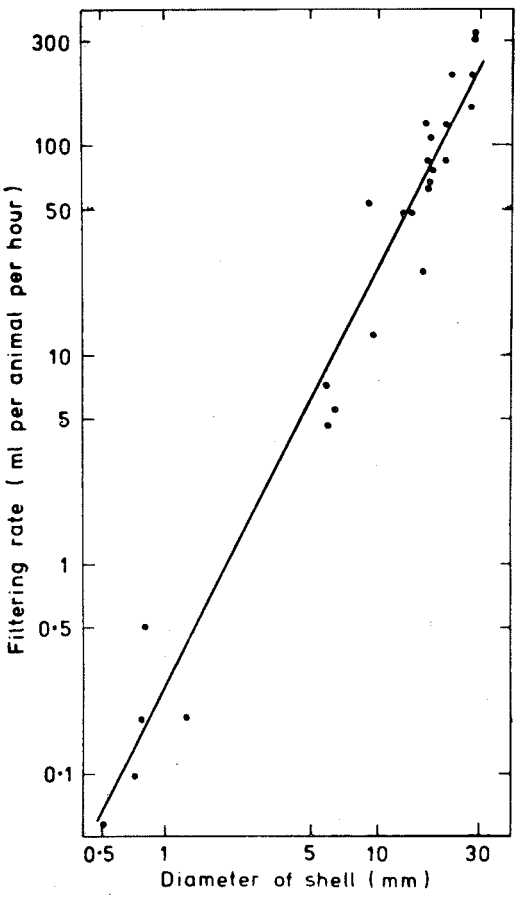

Fig. 1. The filtering rate of *Ostrea edulis* spat in function of shell diameter, tested out with *Isochrysis* at a temperature of 21°C (from Walne 1974).

trying to find solutions to it, this paper reviews briefly, at the risk of incompleteness, the present state of the art on the controlled, semi-controlled or non-controlled growing of postlarval molluscs, with the emphasis on the major bottle-neck: the problematics of the *availability* and the *production* of *algal food*.

Throughout the following enumeration the importance of the five major parameters which regulate algal growth, namely light, temperature, nutrients, pH and turbulence, will be underlined in relation to the nature as well as the quantity of algal food for the molluscs.

a) *Non-controlled sources of food*

The most classic and oldest technique for rearing postlarval bivalves is still to place the collectors with the spat, or the clutchless seed in trays, in a suitable natural environment.

It suffices, however, to quote Ryther and Tenore (1971) to see the limitations of this widespread technique: "Molluscs have two environmental requirements for their growth and commercial culture that may often be mutually exclusive. First the water temperature must be in the proper range for the animals to pump and filter water; second the water must contain enough microscopic food organisms of the proper size and composition to provide food for the shellfish. These two prerequisites are often not present simultaneously in the same environment. Tropical and semitropical waters are naturally poor in nutrients and normally lack the level of primary productivity (i.e. phytoplankton growth) for substantial mollusc growth. The more eutrophic temperate and boreal waters have temperatures too low for feeding and growth of bivalves for at least part and often as much as half the year".

This general statement is fully corroborated by the recent data of Spencer and Gough (1978) who analyzed the growth and survival of hatchery-reared spat of *Ostrea edulis* and *Crassostrea gigas* under three different conditions:

— in trays suspended from a raft moored 12 km offshore Menai Bridge in the U.K.,
— in trays supported on trestles located on the foreshore at low water mark,
— in trays immersed in outdoor tanks with flowing seawater from a large storage tank replenished once or twice a week.

The growth of both species of oysters in the artificial tank was consistently inferior to that of oysters kept at either site in the sea. By October—November growth was little everywhere. The survival was significantly better in the tanks. The poorer results in the controlled land-nursery should be considered in the light of the many variables which influence such a system; Lucas (1977) indeed lists up to 12 factors, technical, physical, chemical, as well as biological, which determine the growth of juvenile molluscs in a land-based nursery.

From Fig. 2 (from Spencer and Gough 1978) it is clear that seasonal growth curves of juvenile bivalves in the natural environment are both temperature and food dependent.

Foster (1972) who cultured oysters, clams and mussels in the warm seawater effluent of a power plant in Maine (USA) observed good growth in late spring, summer, and early fall. In late fall, winter, and early spring the molluscs did not grow at all, despite the suitable water temperatures, by shortage of food.

b) *Food from shallow closed basins*

A first attempt to gain a little more control over the environmental conditions has been to utilize sheltered, closed basins, such as f.ex. the old stone-pit (quarry) of a few hectares in surface and a few meters in depth used by a commercial nursery at the isle of Guernsey. This basin is connected with the sea by a small passage with a sluice through which the seawater can be renewed.

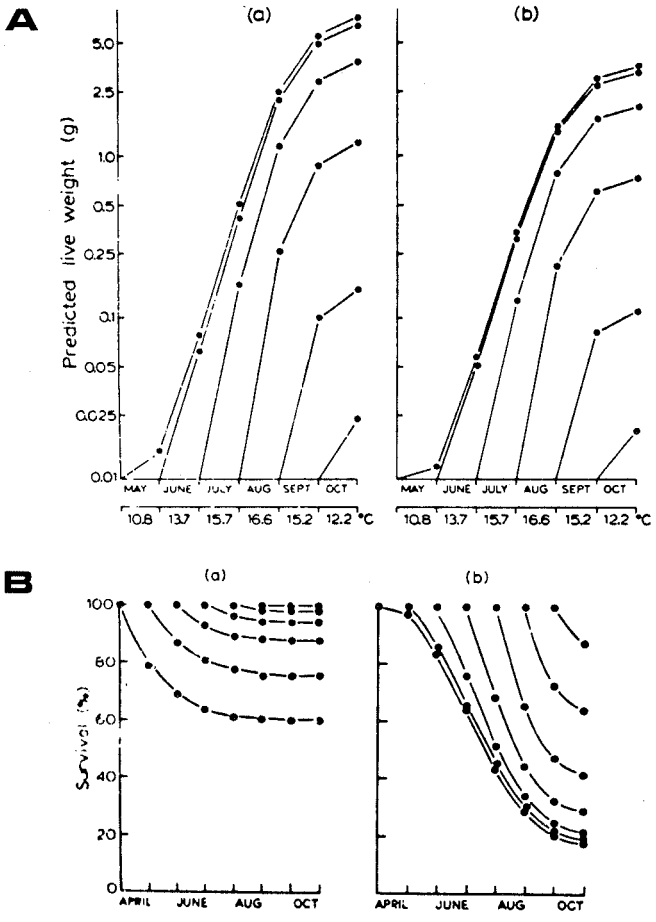

Fig. 2. Seasonal growth (A) and survival (B) of oysters, with an initial live weight of 10 mg, planted in successive months from May to October. (a) *Crassostrea gigas*, (b) *Ostrea edulis*. The average seawater temperature in the Menai Straits (1972—1975) is shown (from Spencer and Gough 1978).

The nursery consists of a raft carrying partially submerged trays with a bottom in mesh. A continuous pumping of seawater through a cylinder with holes, placed inside the trays produces a permanent flow of food through the mesh-bottom of the trays.

Both termperature and primary production of such closed natural basins are somewhat higher than in the open sea which results in a faster growth of the spat.

With regard to the more difficult manipulations on the raft the company recently built an on-shore unit at the same locale with a continuous flow of natural seawater through cylinders the mesh bottom of which is covered

with a layer of mollusc seed. The three dimensional stocking of spat in cylindrical tubes with an upwelling flow through the gauze bottom is now well-known as 3-D system.

Direct pumping of seawater from large shallow outdoor ponds with a relatively high natural phytoplankton productivity to feed postlarval bivalves kept in appropriate culturing units is a technique adopted by several commercial firms in Europe.

Although good growth of the spat is obtained during the warmer period of the year, this technique suffers from the same temperature and food limitations mentioned earlier for open sea conditions, namely almost zero-growth during the winter.

In order to obtain more information on the local possibilities of nursery culturing of bivalve spat with natural food, we recently have set up an experimental unit at the border of the Sluice-dock in Ostend (Belgium). This shallow basin of 86 ha, which is connected via sluices to the harbour and the sea, is well-known for its extremely high productivity resulting in excellent fattening of 18-months oysters seeded on the bottom (Polk 1965).

In our nursery system the seawater from the Sluice-dock is pumped into a storage reservoir where it is preheated before flowing in trays heated at different temperatures.

The goal of our experiments is two-fold:

a) to assess the beneficial effect of a higher temperature on the growth of the postlarvae during the period of the year where the algal production has not yet decreased to too low values,

b) to figure out to which extent the organic fraction of the seston supplements the deficient algal production for growth of the mollusc seed during the winter period.

From the economic point of view the expensive heating of the seawater should be considered in the light of a possible future heating by thermal effluents.

c) *Induced blooming of natural phytoplankton*

Indoors

Greenhouse culturing of natural phytoplankton with agricultural fertilizers is a very old technique and constitutes the backbone of the Wells-Glancy method mentioned earlier.

The onset and the extent of the algal blooming is function in the first place, of the prevailing temperature and light conditions.

In France this technique has been tried out during recent years at a pilot-scale level by Lucas (1976, 1977); the experimental nursery at Le Tinduff in Brittany consists of a greenhouse in transparent plastic sheet provided with 8 tanks of 1.5 m^3 contenance (Fig. 3). Natural seawater is pumped into the tanks and enriched with an algal culturing medium.

The algal suspension produced flows by gravity to raceways containing different types of trays and lanterns.

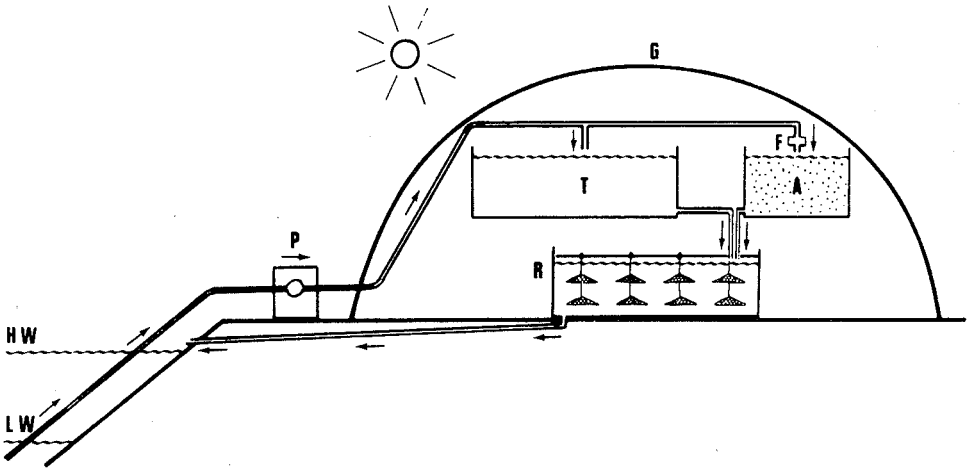

Fig. 3. Diagram of Le Tinduff nursery in France (from Lucas 1977).
HW: high-water mark; LW: low-water mark; P: seawater pump; G: green-house; T: seawater storage tank; A: algal tank; F: filter; R: raceway with bivalve spat.

Lucas tried out only a few times a blooming of natural phytoplankton and is now relying much more on the culturing of monospecific algal strains (see further).

In practice, besides the natural restrictions by a decline in the prevailing light and temperature conditions, the major limitation of greenhouse culturing of algae is the cost of the upscaling of the facility, especially of the greenhouse, to match the food demand of many millions of mollusc seed.

Outdoors

In 1971 a group of scientists from the Woods Hole Oceanographic Institution in Massachusetts (USA) started a most interesting series of experiments aiming at the mass porduction of marine microscopic algae in outdoor ponds, to feed commercially important bivalves. This has led to an impressive number of publications on the fundamental as well as the applied problematics of "short aquaculture food chains": Dunstan and Menzel 1971, Dunstan and Tenore 1972, Goldman and Stanley 1974, Goldman and Ryther 1975, Goldman and Ryther 1976, Goldman and Carpenter 1974, Goldman and Williams 1975, Huguenin 1975, Ryther 1971, Ryther et al. 1972, Ryther et al. 1975, Ryther 1975, Ryther and Goldman 1975, Ryther and Tenore 1975, Tenore and Dunstan 1973a, Tenore and Dunstan 1973b, Tenore and Dunstan 1973c, Tenore et al. 1973.

It was emphasized by Ryther and Tenore (1975) that the amounts of inorganic salts required to produce phytoplankton blooms at a level of two

orders of magnitude higher than the average production in the sea are quite substantial. Indeed 70 tons per hectare per year of fertilizer containing 10% nitrogen are necessary to maintain an algal productivity of 100 g/m^2/day (wet weight).

Recently similar experiments have been performed on a smaller scale at the Isles "Les Embiez" in France by a French team (Riva and Vicente 1978, Nival et al. 1978, Lelong and Riva 1978).

Seawater of the lagoon is pumped continuously through an algal tank of 20 m^3 contenance, and enriched with agricultural fertilizers.

According to the authors, the increased algal production (17 mg/m^3 chlorophyll *a* in the pond versus 0.42 mg/m^3 in the lagoon) leads to substantially better results in the growth of young clams (*Venerupis semidecussata*).

As an alternative source of nutrients the Woods Hole group has been focusing on domestic wastes, which after treatment still contain high nitrogen and phosphorous levels. For many years this concept has been used successfully by the team of Oswald in California (USA) for the mass culturing of freshwater algae in shallow ponds of several hectares of surface, in view of a simultaneous treatment of wastes and protein production.

Outdoor cultures were started in 1972 in Woods Hole in 2 m^3 tanks in a 1:1 seawater-secundary treated sewage medium with a turn-over of 75% of the total volume each day. Maximum algal yields of these cultures, which were always dominated by diatoms, reached 50 g/m^2/day wet weight of algae during the summers of 1972 and 1973.

The algal suspensions diluted with seawater were fed to tanks containing trays with juvenile oysters and clams.

With the prevailing technology the molluscs removed more than 80% of the algal food with a conversion efficiency of 10—20%.

At the end of 1973 the project was scaled up to a level of six 130 m^3 algal ponds of 1 m depth and 225 m^2 surface and eight 13 m long mollusc raceways. Gentle mixing of the algal suspension in these ponds was accomplished by recirculation with a centrifugal pump (1/3 HP). The water in the ponds could be heated to about 15°C above ambient by recirculation through large capacity heat exchangers.

During a 16 month experiment (from January 1974 till May 1975) Goldman and Ryther (1976) grew natural populations of phytoplankton on a continuous basis at a dilution rate of about 0.3/day on the seawater-secundary treated wastewater mentioned previously.

A most interesting aspect of this remarkable experiment is that a comparison could be made between algal growth in non-heated versus heated ponds during the winter period. The first conclusion of importance of this long term test was that, despite the continuous addition of seawater with many species of natural phytoplankton, usually one single species became dominant rapidly. Qualitative changes in this dominance occurred only on a seasonal basis.

The second conclusion was that "most marine species that appear in mass cultures exhibit eurythermal qualities". However, many of the typical algal species mentioned earlier which are utilized for the culturing of bivalve larvae and which are eurythermal as well "are never found in outdoor mass cultures simply because in each temperature region they are outperformed by another species that has a more favorable, but limited, response to temperature".

Thirdly, the dominant role of diatoms in outdoor cultures was again clearly demonstrated with different species taking over at different temperatures: *Skeletonema costatum* was always the dominant species below 10°C and *Phaeodactylum tricornutum* between 10 and 20°C. Between 20° and 25° *Nitzschia closterium* and *Amphiprora* prevailed but above 25°C other diatom species or green flagellated nannoplanktons take over (Fig. 4).

Last, but not least, it was shown by Goldman and Ryther (op. cit.) that the production of phytoplankton biomass is independent of temperature; heating of the outdoor ponds in winter did indeed not have a significant effect on the biomass products in comparison to non-heated tanks.

The authors conclude that the light intensity and the duration of illumination are much more important limiting factors for outdoor mass culturing than temperature.

Algae produced outdoors on secundary treated sewage were fed by Mann and Ryther (1977) to different bivalve species, in raceways heated to 15 and 20°C. The growth of the six mollusc species on the mixed algal food, largely dominated by *Phaeodactylum tricornutum*, varied very much from one species of bivalve to another.

Some bivalve species grew very well, others but poorly. These results contrast markedly with those obtained by Walne (1970, 1973) and Epifanio et al. (1976) who obtained no growth with any of their bivalve species when *Phaeodactylum tricornutum* was used as sole source of food.

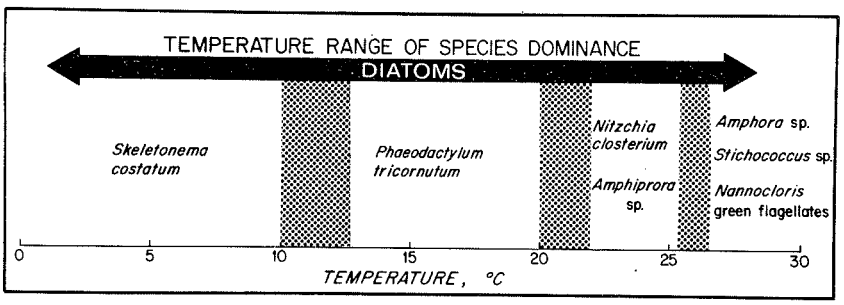

Fig. 4. Dominant phytoplankton species at different temperatures in outdoor cultures started from natural seawater. Shaded areas indicate regions of species instability (from Goldman and Ryther 1976).

Mann and Ryther (1977) therefore conclude that the other phytoplankton species present in their mass cultures probably provide some essential dietary components absent in pure laboratory cultures of *Phaeodactylum tricornutum*.

In our laboratory at the State University of Ghent, experiments are in progress on the outdoor culturing of marine algae on secundary treated animal wastes as a potential food source for juvenile molluscs (De Pauw and De Leenheer 1979). For a detailed description of the culturing units and results already obtained we refer to the paper of De Pauw et al., in this same book.

In the present state of the art the evaluation of the risk of concentration of chemical pollutants and pathogens through the food chain remains an open question and is the subject of intensive research in various countries.

From the sanitary point of view, it now appears that animal manures are less harmful as sources of nutrients than mixed domestic wastes, not at least with regard to viral contamination.

Controlled monospecies culturing

As pointed out pertinently by Epifanio (1976) most of the research on the nutritional value of algae as food for bivalves has been focusing on the growth of larvae. In 1970, however, Walne had already published an excellent report on the value of 25 species of algae for four species of post-larval bivalves showing a definite relationship between the type of algae and the growth rate of the young bivalves.

From Table III (by Epifanio 1976, modified from Walne 1970) it clearly appears that the algal species which have the highest nutritional value for the larvae are also those that give the best growth to the postlarvae. As such it is not surprising that mass culturing of such suitable species is assayed under indoor as well as outdoor conditions.

Indoor culturing

As already mentioned above, Lucas (1976, 1977), in his greenhouse nursery in France, tries to supplement the insufficient phytoplankton biomass of the natural seawater in winter by culturing *Phaeodactylum tricornutum*, *Tetraselmis suecica* and *Dunaliella primolecta* in 40 liter plastic bags, as an inoculum for his larger 1.5 m^3 tanks illuminated by fluorescent tubes and filled with seawater filtered through 5 micron bags.

In the USA, a research team at the College of Marine Studies at the University of Delaware is focusing for a number of years on the development of commercial closed-cycle, controlled environment shellfish mariculture, with the aim of raising bivalve molluscs from egg to market size in a recirculating system on a diet of monospecific algae.

The algae are grown in a large greenhouse in circular tanks, of 9.5 m^3 and 1 m deep, with a total capacity of 76 m^3.

TABLE III

THE GROWTH OF BIVALVE SPAT WHEN FED ON VARIOUS ALGAL FOODS COMPARED WITH THE GROWTH OF CONTROLS ON *ISOCHRYSIS* FOR A PERIOD OF 21 DAYS (EPIFANIO 1976)

A. *Ostrea edulis* Species	Index of food value Individual experiments	Average
Monochrysis lutherii	1.70, 1.03	1.36
Chaetoceros calcitrans	1.28	1.28
Tetraselmis suecica	1.42, 1.06, 1.12	1.20
Skeletonema costatum	0.93, 1.09	1.01
Isochrysis galbana	. . .	1.00
Dicrateria inornata	0.85, 1.04	0.94
Cryptomonas sp.	0.54, 0.74	0.64
Cricosphaera carterae	0.41, 0.83, 0.61	0.62
Chlorella stigmatophora	0.65, 0.56	0.60
Phaeodactylum tricornutum	0.62, 0.43, 0.73	0.59
Olisthodiscus sp.	0.47, 0.71	0.56
Nannochloris atomus	0.60, 0.60, 0.41	0.54
Chlorella autotrophica	0.37, 0.66	0.52
Pavlova gyrans	0.69, 0.32	0.50
Micromonas pusilla	0.50, 0.40, 0.42	0.44
Dunaliella euchlora	0.40	0.40
Dunaliella tertiolecta	0.36, 0.42	0.39
Chlamydomonas coccoides	0.26, 0.33	0.30
B. *Mercenaria mercenaria* Species		
Skeletonema costatum	3.30	3.30
Pyramimonas grossii	1.19	1.19
Tetraselmis suecica	1.17, 1.07, 1.09	1.11
Isochrysis galbana	. . .	1.00
Nannochloris atomus	0.95, 0.23, 1.57	0.92
Olisthodicus sp.	0.72, 0.55, 0.97	0.75
Micromonas pusilla	0.73, 0.76	0.74
Cricosphaera carterae	1.10, 0.50, 0.50	0.70
Dicrateria inornata	0.28, 1.04, 0.70	0.67
Monochrysis lutherii	0.62, 0.63, 0.53	0.59
Phaeodactylum tricornutum	0.44	0.44
Chlorella stigmatophora	0.45, 0.17	0.31
Chlamydomonas coccoides	0.19	0.19
Dunaliella tertiolecta	0.14	0.14

Excellent growth of the algae is achieved on the classic F/2 medium of Guillard (1974) with 2 or 3 doublings per day leading to average algal concentrations of 5×10^6 /ml.

Considering, however, the complexity of this laboratory algal culturing medium as well as the fact that only 20% of the nitrogen present in this complex medium is utilized by the algae, the Delaware scientists are now trying to define the specific nutritional needs of the algal food and to modify the culturing medium accordingly.

The Delaware group is also experimenting with cultures of algae in 400 liter transparent vertical tubes in rigid plastic (3 m in height and 45 cm in diameter), a technique which is now gaining more and more supporters in other hatcheries and nurseries.

The cost of producing pure monospecific algal foods in highly controlled indoor systems such as those worked out in Delaware as well as in commercial hatcheries, are considerable. It is clear that the expenses for filtering, sterilizing, heating and even eventually illuminating, are very substantial.

As far as the algal biomass is concerned, which is required on a daily basis during several months, if not year-round, to run a commercial mollusc nursery, Pruder et al. (1976) have worked out a most interesting graph based on literature data and own results. Figure 5 shows the cumulative number of algae necessary to grow the Pacific oyster *Crassostrea virginica* from egg to market size in 12 months at a temperature of 20—22°C in a controlled system.

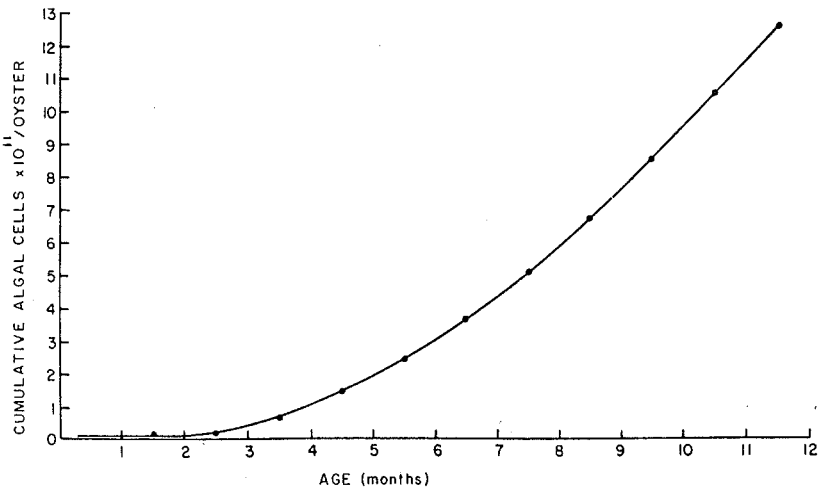

Fig. 5. Cumulative number of algal cells cleared by an oyster, growing from egg to market size in 12 months in the experimental nursery at Delaware, USA, at a temperature of 20—22°C (from Pruder et al. 1976).

Although the results obtained so far by the Delaware group are quite impressive, Epifanio, one of the scientists of the team wrote in 1975: "the major bottleneck to commercialization of intensive bivalve culture systems lays in the inability to economically culture massive quantities of these suitable algal species".

Outdoor culturing

The Cultured Clam Corporation, Division of the Aquacultural Research Corporation in Dennis, Massachusetts (USA) is an enterprise working on the controlled reproduction and growth primarily of the hard clam *Mercenaria mercenaria*. The postlarvae are grown for a few weeks on pure algal strains (mainly *Thalassiosira*) cultured indoors in vertical transparent plastic tubes of 400 liter contentance.

For the further ongrowth *Thalassiosira* is cultured in 23 m^3 outdoor cylindrical concrete tanks of 7 m in diameter, filled to a depth of ±0.5 m. The water used to culture the algae is pumped directly from a brackish water well into the tanks. Prior to inoculation, the whole water body of each tank is first treated with chlorine for 24 hours and then dechlorinated with thiosulfate.

Two tanks with a fairly high algal density (2—3.10^6 cells/ml) are operated on a continuous inflow of water and nutrient (Guillard medium) and outflow of the algal suspension, until the culture collapses by contamination or predation. Simultaneously, algae are grown in batch in two other tanks as emergency substitutes for the continuous cultures. Usually the flow-through cultures can be maintained for 3 to 4 weeks with a harvest of 700 to 900 liter of algae/hour, or a turn-over rate of ±one per day.

Once a day carbon dioxide is blown into the cultures for one hour to lower the pH to ±6.5.

The algal suspension is pumped into a mixing tank where it is diluted with heated ocean water prior to flow to the mollusc nursery located indoors.

As the cost-benefit of this particular experimental clam hatchery-nursery has not been assessed fully yet, the commercial livability of a plant working with this particular technology is still an open question.

From a general point of view, it is, however, a fact that wherever very pure seawater, heat and light are present at very low costs, mass production of suitable monospecific algae and mollusc culturing are possible at an interesting price.

Departing from those premises two commercial ventures have been started during recent years in Hawaii with farming of oysters straight to the commercial size on algae mass cultured in open ponds.

On the island of Oahu several reservoirs of one acre surface have been built by the Kahuku Seafood Plantation. Each of them contains 3000 m^3 brackish seawater pumped up from wells and inoculated with monospecific

algal strains from smaller cultures. 30 to 50% of each tank can be harvested daily, which means a yield of 3.500 kg of plankton cells per reservoir per day.

The algal suspension flows through raceways stacked with trays of clams and oysters.

As a result of the excellent year-round temperature and light conditions, both the algal production and the growth of the molluscs is year-round. The plant will be scaled up in steps to produce (according to Director T. Pryor 1978), an estimated 1 million oysters per month in early 1979.

The second company, "Aquatic Farms Ltd.", is producing oysters and other bivalve molluscs in a semi-closed brackish water intensive-culture system (Burzell 1978). Selected phytoplankton species are cultured on a continuous basis in 2 ponds of 2500 m^2 surface and 1 m depth. Although half of the culture is harvested daily, the algal concentration remains at about 1.10^6 cells/ml. The algal suspension is diluted with very pure water from a salt water well before it is distributed into shallow trenches filled with oyster trays.

From the engineering point of view the design of the whole technology is remarkable. The trenches receiving the oyster trays have been built in a type of cascade system, i.e. 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 at the original level.

The results obtained so far in this relatively new plant are remarkable too. Survival is more than 95% and oysters are reaching market size in approximately seven months. The present production is now in excess of 50 tons of live weight oysters per year.

A last example of outdoor culturing of monospecific algae is the experimental artificial upwelling mariculture system of St. Croix at the Virgin Islands in the Caribbean Sea (Roels et al. 1976).

Deep water, very pure and nutrient-rich, is pumped continuously through a polyethylene pipeline from 870 meter depth into two 50 m^3 concrete pools in which unialgal cultures of planktonic diatoms are grown. Continuous cultures can be maintained up to several weeks with densities of 1.10^4 — 1.10^6 cells/ml and a turn-over rate of more than 1 pool volume per day.

The algal cultures are pumped continuously at metered rates into shellfish tanks where several species of oysters and clams have been raised from small spat to marketable adults in periods from 6 months to 1 year, depending on the species.

If the costs of pumping the deep, nutrient rich water to the surface can be eliminated, f.ex. by coupling the mariculture plant with any type of industry utilising the deep water for energy applications (OTEC: Ocean Thermal Energy Conversion), it is clear that the upwelling type of mass algal production will become one of the most economic ventures, since it is provided

free of charge with light, temperature and nutrients which belong to the 5 major determinants for good algal growth.

CONCLUSIONS

At the present moment it is not possible to draw one straight-line conclusion with regard to the future of mollusc nurseries. There are, however, a number of facts which emerge from the foregoing information:

- the type of technology which shall be selected for the mass production of the algae depends to a very large extent, if not entirely, on the geographical (climatic) location of the nursery;
- outdoor continuous and controlled mass culturing of monospecific algal strains is only possible in some unique, mostly tropical situations, with very pure water pumped either from the deep sea or from brackish water wells. According to the experience of several persons involved in such type of production, this "continuous culturing" means periods of a couple of weeks, maximum one month, after which the cultures are either contaminated by other algae or predators, or collapse;
- indoor controlled mass culturing of one or several high nutritive algal strains in volumes sufficiently large to meet the daily food requirements of a commercial operation is still prohibitive from the economic point of view;
- the utilization of the natural phytoplanktonic food from the sea or closed embayments is restricted to temperate climates and confines the growth period, and thus the commercial operation, to the warmer period of the year;
- induced blooming of natural phytoplankton by fertilization of outdoor ponds leads to mixed cultures dominated by different species according to the ambient temperature. The production of the total biomass being relatively independent of temperature, year-round production is thus possible at sites with good conditions of natural illumination even during winter months;
- considering the relative simplicity to handle induced natural phytoplankton blooms, more research should be focused on the nutritional value of mixed natural algal foods for different postlarval bivalves. This should lead in the end to the selection of those shellfish species which are capable of filtering and assimilating the type of phytoplankton that will dominate in a particular geographical locale;
- the bioengineering of the mass culturing of marine algae as well as that of the mollusc nurseries proper is still in its infancy. Comparative research is needed between different technologies to determine their respective productions and their respective costs.

As a result of the great interest of many countries for nursery culturing of

bivalve molluscs, a proposal for a coordination of research activities in this particular field is now considered by COST (European Cooperation in the field of Scientific and Technical Research).

This proposal will hopefully lead in the near future to a concerted action at the European level and to a rapid implementation of the technologies most promising.

REFERENCES

Burzell, L. A. (1978) 5 technicians from Aquatic Farms, Ltd. Each brings his own specialized skill into the operation, The Commercial Fish Farmer, 4 (3): 20—23.

Cost, 1978, Proposal for a coordination of research activities in the field of mariculture, Report from the secretariat to the Cost Senior Officials Committee, European Cooperation in the field of scientific and technical research, Cost/58/78, 13 pp.

Davis, H. C., and R. R. Guillard (1958) Relative value of ten genera of micro-organisms as foods for oyster and clam larvae, Fishery Bulletin of the Fish and Wildlife Service, 295—304.

De Pauw, N., and L. De Leenheer (1979) Mass culturing of marine and freshwater algae on aerated swine manure, 441—473. In: Cultivation of fish fry and its live food, E. Styczynska-Jurewicz, T. Backiel, E. Jaspers, G. Persoone (Eds.), European Mariculture Soc., Special Publication no. 4, 534 p.

De Pauw, N., H. Verlet, and L. De Leenheer Jr. (1980) Heated and unheated outdoor cultures of marine algae with animal manure. In: G. Shelef, C. J. Soeder and M. Balaban (Eds.), Algae Biomass, Elsevier, pp. 315—341.

Dunstan, W. M., and D. W. Menzel (1972) Continuous cultures of natural populations of phytoplankton in dilute, treated sewage effluent, Limnol. Oceanogr., 16: 623—632.

Epifanio, C. E. (1976) Culture of bivalve molluscs in recirculating systems: Nutritional requirements, Proc. of the first Internat. Conf. on Aquaculture Nutrition, October 1975, Lewes, Rehoboth, Delaware, K. S. Price, Jr., W. N. Shaw, and K. S. Danberg (Eds), College of marine Studies, University of Delaware, Newark, Delaware, USA, 173—180.

Epifanio, C. E., C. M. Logan, and C. Turk (1976) Culture of six species of bivalves in a recirculating seawater system, Proc. 10th European Symposium on Marine Biology, Ostend, Belgium, September 1975. G. Persoone, and E. Jaspers (Eds), Universa Press, Wetteren, Belgium, Vol. 1, 97—108.

Foster, W. (1972) Fourth Annual Report Environmental Studies, Maine Yankee Atomic Power Co., Augusta, Maine, Maine Dept. Sea and Shore Fisheries, Mason Station Aquaculture.

Glude, J. (1976) Oyster culture — A world review, FAO Technical Conference on Aquaculture, Kyoto, Japan, FIR: AQ/Conf./76/R.16, 1—11.

Goldman, J. C., and E. J. Carpenter (1974) A kinetic approach to the effect of temperature on algal growth, Limnol. Oceanogr., 19: 756—766.

Goldman, J. C., and J. H. Ryther (1975) Nutrient transformations in mass cultures of marine algae, J. Env. Eng. Div. A.S.C.E., 101: 351—364.

Goldman, J. C., and J. H. Ryther (1976) Waste reclamation in an integrated food chain system, In: J. Tourbier and R. W. Pierson, Jr. (Eds.) Biological Control of Water Pollution, Univ. Penn. Press.

Goldman, J. C., and H. I. Stanley (1974) Relative growth of different species of marine algae in wastewater-seawater mixtures, Mar. Biol., 28: 17—25.

Goldman, J. C., and L. D. Williams (1975) Mass production of marine algae in outdoor cultures, Nature, 254: 594—595.

Guillard, R. L. (1974) Culture of phytoplankton for feeding marine invertebrates. In: Culture of marine invertebrate animals, Plenum Pub. Co., New York, N.Y., 29—60.

Huguenin, J. (1975) Development of a marine aquaculture research complex, Aquaculture, 5: 135—150.

Imai, T. (1977) Aquaculture in shallow seas. In: Progress in shallow sea culture, 615 pp., translated from Japanese Published for N.M.F.S., NOAA and NSF by Amerind Publishing Co., Pvt. Ltd., New Delhi.

Kinne, O., and H. Rosenthal (1977) Cultivation of animals. Commercial Cultivation (Aquaculture), In: Marine Ecology Volume III part 3, Ed. O. Kinne, Wiley, Chichester — New York, Brisbane, Toronto, 1321—1398.

Lelong, P., and A. Riva (1978) Alimentation et croissance de bivalves filtreurs en bassins aménagés, Actes de Colloques du CNEXO, 1978 (in press).

Loosanoff, V. L. (1971) Development of shellfish culture techniques, Proc. Conf. on artificial propagation of commercially valuable shellfish, K. S. Price and D. L. Maurer (Eds.) College of marine studies, University of Delaware, Newark, Delaware, USA, 9—40.

Lucas, A. (1976) A new type of nursery for rearing bivalve postlarvae. Construction, equipment and preliminary results, Proc. 10th European Symposium on Marine Biology, Ostend, Belgium, September 1975. G. Persoone, and E. Jaspers (Eds.) Universa Press, Wetteren, Belgium, Vol. 1, 257—270.

Lucas, A. (1977) Culture of the Manilla clam (*Venerupis semidecussata* REEVE) from hatchery-reared spat, Actes de Colloques du CNEXO, 4: 317—330.

Mann, R., and J. H. Ryther (1977) Growth of six species of bivalve molluscs in a waste recycling-aquaculture system, Aquaculture, 11: 231—245.

Nival, P., G. Malara, P. Lelong, C. Cellario, R. Charra, and A. Riva (1978) Evolution du phytoplancton dans les bassins de culture, Actes de Colloques du CNEXO, 1978 (in press).

Pillay, T.V.R. (1976) The status of aquaculture 1975, FAO Technical Conference on Aquaculture, Kyoto, Japan. FIR:AQ/Conf./76/R36, 1—13.

Polk, P. (1965) Productivity of the Bassin de Chasse in Ostend, Nature, 205: 101—103.

Pruder, G., E. Bolton, E. Greenhaugh, and R. Baggaley (1976) Oyster growth and nutrient nitrogen cost in bivalve molluscan mariculture, Delaware Sea Grant Report 11—76, 20 pp.

Pryor, T. A. (1978) 'Systemculture' works on Oahu. Culture of phytoplankton provides feed for oyster farm, The Commercial Fish Farmer, 4(3), 24—26.

Riva, A., and N. Vicente (1978) Situation et aménagement du site des Embiez, Actes de Colleques du CNEXO, 1978 (in press).

Roels, O. A., K. C. Haines, and J. B. Sunderlin (1976) The potential yield of artificial upwelling mariculture, Proc. 10th European Symposium on Marine Biology, Ostend, Belgium, September 1975. G. Persoone, and E. Jaspers (Eds), Universa Press, Wetteren, Belgium, Vol. 1, 381—390.

Ryther, J. H. (1975) Preliminary results with a pilot plant waste recycling-marine aquaculture system, Proc. Internat. Conf. Renovation and Reuse of Wastewater through aquatic and terrestrial systems, Bellagio, Italy (in press).

Ryther, J. H. (1971) Recycling human wastes to enhance food production from the sea, Environmental Letters, 1:79—87.

Ryther, J. H., and J. C. Goldman (1975) Microbes as food in mariculture, Ann. Rev. Microbiol., 29:429—443.

Ryther, J. H., and K. R. Tenore (1971) Integrated systems of mollusc culture (unpublished manuscript)

Ryther, J. H., W. M. Dunstan, K. R. Tenore, and J. E. Huguenin (1972) Controlled eutrophication — increasing food production from the sea by recycling human wastes", Bioscience, 22(3):144—152.

Ryther, J. H., J. C. Goldman, C. E. Gifford, J. C. Huguenin, A. S. Wing, J. P. Clarner, L. D. Williams, and B. E. Lapointe (1975) Physical models of integrated waste recycling — marine polyculture systems, Aquaculture, 5:163—177.

Spencer, B. E. and C. J. Gough (1978) The growth and survival of experimental batches of hatchery reared spat of *Ostrea edulis* L. and *Crassostrea gigas* Thunberg, using different methods of tray cultivation, Aquaculture, 13:293—312.

Tenore, K. R. and W. M. Dunstan (1973) Growth comparisons of oysters, mussels and scallops cultivated on algae grown with artificial medium and treated sewage effluent", Chesapeake Sci., 14(1): 64—66.

Tenore, K. R. and W. M. Dunstan (1973) Comparison of rates of feeding and biodeposition of the American oyster, *Crassostrea virginica* Gmelin, fed different species of phytoplankton, J. exp. mar. Biol. Ecol., 12:19—26.

Tenore, K. R. and W. M. Dunstan (1973) Comparison of feeding and biodeposition of three bivalves at different food levels", Mar. Biol., 21:190—195.

Tenore, K. R., J. C. Goldman and J. P. Clarner (1973) The food chain dynamics of the oysters, clams, and mussels in an aquaculture food chain, J. exp. mar. Biol. Ecol., 12:157—165.

Ukeles, R. (1971) Nutritional requirements in shellfish culture, Proc. Conf. artificial propagation of commercially valuable shellfish. College of Marine Studies, University of Delaware, Newark, 43—64.

Ukeles, R. (1976) Views on bivalve larvae nutrition, Proc. of the first Internat. Conf. on Aquaculture Nutrition, October 1975, Lewes, Rehoboth, Delaware, K. S. Price, Jr., W. N. Shaw, and K. S. Danberg (Eds), College of Marine Studies, University of Delaware, Newark, Delaware, 127—162.

Walne, P. R. (1970) Studies of the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus*, Fish. Investigations London (2), 25(5), 62 pp.

Walne, P. R. (1973) Growth rates and nitrogen and carbon hydrate contents of juvenile clams, *Saxidomus giganteus*, fed three species of algae, J. Fish. Res. Bd Can., 30: 1825—1830.

Walne, P. R. (1974) Culture of bivalve Molluscs, 50 years Experience at Conwy, Fishing News (Books) Ltd., Surrey, 100—124.