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INTENSIVE METHODS OF PRODUCING ALGAE CULTURES

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This article describes an intensive culture system for unicellular marine microalgae, which are grown as food for various stages of hatchery culture of commercially valuable molluscs.

Until recently, live algae were the only source of food for bivalve larvae and juveniles. However, this is now beginning to change as a result of recent research into the development of non-live and artificial diets.

However, the production of live algae remains a critical aspect of successful hatchery management, as they can be used as a live food supplement for innovative food products.

In addition, the need for microalgae cultivation arises because the natural phytoplankton content of seawater used in hatcheries is insufficient to support optimal growth of larvae and juveniles grown at high densities.

The basic methods of algae cultivation have changed little over the years. Incubators choose either indoor, intensive culture with artificial lighting, usually external to the culture vessels, or open, extensive culture in large tanks or ponds using natural lighting.

The article describes intensive methods for obtaining algae cultures on the basis of a specialized enterprise for the propagation of European oysters Culture blue AS (Norway).

As a result of using the methods considered, the maximum yield point can be reached within a 48-hour interval, so the enterprise has introduced a system of automated nutrient medium supply.

Since the cultures may not grow, or become excessively contaminated by competing microorganisms, or will disintegrate, the article presents a number of shortcomings in the system for verification in order to determine the source of discrepancies.

Therefore, the system considered is carefully controlled and highly productive.

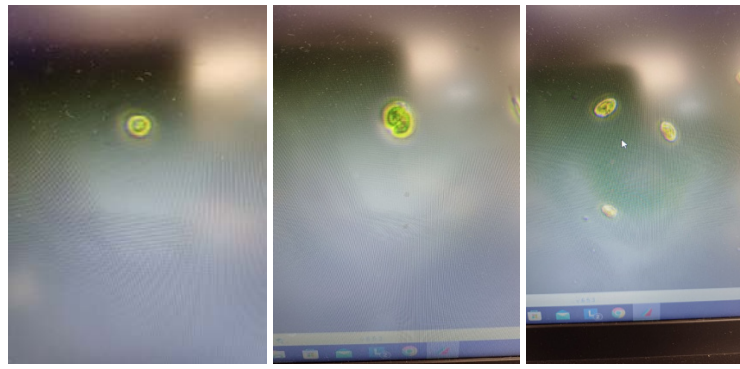
When used in modern mariculture, this system is quite capable of providing food for larvae, small fry and broodstock kept in artificial incubators.

Keywords: cultivation, marine microalgae, mollusc, food chain, food additive, incubator, mariculture.

Analysis of recent research and publications. Unicellular marine microalgae (Figure 1) are grown as food for various stages of hatchery culture of commercially valuable molluscs.

Until recently, live algae have been the only food source for bivalve larvae and juveniles. This is now beginning to change as a result of recent research into the development of suitable inanimate and artificial diets.

However, the production of live algae will remain a critical aspect of successful hatchery management for the foreseeable future, at least as a live food supplement to innovative food products.



a *b* *c*

Fig. 1. **Photomicrographs of algae commonly cultivated in hatcheries, *Isochrysis sp.* (a), *Tetraselmis sp.* (b) та *Rhodomonas baltica* (c), which show relative differences in cell size (Photo – Olifirenko V.V.)**

Flagellated and diatom species, among the microalgae, are the primary producers at the base of the marine food chain. They produce organic cellular components by absorbing carbon dioxide and nutrients from seawater, using light as an energy source in a process called photosynthesis.

They are typically cultured in hatcheries in appropriately treated natural seawater enriched with additional nutrients that include nitrates, phosphates, essential trace elements, vitamins and carbon dioxide as a carbon source. Synthetic seawater can be used, but it is prohibitively expensive, except on a small laboratory scale.

The need for microalgae cultivation arises because the natural phytoplankton content of the seawater used in the hatchery is insufficient to support optimal growth of larvae and juveniles reared at high density.

In particular, in larval culture, artificial algae cultures are used to replace all natural phytoplankton. Artificially grown algae should consist of cultures of species with high nutritional value.

In this context, and in providing appropriate feed rations for breeding stock and young, only a few of the very many natural algae have good nutritional value

for bivalve molluscs, and not all of them are amenable to artificial cultivation on a sufficiently large scale.

A list of the most commonly used species in bivalve hatcheries is given in Table 1. The parameters of cell size and composition are also shown.

Table 1. **Cell volume, organic mass and gross lipid content of some of the most commonly cultivated algae species used as food for bivalve larvae and spars**

Type	Average cell volume (μm^3)	Organic mass (μg 10^{-6} cells)	Lipids, %
Flagellates			
Tetraselmis suecica	300	200	6
*Dunaliella tertiolecta**	170	85	21
Isochrysis galbana *Isochrysis (T-ISO)* *Pavlova lutherii*	40-50	19-24	20-24
Diatoms			
Chaetoceros calcitrans	35	7	17
Chaetoceros gracilis	80	30	19
Thalassiosira pseudonana	45	22	24
Skeletonema costatum	85	29	13
*Phaeodactylum tricornutum**	40	23	12

*Note: types marked * have relatively low nutritional value*

Algae culture accounts for about 40 % of the cost of rearing juvenile bivalve molluscs to a shell length of about 5 mm in a hatchery.

For example, 1 million juvenile oysters with a shell length of 5 mm will consume 1400 l of high-density cultured algae daily, at an optimal rearing temperature of 24 °C. Smaller daily volumes are required for feeding broodstock and larvae.

Result and discussion. The basic methods of algae cultivation have changed little over the years.

Hatcheries choose either indoor, intensive culture with artificial lighting, usually external to the culture vessels, or outdoor, extensive culture in large tanks or ponds using natural light.

The specialist European oyster breeding company Culture blue AS (Norway) uses intensive methods to obtain algae cultures.

Intensive methods are satisfactory in terms of reliability and productivity, but are expensive in terms of capital expenditure and labour, while extensive methods are generally less reliable and sometimes not very productive.

Medium-scale cultures (usually 4 to 20 l in volume) can be used as food for larvae or to start large-scale cultures. Large cultures are usually at least 50 l in volume and are often much larger in volume. The plant uses 300-liter cultivator columns.

Starter culture management. The plant's inoculum maintenance procedures are virtually identical to standard ones. These cultures were specifically grown to provide inoculum for growing larger volumes of crops needed for food production (Figure 2).

Fig. 2. **Photographs showing typical conditions for inoculating algae cultures (Photo – Olifirenko V.V.)**

First, a line of starter cultures was created from the stock of the desired species. Starter cultures, like rootstocks, were grown in 500 ml flasks, and later in 2000 ml of nutrient medium. Since they are needed to provide the inoculum, they must be grown quickly. They are cultivated at a temperature of 18 to 22 °C at a distance of 15-20 cm from fluorescent lamps with a power of 65 or 80 W. This gives an illumination level on the surface of the culture of 4750 to 5250 1ux.

Starter cultures are grown for various periods of time before use. In the case of diatom species that have a short generation time, this period is from 3 to 5 days. For most flagellates, it is from 7 to 14 days. When the starter culture is ready for use, it is subcultured using sterile methods, as described earlier.

Between 20 and 50 ml, (depending on the type and density of the culture), is transferred to a fresh 250 ml culture to maintain the culture starter line. The remainder is used as inoculum for larger cultures (up to 25 litres) grown for feeding, or as an intermediate step in a large-scale cultivation process, where it in turn acts as an inoculum for much larger cultures.

Inoculation of larger volume crops may require larger volume starter crops. For the sake of clarity, crops with a volume of 2 to 25 litres will be referred to as medium-scale crops. For example, a 200-litre production culture first starts with

a 250 ml starter of the required algae species, which is transferred when it grows to a larger volume of 2 to 4 litres. When the 300 litre culture is concentrated, the inoculum of 2 to 4 litres is used to start a new culture of similar volume.

The company operates a medium-scale algae production culture. This is the so-called batch culture.

Batch culture involves inoculating the culture medium with the desired species of algae in plastic bags. The culture is then rapidly grown until further cell density is inhibited by insufficient light penetration into the culture, after which the culture is completely harvested, the container washed and sterilized, or simply the plastic bag is replaced and a new culture is started. Batch culture is typically used for delicate species and fast-growing diatoms (Figure 3).

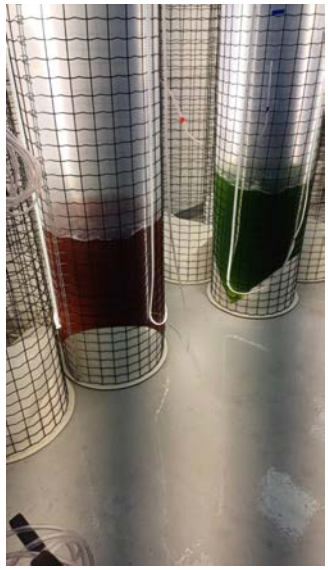


Fig. 3. **Batch culture**
(Photo – Olifirenko V.V.)

Batch harvesting is usually carried out at the peak of exponential growth, when the crops are entering the stationary phase.

The complexity of the cultivation operation depends on the demand for algae and the cost constraints within which the system must operate.

In its simplest form, a cultivation system can be simply a scaled-up version of a starter, using 2 litre to 25 litre glass flasks, and then transferring the algae culture to large volume columns. These are partially filled with a growth medium – in this case, sterile, nutrient-enriched seawater – and then inoculated with the required algae species.

The air is filtered through a 0.2 m pore cartridge or membrane filter to remove most airborne contaminants and competing microorganisms.

The growth medium was prepared from seawater filtered through a reverse osmosis filter.

To obtain maximum productivity from some algae species, it may be necessary to dilute seawater with clean (distilled) freshwater (or from an uncontaminated source) before filtering or autoclaving. The rate of cell growth and division *Chaetoceros calcitrans*, *Thalassiosira pseudonana i Skeletonema costatum* is optimal at a salinity of about 20 ppm.

The performance of many flagellates was optimal at salinities of 25 to 32 ppm.

Lighting for culture growth is provided by fluorescent lamps, usually mounted outside the culture columns. The number of lamps used is determined by the height and diameter of the culture vessels, with the aim of providing 15,000 to 25,000 lux, measured at the center of the empty culture container. Two 65 or 80 W lamps are sufficient to illuminate 3 L glass flasks with a diameter of about 18 cm, while 5 lamps of the same light output are required for vessels of about 25 l (diameter 35 cm). Optimal growth is at a temperature of 18 to 22 °C for most species.

By manipulating the culture conditions of some species, such as *Tetraselmis*, it is possible to change the size of the cells so that the cells can be more easily consumed by the larvae. Small-scale cultivation systems can be technically improved to increase their productivity by using them as chemostats. However, if the goal is solely to produce more food, a better solution would be to turn to large-scale cultivation methods.

Large-scale culture method. Commercial bivalve hatcheries must produce large volumes of high-quality, high-nutritional algae daily to sustain larval production on a sufficient scale.

Examples of some systems currently in use in Europe and North America are based on the use of polyethylene bags suspended or supported by a cylinder of plastic or galvanized steel mesh. All have in common that the culture is contained in a tall, narrow cylinder, which is the most efficient configuration.

The exception is hatcheries, mainly on the west coast of North America. They continue to use large, circular tanks illuminated by high-efficiency metal halide lamps. The highest efficiency is achieved when the lighting lamps are mounted inside the cultures (Figure 4) rather than outside in the form of a block of fluorescent lamps.

Culture blue AS uses polyethylene bags for algae cultivation. Polyethylene is available in large diameter tubes of various widths and in rolls containing convenient lengths. By cutting the appropriate length and joining the ends by heat sealing, a sterile, flexible cultivation container can be formed, either in the form of a cylinder or in the form of an oblong bag. The containers thus formed

can be reinforced by supporting them in a frame of plastic-coated steel mesh. Alternatively, the cylinders can be suspended, with or without a side support mesh, if the diameter of the bag is less than 30 cm and the height is less than 200 cm. The method used at the company is shown in Figure 5.

Fig. 4. **Large-scale culture was often carried out in large, round or rectangular tanks with overhead lighting**

Fig. 5. **Examples of plastic bags and algae cultivation systems (Photo – Olifirenko V.V.)**

Bags are the least expensive way to produce large-scale cultivation containers. These containers can be used indoors with artificial lighting or outdoors to take advantage of natural light. The bags shown are formed from

10,000 mm high-strength polyethylene tubing, 90 cm wide. They are supported by welded steel mesh frames and have a capacity of 300 litres with a large surface area for light penetration. Large cultures of this type can be illuminated by vertically mounted 1.8 m long, 80 W fluorescent lamps.

The bag systems shown in the figure are made of a polymer material but are supported by a strong metal mesh.

In general, the larger the diameter of the culture tank, the lower the maximum cell density possible at a fixed light level. However, these bags outperform similarly sized rectangular, fibreglass or plastic tanks, which are sometimes still used for mass cultivation. But they are inefficient compared to indoor light cultivation.

The polyethylene bags themselves have a relatively short lifespan, as the inner surface attracts culture residue and bacteria, which together reduce light penetration and are a source of contamination. After the culture is finished, the bag must be renewed. Large diameter bags are inefficient, but bags with a diameter of about 30 cm can be effective, as the surface area to volume ratio for light penetration is improved.

The maximum yield point can be reached within a 48-hour interval, so we have implemented an automated medium supply system at the company. The solution is to work with the cultures continuously, i.e., harvest continuously. algae This solution was made possible by optoelectronic cell density monitoring. A diagram of the automated system we developed and used in production conditions is shown in Figure 6.

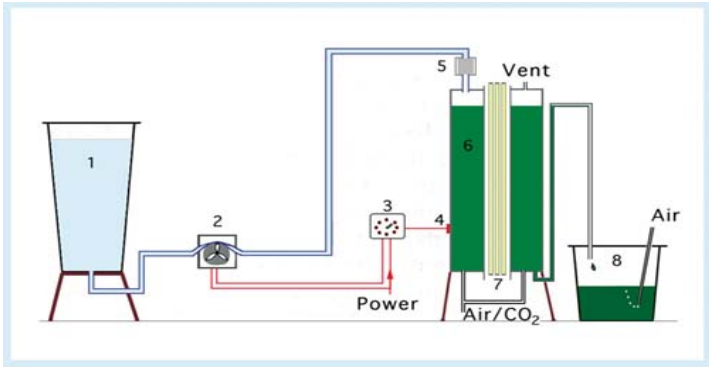


Fig. 6. **Schematic of a Continuous Algae Culture System**

The principle of automatic operation is not new. Chemostats or turbidostats that use external light sources to produce microalgae species have been described previously. The system described above is an updated and more efficient version of the concept (Figure 7).

Fig. 7. **System elements used in production by Culture blue AS (Photo – Olifirenko V.V.).**

Troubleshooting an Automated Algae Cultivation System. Cultures may fail to grow, become overly contaminated with competing microorganisms, or collapse even in the best incubators. Below are a few of the system deficiencies we have identified to check to determine the source of such failures.

1. Air Supply. Is there enough air being supplied to the cultures? Are the cells settling to the bottom of the culture vessel? This can happen when cultivating certain diatoms, in which case the air flow rate should be increased. This should not happen with widely cultivated flagellates. If so, the problem lies elsewhere.

2. Temperature. Check the minimum/maximum thermometer. Has the temperature in the algae culture room increased or decreased in the past 24 hours?

Most commonly cultivated algae species cannot tolerate temperatures above 26 ºC for long periods of time – or temperatures below 12 ºC. Temperatures between 18 and 22 ºC are ideal.

3. pH. Check CO_2 supply. Check the pH of the algae cultures with a pH probe. Is the pH too high (above 8.5). Is the pH too low (below 7.5)? Adjust the CO_2 supply accordingly.

4. Nutrients. Check records of the last time the cultures were fed nutrients. This is especially important for semi-solid cultures.

5. Fouling. The walls of the culture container, especially at the water/air interface, are visibly foaming or fouled with what appears to be detritus. If so, the culture is nearing the end of its useful life and needs to be replaced. If this is a persistent problem early in the culture cycle with a particular species, check the starter culture for signs of organism infestation and replace as necessary.

Not all types can be successfully cultured throughout the season. Some have specific "windows of opportunity" when they can be reliably grown. However, there is no consistency between hatcheries as to when a given species will grow well and when it will not. This must be learned from experience and emphasizes the importance of careful record keeping.

Conclusions. Thus, the intensive culture system described in the article is a carefully controlled and highly productive one.

When used in modern mariculture, it is quite capable of providing food for larvae, small fry and brood stock kept in artificial incubator conditions.

ІНТЕНСИВНІ МЕТОДИ ОТРИМАННЯ КУЛЬТУР ВОДОРОСТЕЙ

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У статті описана система інтенсивного культивування одноклітинних морських мікроводоростей, які вирощують як їжу для різних етапів інкубаторної культури комерційно цінних молюсків.

До недавнього часу живі водорості були єдиним джерелом їжі для личинок двостулкових молюсків і молоді. Але на даний час ситуація починає змінюватися в результаті недавніх досліджень з розробки неживих і штучних раціонів.

Проте виробництво живих водоростей залишається критично важливим аспектом успішного управління інкубаторією, так як вони можуть використовуватись в якості живої харчової добавки до інноваційних харчових продуктів.

Крім того, потреба в культивуванні мікроводоростей виникає тому, що природний вміст фітопланктону в морській воді, що використовується в інкубаторі, недостатній для підтримки оптимального росту личинок і молоді, що вирощується з високою щільністю.

Основні методи культивування водоростей мало змінилися протягом багатьох років. Інкубатори вибирають або внутрішню, інтенсивну культуру зі штучним освітленням, як правило, зовнішнє по відношенню до судин для культивування, або відкриту, екстенсивну культуру у великих резервуарах або ставках, що використовують природне освітлення.

В статті описано інтенсивні методи отримання культур водоростей на базі спеціалізованого підприємства з розмноження європейської устриці Culture blue AS (Норвегія).

У результаті використання розглянутих методів точка максимального врожаю може бути досягнута протягом 48-годинного інтервалу, тому на підприємстві запроваджено систему автоматизованої подачі живильного середовища.

Так як, культури можуть не вирости, або стати надмірно забрудненими конкуруючими мікроорганізмами або розпадуться в статті наведено низку недоліків у роботі системи для перевірки з метою визначення джерела невідповідностей.

Отже, розглянута система є ретельно контрольованою та високопродуктивною.

При використанні даної системи в сучасній марикультурі вона цілком здатна забезпечити їжею личинок, дрібну молодь та маточне поголів'я, що утримуються в штучних умовах інкубаторів.

Ключові слова: культивування, морські мікроводорості, малюск, харчоваий ланцюг, харчова добавка, інкубатор, марикультура.

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