THE USE OF ARTIFICIAL DIETS IN THE
HATCHERY REARING OF BIVALVE MOLLUSKS

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ABSTRACT

Hatchery rearing of bivalve mollusks depends on the production of live microalgae, which is costly and often unpredictable. Therefore, the development of a cost-effective artificial diet would greatly reduce the operating costs and improve the efficiency of bivalve seed production. This paper reviews the results reported so far in literature on the use of the various alternatives to on-site algal production. Furthermore, an overview is presented of the results obtained by the authors using manipulated yeasts as an algal substitute in feeding trials with juveniles of various commercially important bivalve species. Literature data and experimental findings were compared with experience "from the field" through an international inquiry among the operators of 50 commercial and research hatcheries. Despite the extensive research efforts, artificial diets are rarely applied in the routine process of bivalve seed production and are mostly considered as a useful backup diet.

INTRODUCTION

Intensive rearing of bivalves has so far relied on the production of live algae, which generally accounts for about 30% of the total seed production cost. Unicellular algae are an indispensable food source for the conditioning of the broodstock, the rearing of the larvae till settlement and the subsequent growing of the postset until they reach a size of 1 to 2 mm. From the latter stage onwards they are fed partially, or in some cases exclusively, natural phytoplankton. Once the seed attains planting size (5-10 mm), they are transferred to grow-out areas, where they reach market size feeding solely on natural food (Claud, 1981; Manzi, 1985; Hehm, 1990).

The variable quality and availability of natural phytoplankton has prompted most bivalve hatcheries operators to use monoculture algal cultures. The relative algal requirements of the various culture phases in the hatchery depend on whether the operation aims at the mass-production of larvae for remote setting or growing millions of seed till planting size (Fig. 1). In either case, the juveniles, representing the largest biomass in the hatchery and demanding the highest weight-specific rations, consume the largest volumes of algal culture.

Algal requirements in bivalve hatcheries
distribution among culture phases

(10%) (50%)
(40%) (0%)
broodstock larvae postset

Fig. 1: The relative requirements for cultured algae of the broodstock, the larvae and the postset stages in a bivalve hatchery.
Suitable algal species have been selected on the basis of their mass-culture potential, cell size, digestibility, and overall food value. Various culture systems have been developed to grow these food species on a large scale, ranging from extensive to highly controlled monocentric intensive cultures. The latter are more preferably by aquaculture as they allow more control over contamination and culture conditions, which are known to affect the nutritional value of the alga. However, the requirement for space, energy, nutrients and skilled labor to produce algae intensively results in production costs ranging from US $ 100 to more than US $ 200 per kg of dry biomass (De Paauw and Pernouse, 1980). Furthermore, culture crashes due to contamination and temporal variations in the algal food value will pose problems for any aquaculture operation depending on mass-cultures of unicellular algae.

To overcome or reduce the problems and limitations associated with algal cultures, various investigators have attempted to replace algae by using artificial diets either as a supplement or as the main food source. Different approaches are being applied to reduce the need for on-site algal production, i.e. dried, heterotrophically grown algae, preserved algal pastes, micro-encapsulated and micro-pelletized diets, and yeast-based feeds.

**CENTRIFUGED AND DRIED ALGAE**

As algae are the natural food of bivalves, as an alternative to on-site algal culture would be the distribution of preserved algae that are produced at relatively low cost in a large facility under optimal climatological conditions and using the most cost-effective production systems (Anonymous, 1993). Centrifugation of algae into a paste form and subsequent refrigeration until required may facilitate hatchery management and was believed to have an impact on the oyster industry in North America of the same significance as the remote setting of eyed larvae (Watson, 1986). However, the limited shelf-life and the high prices of the presently available algal pastes (US $ 500 and more per kg D2O) have discouraged many growers from using them. Recently, the development of preservation techniques extended the shelf-life of Thalassionema nitzschioides concentrates from about 10 days to more than one year, which makes it possible to valorize excess and off-season algal production (Donaldson, 1991).

Outdoor pond production on a large scale has led to the bulk availability of a limited number of ‘algal meals’, such as spray-dried Spirulina and a spray-dried extract of Dunaliella saltas. Recent work showed that the latter algal meal improved the growth of Sydney rock oyster larvae when it was supplemented to live algae (Numagauchi and Nii, 1993).

Recently, techniques have been developed for the large scale production of marine micro-algae under heterotrophic growth conditions, i.e. utilizing organic carbon instead of light as an energy source. Heterotrophic algal cultures can attain up to 1,000 times higher densities than phototrophic cultures and are preserved by spray-drying (Glibert, 1991). Although the latter author projected costs of producing algae in industrial fermentors between US $ 5 and 25 per kg, dried heterotrophically-grown Trichodesmium narvae was launched in 1989 by a British company at US $ 170/kg. Recent trials have demonstrated that growth of bivalve larvae and juveniles fed dried T. narvae is comparable to that obtained for live, light-grown T. narvae. Although the performance of dried algae was always inferior to that of controls fed an algal mixture, they offer interesting possibilities as a partial substitute for live algae (Laing et al., 1999; Laing and Verdugo, 1991; Laing and Milligan, 1992). Unfortunately, heterotrophic mass-production has been realized for very few algal species, and most of the species that are known to be of high nutritional value for bivalves (e.g. Chondromyces, Laminaria, Phaeocystis, Thalassiosira, Mononema) are not capable of growing in the dark (Glibert, 1991). Furthermore, heterotrophic conditions resulted in a drastic change in the gross composition as compared to light-grown T. narvae (Laing et al., 1999) and reduced (n-3) HUFA content (Pohl and Zurchal, 1970). Nevertheless, further developments in this rather new technology may improve the biochemical composition and the range of dried algae available.

**MICRO-PARTICULATE AND MICRO-ENCAPSULATED DIETS**

Multi-component diets consisting of both particulate and disintegrated nutrients have been used to study the nutritional requirements in bivalves (Castell and Trieder, 1974; Trieder and Castell, 1980; Langdon, 1983) or as a substitute for oysters (Nii, 1985; Nii and Walsey, 1986). Furthermore, several authors have reported the use of starch supplementation to increase the condition index and gonad size in oysters (Haven, 1965; Turgeon and Haven, 1978; Wulley and Reid, 1978). These powder diets, apart from being nutritionally incomplete, caused water quality problems and subsequently promoted bacterial proliferation in the culture systems. Through micro-encapsulation techniques dietary ingredients can be encapsulated within digestible capsules and delivered to suspension-feeders without losses of nutrients to the aqueous medium (reviewed by Jones et al., 1984; Langdon and Siegfried, 1984). Juvenile bivalves fed microcapsules or microgel particles showed growth up to 50% of that obtained for controls fed live algae (Laing, 1987; Langdon and Siegfried, 1984).

Furthermore, feeding microcapsules high in n-3 and n-6 fatty acids is currently of interest since the reproductive output of oyster broodstock (e.g. Southgate et al., 1996) or eels (Nii et al., 1993) is enhanced by feeding certain dietary fatty acids. The reproductive output of oyster broodstock (e.g. Southgate et al., 1996) or eels (Nii et al., 1993) is enhanced by feeding certain dietary fatty acids.

**YEAST-BASED DIETS**

Because of their high protein content, cells have interesting characteristics as a food for alternative to live algae, yeasts can be mass-produced and can substitute up to 50% of the algal diet of oysters (Ruffolo, 1979; Alatalo, 1982). Yeast can be highly digested and is a rich source of essential amino acids for bivalves and is an excellent alternative to algal diets (Ruffolo, 1979). yeast are presumably feeding in the digestive system of oysters and of other bivalves and may be used as a substitute for algal diets. The use of yeast has been evaluated in growth experiments run at various research centers and at the University of Sydney, which have used yeast as a substitute for algal diets. Yeast are generally fed as a paste or as a concentrate, which is a more concentrated form of yeast. Yeast are fed at about 15% of the diet, which is equivalent to 5% of the diet, which is equivalent to the amount of algal meal. Yeast are generally fed as a paste or as a concentrate, which is a more concentrated form of yeast. Yeast are fed at about 15% of the diet, which is equivalent to 5% of the diet.

**MANIPULATED YEASTS**

Research at the University of Oxford, UK, has shown that yeast can be manipulated to increase the digestibility of the yeast. This manipulation has resulted in a product with high potential as a feed. The product has been shown to be a valuable algal substitute (Ruffolo et al., 1996). The use of yeast as a substitute for algal diets has been reported in several trials and has been shown to be a valuable alternative to algal diets. The use of yeast as a substitute for algal diets has been reported in several trials and has been shown to be a valuable alternative to algal diets.

A preliminary experiment, which was conducted, revealed that an 80% replacement of the rate of 50% of that obtained in the yeast-based diet supported similar growth as the 100% algal diet, which was reduced (50% of that obtained in the yeast-based diet). A preliminary experiment, which was conducted, revealed that an 80% replacement of the rate of 50% of that obtained in the yeast-based diet supported similar growth as the 100% algal diet, which was reduced (50% of that obtained in the yeast-based diet).

Furthermore, reducing the 50% of that obtained in the yeast-based diet supported similar growth as the 100% algal diet, which was reduced (50% of that obtained in the yeast-based diet). A preliminary experiment, which was conducted, revealed that an 80% replacement of the rate of 50% of that obtained in the yeast-based diet supported similar growth as the 100% algal diet, which was reduced (50% of that obtained in the yeast-based diet). A preliminary experiment, which was conducted, revealed that an 80% replacement of the rate of 50% of that obtained in the yeast-based diet supported similar growth as the 100% algal diet, which was reduced (50% of that obtained in the yeast-based diet).
Furthermore, feeding microencapsulated high in (n-3) HUFA as a supplement to live algae can improve growth of oyster seed fed algae that are deficient in these essential fatty acids (Langdon and Waldvogel, 1983) and increase the reproductive output of oyster broodstock (Lanc, 1989). Very recently, promising results are reported by Soulehan (in press) using protein-walled microcapsules (lab-scale production cost of AUS $20/kg DW) for larval feeding of the giant clam and the Sydney rock oyster. As the microcapsules can be stored for several months as a sterilized slurry, detrimental effects of drying on the capsule wall stability and buoyancy is avoided (Soulehan, 1991).

The main problems arising from the use of microencapsulated feeds are settling, clumping and bacterial degradation of the particles, leaching of nutrients, and low digestibility of the wall material (Langdon and Bakun, 1984; Chu et al., 1987; Langdon, 1989). In this regard, low susceptibility to bacterial attack and high digestibility for the bivalve may be conflicting requirements for a capsule wall, as demonstrated by Langdon and DeBruyn (1989) for two types of protein-walled microcapsules ingested by the Pacific oyster. So far, microencapsulated diets for bivalves mostly remain at the research level due to cost and difficulty in producing capsules of the correct small size on a large scale.

YEAST-BASED DIETS

Because of their high protein content, suitable particle size and high stability in the water column, yeast cells have interesting characteristics as a food for filter-feeders. Furthermore, as opposed to most of the other alternatives to live algae, yeast can be mass produced at a relatively low cost. Dried feed yeast (Candida utilis) can substitute up to 50% of the algal diet of certain bivalve species without a significant drop in growth rate (Epilast, 1979; Alatalo, 1980). The extremely low nutritional value of some non-algal single-cell proteins could not be explained by differences in dietary composition and has been attributed to the low digestibility of the microbial cells (Candida utilis for the American oyster, Epilast, 1979; S. cerevisiae and Methylomonas clare for the Sydney rock oyster, Neil, 1985). However, carbon and nitrogen assimilation efficiencies of C. renouss fed algae were only slightly greater than the efficiencies with which oysters utilized yeast (Alatalo, 1980). By contrast, Porretas (1988) observed by means of transmission electron microscopy that multiplying yeasts are preferentially lysed in the digestive system of the Manila clam and found an assimilation efficiency for yeast of only 30% using radiotracer techniques. Alternatively, a deficiency or imbalance of nutrients in yeast may explain its poor nutritional value. Urban and Langdon (1984) thus greatly improved the growth of C. renouss on a 50/50 algae/C. utilis diet by supplementing rice starch and kaolin.

MANIPULATED YEASTS

Research at the University of Ghent, Belgium, leading to the development of techniques to improve the digestibility (Creten et al., 1990) and the nutritional composition (Leger et al., 1985) of yeast-based diets, resulted in a product with great potential as a substitute for microalgal algae. A similar manipulated yeast product has proven to be a valuable algal substitute in the larval culture of marine shrimp (Nanassinopus pusillus; 1990). The use of yeast as a food source in the juvenile rearing of bivalve mollusks was evaluated in growth experiments run at various locations, using different species of clams and oysters. The juvenile bivalves were batch-fed in recirculating systems of varying size and each experiment contained a control treatment which was fed the full standard algal ration, consisting of a mixture of different algal species. A preliminary experiment, which was performed with T. philippinum at a Spanish commercial hatchery, revealed that an 80% replacement of the algal control diet by the yeast product yielded a daily growth rate of up to 93% of that obtained in the algae-fed controls over a 4-week culture period. A 50/50 algae/yeast diet supported similar growth as the 100% algal diet. However, growth of clams fed a sole diet of yeast was strongly reduced (Fig. 2; Athenoia et al., 1989). The promising results of this experiment were verified under more standardized conditions in a sequence of tests carried out at the South Carolina Wildlife and Marine Resources Department, USA with seed of the hard clam Meretrix manrocasta. During 2-weeks experiments, it was shown that replacing 50% of the algal ration by the yeast diet did not affect growth rate of M. meretrix fed a mixture of C. polyso and I. pacifica (clone T-18). Clams fed a 100% algae/yeast diet exhibited a daily growth rate of 75-84% of that observed in algae-fed controls (Fig. 2; Creten et al., 1991). In a second hatchery trial performed at Guernsey Sea Farms Ltd., UK with seed of T. philippinum and the pacific oyster Crassostrea gigas, demonstrated that replacing 80% of the algal diet yielded an average daily growth rate of 70-80% of that measured in the algal control treatments during a 3-week experimental period. Furthermore, the latter experiments could not detect a significant difference between the nutritional value of the yeast diet and that of a directly equivalent mixture as an 80% replacement diet for live algae. The use of dried Cryptosporis sp. as an 80% algal substitute for C. gigas resulted in slower growth compared to that obtained with the yeast diet under the same conditions (Courtaud et al., in press).
The results reported for the manipulated yeasts confirmed the literature data with regard to the successful use of yeasts as a 50% algal substitute for rearing bivalve seed, and demonstrated a considerably better performance at higher levels of partial substitution. This may be ascribed to the improved digestibility and nutritional value of the manipulated yeast diet. However, the observation of a decreased growth rate in postset fed a mixed diet of algae and artificial diets after 1-2 weeks of culture compared to that of the algae-fed control, indicates that the nutritional value of the mixture is still inferior to that of full ration of live algae.

As a result, although manipulated yeasts offer interesting possibilities as a cheap, partial substitute for live algae in bivalve seed rearing, further research is required to improve their nutritional value and to document their application under commercial culture conditions.

INTERNATIONAL SURVEY

Except for the sporadic reports at international meetings (Helm and Hancock, 1990), the extent to which algal substitute diets have been tried, and rejected or retained, by bivalve hatchery operators is poorly documented. Furthermore, in order to direct future research efforts, it is essential to know the selection criteria of the farmer for an algal substitute which is eventually to be used in the daily practice of bivalve seed production. In this way, depending on the bivalve species and the applied production technology, either a cheap bulk feed or a more expensive, high quality diet may be preferred. An international inquiry, organized in 1991, allowed the collection of data concerning the requirement for live algae and its associated costs encountered in 50 commercial and experimental hatcheries from all over the world. Furthermore, the hatchery operators were questioned about their experience with alternatives for live algae (Couteau and Storgård, in press).

The capacity of the algal production facilities mostly ranged between 0.1 and 0.5 kg dry algal biomass per day, and exceeded 10 kg dry weight for one commercial hatchery. The total algal production reported by 37 hatcheries amounted to about 500 m³ algal culture day⁻¹, which is equivalent to about 50 kg of dry biomass (Fig. 3). The total cost of algal production in 1990 reported by 25 hatcheries approximated US $ 780,000 and averaged about 30% of the total seed production cost. The estimates for the algal production cost per unit dry weight ranged from US $ 50 to 400 kg⁻¹ (Fig. 4). Lower estimates, ranging between US $ 50 and 100, were reported by hatcheries producing relatively large quantities of algae. About a third of the questioned operators considered the algal production as a limiting factor in the rearing of bivalve seed, whereas over 50% planned an expansion of the algal cultures and more than 90% was interested in the use of a suitable artificial diet.
Fig. 3: Daily algal production in 42 bivalve hatcheries. Most operators produce daily between 0.1 and 0.3 kg of dry algal biomass. Five hatcheries, which all reared giant clam seed, did not grow any algae at all. A distinction was made between commercial and academic hatcheries (from Coutteau and Sergeant, in press).

Fig. 4: Algal production cost as a function of the production capacity for 8 bivalve hatcheries. The production cost was lowest for algae produced in large scale facilities. Dotted line connects estimates from one company. Cost estimates from commercial and academic hatcheries were indicated by unfilled and filled symbols, respectively (from Coutteau and Sergeant, in press).
The large interest for alternatives to on-site algal production was further demonstrated by the fact that more than 50% of the operators claimed to have experimented with artificial diets. The limited number of algal substitute diets reported in this study are presented in Table 1, classified either as dried algae, algal pastes, micro-encapsulated diets, yeast-based diets, or miscellaneous. The experience recorded for the various bivalve species, culture phases and substitute diets is summarized in Table 2. Despite these efforts, artificial diets are included in the routine production process of only a few hatcheries and are more often considered as a useful backup diet. In either case, the live algae could only be partially replaced by dried Tetraselmis suecica (up to 25-50%) or a preserved algal paste (up to 75%).

It is interesting to note that the results obtained with the same artificial diet greatly vary between experimenters and are often inferior to those reported in scientific papers. In this way, the unsatisfactory results, reported by some hatchery operators for micro-encapsulated diets fed to spat of the Manila clam and the Pacific oyster, conflict with the successful experiments performed by Laing (1987). The latter author obtained for the same bivalve species a similar growth as the algal feed controls when substituting up to 80-85% of the algal diet by microcapsules. Also, the limited replacement of live algae by dried T. suecica is in contrast with reports of successful substitution of up to 50% and 90% of the live algae in the spat rearing of, respectively, Crassostrea virginica (Helm and Hancock, 1990) and Pecten philippiensis (Laing and Millican, 1992). Although various authors have demonstrated through laboratory experiments that live algae could be substituted for up to 50% by dried yeast (Canadase unit) in the juvenile rearing of several bivalve species (Epiphanes, 1979, Alabino, 1983, Urbas and Landos, 1984), no confirmation of this was revealed in the survey. The inconsistent performance of artificial diets may have several explanations. Certain products, such as the dried algae, appeared to be difficult to use and may not always have been presented in the optimal form to the animals. Alternatively, the experimental conditions, including quality and quantity of the algal control diet, stocking density, water quality, and scale of the experiment, may affect the performance of the artificial diet. In this regard, the specific conditions of laboratory experiments can be expected to differ from those encountered in a hatchery.

### Table 1: Inventory of algal substitute diets reported in an international questionnaire among operators of bivalve hatcheries

<table>
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<th>classification</th>
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<th>source</th>
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<td>algal concentrates</td>
<td>- Close oyster diet 1 (C)</td>
<td>Close oyster Co., WA, USA</td>
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<td>- algal paste (E)</td>
<td>SeaAg Inc., FL, USA</td>
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<td></td>
<td>- algal paste (C)</td>
<td>Innovative Aquaculture, BC, Canada</td>
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<td></td>
<td>- algal paste</td>
<td>refrigerated, centrifuged from excess production</td>
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<td>dried algae</td>
<td>- Tetraselmis suecica (C)</td>
<td>Cell Systems Ltd., UK</td>
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<td></td>
<td>- Nitzchia sp. (E)</td>
<td>Marth Corp., MA, USA</td>
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<td>- Axopora (C)</td>
<td>Eastrise Farms, CA, USA</td>
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<td>microcapsules</td>
<td>- Frappier Booster (C)</td>
<td>Sanofi, F</td>
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<td>- micro-encapsulated diet (E)</td>
<td>James Cook University, Australia</td>
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<td>yeast-based diets</td>
<td>- Tetra (C)</td>
<td>Artesia Systems N.V.-S.A., Belgium</td>
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<td></td>
<td>- manipulated yeast diets (E)</td>
<td>University of Ghent, Belgium</td>
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<td></td>
<td>- various brands of dried baker's yeast (C)</td>
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<td>miscellaneous</td>
<td>- corn flour</td>
<td>maize, not specified</td>
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<td></td>
<td>- corn starch</td>
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Table 2: Inventory of the experience with various algal substrates reported in an international questionnaire among operators of bivalve hatcheries. The experience is ordered according to the bivalve species and stage (B = broodstock, L = larval, S = spat), and the type of algal substrate. The use of algal substrates as a backup diet or in routine culture is denoted with § and ‡, respectively.

<table>
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<tr>
<th>Bivalve species</th>
<th>ARTIFICIAL DIETS</th>
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<td>yeasts</td>
<td>microalgae</td>
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