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Substitutes for live microalgae in mariculture: a review

René Robert [1] and Pascal Trintignac [2]

[1] *IFREMER, Centre de Brest, Laboratoire de Physiologie des Invertébrés marins, BP 70, 29280 Plouzané, France.*
E-mail : rrobert@ifremer.fr

[2] *SMIDAP, Syndicat Mixte pour le Développement Aquacole en Pays de la Loire, Structure de Conseil Aquacole, 3 rue Célestin Freinet, Bât. B Sud, 44200 Nantes, France.*

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Abstract

In mollusc hatcheries and nurseries, live microalgae are traditionally used as feed for bivalves. Despite recent improvements in efficiency, such intensive culture of microalgae represents major cost factors in the production of bivalve larvae and spat. In an attempt to provide cost-effective alternatives and to simplify hatchery-nursery procedures, non-living algal food has been developed over the past few years. Substitution products such as bacteria, yeast, vitamin or docosahexaenoic acid enriched powders, dry and concentrated microalgae and microcapsules have been tested and evaluated on an experimental scale. The present article reviews major research aimed at developing non living diets in mariculture, which generally resulted in lower growth and higher mortalities than that recorded for controls fed live microalgae. At the present time, concentrated and dried microalgae appear to be the best alternative products for bivalves, for juveniles essentially, while microcapsules provide an excellent research tool to study their nutritional requirements.

Keywords: Mollusc rearing, artificial feed, microalgae, hatchery, nursery.

Les aliments de substitution en écloserie de mollusques.

Résumé

En écloserie et nourriceries, les microalgues fraîches sont traditionnellement utilisées pour l'alimentation des bivalves. Malgré des progrès incontestables pour en améliorer la productivité, le coût de revient des cultures intensives de microalgues reste élevé. De ce fait, mais aussi pour faciliter les procédures d'élevage, d'autres types d'aliments (levures, bactéries, poudres solubles vitaminées ou riches en acide docosahexanoïque, algues séchées, pâtes d'algues et microcapsules) ont été recherchés et testés au laboratoire. La synthèse des principaux travaux ainsi que les résultats de nos propres investigations, sont rapportés dans cette étude. La plupart des produits de substitution ne permettent pas, pour l'instant, d'induire de façon reproductible, une croissance supérieure ou égale à celle obtenue avec des algues fraîches. Actuellement les microalgues séchées et les pâtes d'algues apparaissent comme les meilleurs produits de complément, essentiellement pour les juvéniles, tandis que les microparticules constituent un outil de recherche performant pour étudier les besoins nutritionnels des bivalves à tous les stades de développement.

Mots-clés: Bivalve, nutrition, algues, aliments de substitution, écloserie, nourricerie.

INTRODUCTION

The controlled culturing of microphytes is, to date, the most widely used feeding technique in the hatchery-nursery rearing of bivalve genitors, larvae, postlarvae and spat. The culturing and utilisation of fresh microalgae, however, present a number of important constraints. Indeed, to satisfy the molluscs' nutritional needs, large phytoplankton volumes must be produced and the production of algae is expensive. When using intensive microalgal cultures (cell density $\approx 8 \times 10^6$ ml^{-1}), 0.5 to 2 l, 15 to 20 l and 1 000 to 1 500 l are required daily per genitor, per 10^6 larvae, and per 10^6 postlarvae ≤ 3 mm, respectively (Chew *et al.*, 1987; Helm, 1990). For sizes exceeding 3 mm, the animals are usually transferred to the natural environment or are first placed in open-air nurseries. In these latter facilities, daily consumption is even greater reaching 40 to 100 m^3 of extensive microalgal cultures (cellular density $\approx 10^6$ ml^{-1}) for 10^6 juveniles (Baud and Bacher, 1990). Moreover, as light is one of limiting factors for this method of microalgal production, the culture tanks are generally shallow and wide. As a result, the surface area of a bivalve hatchery-nursery allocated to the production of microalgae is quite large and microalgal culturing is very time consuming. Finally, despite the preventive measures which are usually employed, the culture of phytoplankton can be subject to various types of contamination leading to sudden culture mortality and/or unsuitability as a food source.

In an attempt to provide cost-effective alternatives and to simplify hatchery-nursery procedures, non-living algal food has been developed over the past few years. In the 1960s, new substitute feed were sought out in an attempt to overcome these inconveniences (Hidu and Ukeles, 1962) although the interest generated by such studies was only fully felt during the 1980s and 1990s with the creation of a large number of commercial hatcheries. A survey conducted among these businesses revealed that the three main criteria required of a substitute feed are: a high nutritive value, low cost and simple utilisation and storage (Coutteau and Sorgeloos, 1992). Firstly, the feed must meet certain demands; it must be easily ingested, have a high digestibility and be non toxic. In addition, it must not aggregate or easily break apart. Finally, it must not harbour sources of bacterial contamination. A certain number of products such as bacteria, yeast, dried microalgae, microalgal pastes and microcapsules have been studied experimentally. In the present paper, the main results, from the literature as well as some of our own results, are presented and an attempt is made to define the possible role of these different products in the controlled rearing of bivalves.

BACTERIA

In hatcheries and nurseries, bacteria are present in the rearing water and in the microalgal cultures. They are therefore capable of contributing to bivalve nutrition and several studies have demonstrated their importance to the larval (Hidu and Tubiash, 1963; Martin and Mengus, 1977; Prieur, 1982; Douillet and Langdon, 1993) and postlarval stages (Langdon and Bolton, 1984) under controlled conditions (Table 1). The same is true in the natural environment (Baldwin and Newell, 1991). Bacteria can furnish part of the metabolic requirements by directly providing organic molecules (Langdon and Bolton, 1984) and vitamins (Moal *et al.*, 1996). They may also act indirectly on the breakdown of proteins thus releasing amino acids which can be assimilated by the bivalves (Manahan, 1983) or may facilitate digestion by releasing exoenzymes such as proteases and lipases into the surrounding medium (Prieur, 1982; Nicolas, 1997 pers. com.).

These results should be considered with caution as it has been demonstrated that, under conditions close to those observed in rearing (10^6 bacteria ml^{-1}, Jeanthon *et al.*, 1988), the bacterial organic input represents only 3 to 15% of the microalgal contribution to *Pecten maximus* larvae (Moal *et al.*, 1996) and 0.5 to 2% for *Crassostrea virginica* juveniles (Langdon and Bolton, 1984). Their role is therefore not quantitative but may be qualitative. Very few studies, however, have demonstrated that the bacteria do indeed have a positive effect on larval growth. Indeed, among the many bacteria that have been isolated in hatcheries, no species have been found to be beneficial for larvae (Douillet and Langdon, 1993). Lastly, most of bacteria contain neither polyunsaturated fatty acids nor sterols (Brown *et al.*, 1996; Perry *et al.*, 1979) which are essential compounds in the growth of marine molluscs (Brown *et al.*, 1989; Holden and Patterson, 1991; Marty *et al.*, 1992). Feeding of molluscs during their larval and postlarval stages using selected bacteria does not appear to be possible at the present time although this concept continues to interest several authors (Brown *et al.*, 1996). Finally, it is important to note that, in hatcheries, bacterial contaminations are often responsible for drastic larval mortalities (Elston, 1990) and, as a result, physical and chemical methods are often utilised to limit their development. These precautionary measures would therefore seem to invalidate the use of bacteria as a food source in hatcheries.

Table 1. – Reported experiences on growth development of bivalve larvae (L) and spat (S) or on bivalve broodstock conditioning (B) with non living algal subsistutes in academic hatchery and nursery.
– no effect; + moderate effect (greater than unfed control); ++ noticeable effect (similar to or greater than the standard algal control).
(%): ratio of non living food supplemented to algae to reveal a positive effect. Numbers refered to the author cited.

Bivalve species	Bacteria		Yeast		Dried algae		Algal paste		Microencapsulated diets and lipid vesicles	
Clams										
Mercenaria mercenaria	L+	13	S+ (50%)	12; 6	S–	20			L++ (50%); S++ (85%)	8; 16
Ruditapes philippinarum			S+ (50 to 80%)	1; 5	L+; S+; S++ (70-90%); S+ (40%); S+ (80%); G+	17; 20; 18; 19; 5; 10; 21	S+	7	L++ (50%); S++ (85%)	14; 16
Rudipates decussatus					S+	20				
Tridacna gigas									L++	32
Mussels										
Mytilus edulis	L+	28	S+ (50%)	12						
Mytilus galloprovincialis	L+	24								
Oysters										
Crassostrea gigas	L+	11	S+ (80%)	5	S+; S+ (50%); S (20%)	20; 19; 15			L+ (50%); S+ (40%); B+;	14; 16; 29, 30
Crassostrea virginica	L+	13	S–	12					L+; L+; S+; S+; S+ (40%)	3; 4; 22; 23; 16
Ostrea edulis					L–; S+; S+ (70%)	17; 20; 19			S+ (40%)	16
Saccostrea commercialis	L–	2	S+ (80%)	26; 2	L+ (70%)	27	L++	25	L++; L+ (30%)	33; 27
Scallops										
Argopecten irradians			S+ (50%)	12						
Argopecten purpuratus									B+	9
Chlamys nobilis									L+ (50%)	34
Pecten maximus							L+	31		

Albentosa *et al.*, 1989 (1), Brown *et al.*, 1996 (2); Chu *et al.*, 1982 (3); Chu *et al.*, 1987 (4); Coutteau *et al.*, 1993 (5); Coutteau *et al.*, 1994*a* (6); Coutteau *et al.*, 1994*b*, (7); Coutteau *et al.*, 1994*c* (8); Coutteau *et al.*, 1996 (9); Curatolo *et al.*, 1993 (10); Douillet and Langdon, 1993 (11); Epifanio, 1979 (12); Hidu and Tubiash, 1963 (13); Kean-Howie *et al.*, 1994 (14); Knauer and Southgate, 1996 (15); Laing, 1989 (16); Laing *et al.*, 1990 (17); Laing and Millican, 1991 (18); Laing and Millican, 1992 (19); Laing and Verdugo, 1991 (20); Laing and Lopez Alvarado, 1994 (21); Langdon and Bolton, 1984 (22); Langdon and Siegfried, 1984 (23); Martin and Mengus, 1977 (24); Nell and O'Connor, 1991 (25); Nell *et al.*, 1996 (26); Numaguchi and Nell, 1991 (27); Prieur, 1982 (28); Robinson, 1992*a* (29); Robinson, 1992*b* (30); Robert *et al.*, 1996 (31); Southgate *et al.*, 1992*a* (32); Southgate *et al.*, 1992*b* (33); Teshima *et al.*, 1982 (34); Urban and Langdon, 1984 (35).

YEAST

Yeast were considered very early to be a potential food substitute in aquaculture due to their small size, high protein content and the ease with which they are cultured. Despite an early study by Loosanoff in 1944 in which larval cultures were provided yeast (Hidu and Ukeles, 1962), the actual importance of this food source was determined by Epifanio in 1979 using spat. An absence of growth was observed by this last author for four species of bivalves which led him to advocate that yeast should not be used as a sole food source. With the exception of *Crassostrea virginica*, however, a 50% yeast supplement supported good growth rates of bivalve juveniles (Epifanio, 1979; Table 1). Urban and Langdon (1984), however, observed that oyster growth depends essentially on the proportion of microalgae present in the food mixture. In addition, at a ration above 10.4 mg of yeast per day, growth ceased. The latter authors believed that the low nutritive value of yeast is due to their deficiency in polyunsaturated fatty acids (of the ω3 family) and water-soluble vitamins (Urban and Langdon, 1984). In addition, the thickness of their cell wall does not facilitate digestion (Epifanio, 1979).

The elaboration of a chemical treatment solved this problem and, as a result, this treated food resource was examined on *Ruditapes philippinarum* spat (Albentosa *et al.*, 1989) which confirmed the nutritional deficiency of yeast in essential compounds. Using a technique for the incorporation of ω3 type fatty acids, Coutteau *et al.* (1993, 1994*a*) revealed that yeast treated in this manner supported good growth of *Ruditapes philippinarum* and *Mercenaria mercenaria* juveniles : an 80/20 yeast to fresh microalgae ratio led to growth levels which represented 70 to 90% of those

observed for the fresh microalgae fed controls. Moreover, replacing 50% of the algal ration by yeast did not result in a significant decrease in *M. mercenaria* spat growth rate relative to controls (Coutteau *et al.*, 1994*a*). Poorer results were observed for *Crassostrea gigas*, however, for which 50% of control growth was recorded (Coutteau *et al.*, 1993). A high yeast ratio was shown to cause significant pseudofaeces production and a growth decrease in this species which is in agreement with the observations of Urban and Langdon (1984). Conversely, a high yeast ratio brought about a growth rate increase in *Ruditapes philippinarum* (Coutteau *et al.*, 1993) which would seem to indicate the existence of different nutritional needs. Indeed, as pointed out by Laing *et al.* (1990) the clam seems to be able to produce the fatty acids necessary for its development by elongation and desaturation, a capacity which does not exist in the Ostreidae. As a result, the clam only requires a minimal microalgal ration to meet its essential fatty acid needs. For most of the Veneridae, a complementary microalgal and transformed yeast diet is therefore conceivable at the juvenile stage, while the *Tridacna* clams are even capable of development when fed only yeast (Coutteau and Sorgeloos, 1992). The utilisation of transformed yeast seems to be more limited in the Ostreidae. Nevertheless, recent studies by Nell *et al.* (1996) on *Saccostrea commercialis* spat fed six different yeast species (not transformed) and three yeast-based diets (Microfeast) reported that dry weight gain can reach 67% (for the best live yeast diet) to 76% (for dry yeast-based diets) of that observed in controls (live microalgae) at an 80% substitution level.

The value of such a supplemental feed in other bivalves (Pectinidae for example) remains to be determined for the juveniles as it does for the larval stages of all molluscs. Although some results have been reported for the Veneridae (Coutteau and Sorgeloos, 1992), their role in the conditioning of genitors remains to be determined. Because they lack polyunsaturated fatty acids untreated yeast is unsuitable as a complete diet for larvae and spat of bivalves. Further studies are therefore required to fully evaluate the importance of transformed yeast as a complementary mollusc food source.

DRIED MICROALGAE

The first studies to use dried microalgae as a mollusc food source date back to the 1960s at which time Hidu and Ukeles (1962) observed larval growth rates in *Mercenaria mercenaria* which were comparable to those obtained using fresh microalgae. Similar experiments were subsequently sporadically carried out on oysters with poorer success (Walne, 1974) but it was not until the 1990s that further research in this area was performed. The resumption of these research activities was made possible by the mastering of drying methods in addition to the development of a new procedure for the industrial production of marine microalgae under heterotrophic conditions (Laing, 1989). The research involved larvae, juveniles and genitors and, as a result, the potential role of this food source became more clearly defined (Table 1).

Only two marine microalgae treated as described above were tested in detail as feed for *Ruditapes philippinarum* larvae, namely *Nannochloris atomus* and *Tetraselmis suecica*. This latter was only used for the umbone larvae (mean length > 120 µm) due to its cell size which is in the order of 8 to 12 µm (Laing *et al.*, 1990). A growth rate comparable to that obtained with fresh *Tetraselmis* was obtained for larvae fed the dried version of this microalgae. Conversely, a better development leading to metamorphosis in 21 days was obtained with dried *Nannochloris* whereas a zero growth rate was observed with fresh microalgae. A much better larval performance, however, was seen in controls provided a mixture of two live microalgae species with clam metamorphosis occurring after 8 to 10 days after hatching. Decreasing growth rates accompanied by high mortality levels were also observed in *Ostrea edulis* when the larvae of this species were exclusively fed dried *Tetraselmis* (Laing *et al.*, 1990).

On the other hand, Numaguchi and Nell (1991) reported that growth rates of *Saccostrea commercialis* larvae fed live algae at 0.05 $mg.l^{-1}$ were significantly increased when supplemented dried extract of *Dunaliella salina* was added at a ration of 0.123 $mg.l^{-1}$.

In young spat (< 2.5 mg), the mollusc's size does not preclude the use of *Tetraselmis suecica* and a greater number of studies have been carried out (Table 1). Laing and Verdugo (1991) showed, on the one hand, that growth rates of *Ruditapes philippinarum*, *R. decussatus* and *Crassostrea gigas* fed dried *Tetraselmis* did not vary substantially from those observed using live *Tetraselmis* (Fig. 1) and, on the other hand, that dried *Tetraselmis* allowed higher growth rates in *Ostrea edulis* but lower growth rates in *Mercenaria mercenaria*. However, in either case, postlarval performance was lower than that recorded for controls fed live *Chaetoceros calcitrans* forma *pumilum* or a mixture of live *C. calcitrans* forma *pumilum* and *Tetraselmis suecica* (Laing and Verdugo, 1991).

Moreover, Laing and Millican (1991, 1992) reported that dried *Tetraselmis* used as a partial algal substitute, might resulted in similar postlarval growth rates than the controls in *Ostrea edulis*, *Crassostrea gigas* and *Ruditapes philippinarum*. Here again, the clam distinguishes itself by possessing a particularly low need for fresh microalgae in the mixture (10%) as compared to the oyster for which proportions are in the order of 30 to 50% for the flat and cupped oysters, respectively (Laing and Millican, 1992). For the clam, a slightly higher fresh microalgae contribution (20%) is reported by Coutteau *et al.* (1993) as they claim that the differences observed are due to the difference in experimental methods used and, in particular, in the way the

animals were fed. Nevertheless, both works conflict with the results of Curatolo *et al.* (1993) who found that supplements of dried diet of up to 40% did not produce satisfactory results for clam spat due to deposition and decay of the dried product, leading to bacterial contamination.

The nutritional value of spray-dried freshwater alga, *Spongiococcum excentricum* has been recently assessed on *Crassostrea gigas* spat (Knauer and Southgate, 1996). These authors showed that neither the dry weight, the ash free dry weight nor the proximate composition of spat fed an 80% *Chaetoceros muelleri* : 20% *S. excentricum* mixture were significantly different from than of spat fed an 100% *C.* diet, which clearly demonstrates that dried *S. excentricum* was suitable as a substitute for live C. *muelleri* at a level not exceeding 20%.

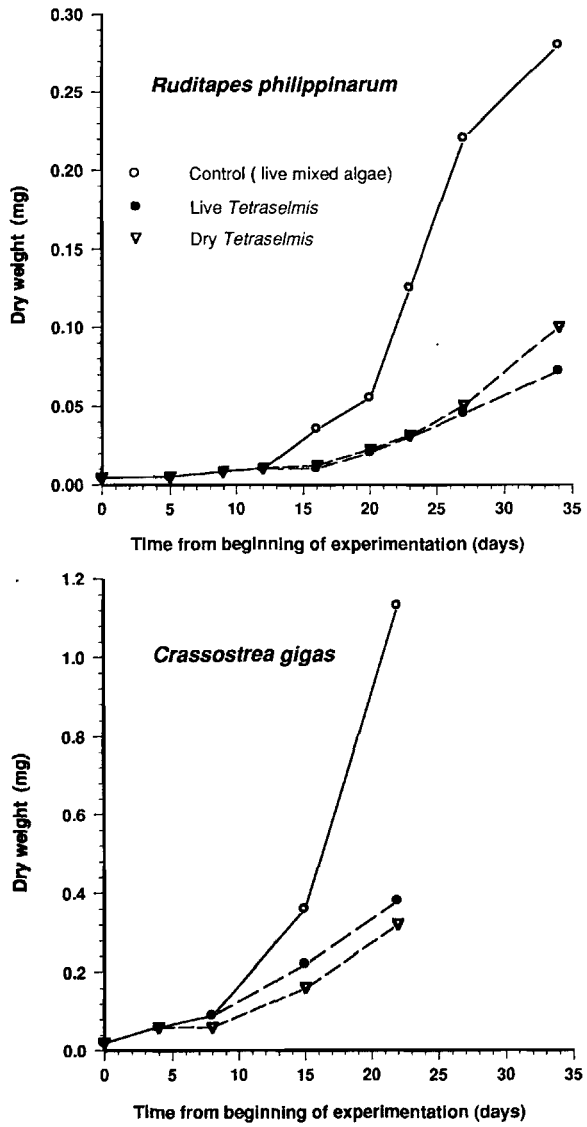

Figure 1. – Growth of *Ruditapes philippinarum* (**a**) and *Crassostrea gigas* (**b**) juveniles fed live *Tetraselmis suecica*, dried *T. suecica* or a mixed diet of live *Chaetoceros calcitrans* and live *Tetraselmis suecica* (from Laing and Verdugo, 1991).

Some interesting results have also been generated in the conditioning of genitors for which studies have been carried out using *Tetraselmis suecica* and the diatom *Cyclotella cryptica* (Table 1). The maturation of *Ruditapes philippinarum* occurs in the same period of time (6 weeks) regardless of whether the microalgae used are fresh or dried, alone or within a mixture (Laing and Lopez Alvarado, 1994). When clams are provided a dried microalgae diet, however, a lower fecundity is observed which represents only 35 to 40% of that recorded for controls fed live algae. No other effects were subsequently observed other than a difference in the size of the D larvae at formation; larval growth rate remained identical regardless of the feed provided during broodstock conditioning.

Mediocre results in the rearing of larvae (*Ostrea edulis, Mercenaria mercenaria*), postlarvae (*Ruditapes philippinarum, Crassostrea gigas, Patinopecten yessoensis*) and in genitor conditioning (*M. mercenaria*), however, have been reported by a number of hatcheries (Coutteau and Sorgeloos, 1992).

Dried microalgae, therefore, appear to be a good feed supplement but cannot fully replace live microalgae. They have the advantage of possessing a weakened cell wall due to the drying process and are therefore easily assimilated by bivalves (Hidu and Ukeles, 1962). In addition, they are easy to store even in the long term. However, due to the drying process only, these treated microalgae may be deficient in several components, namely proteins and vitamins (*e.g.* ascorbate) and ω3 fatty acids which are thus only found in live microalgae. Moreover, the drying process can make the cells "leaky" and hence they can rapidly leach water-soluble nutrient upon resuspension.

As they seem to require only a 10 to 20% fresh microalgae supplement to obtain performance levels similar to controls, the Veneridae are good candidates for this food source. The *Tridacna* also stand out once again as larval and juvenile development in this family can be obtained using dried microalgae as the sole food source (Coutteau and Sorgeloos, 1992).

In the Ostreidae, the use of this food type remains attractive despite the fact that 50% minimum of live fresh microalgae are necessary to allow good growth rates.

Nutritional needs are particularly important from a quantitative point of view during the postlarval and juvenile stages (Baud and Bacher, 1990; Helm, 1990) and it is conceivable that these supplement products could be preferentially used in nurseries. In this way, Laing and Millican (1992) estimated this complement feed used with fresh algae for clams to be in the order of 0.2 $g.g^{-1}$ $week^{-1}$ for juveniles as a higher ration (0.4 g) was not noticeably more efficient in obtaining

3 mg spat (wet weight). A high feeding cost of 170 US \$ kg^{-1}, however, has been put forward by Coutteau and Sorgeloos (1992) while a cost of 60 US \$ kg^{-1} (0.10 £ g^{-1}) was proposed by Laing and Millican (1992). The cost of fresh microalgae production is variable and depends mainly on the species cultured, the production volume, the technique used (bloom, semi-continuous, continuous), the initial nutrient levels in the water (saline groundwater or not) and the climatic conditions experienced by open air cultures. A wide range of estimations are thus present in the literature and their mean, all conditions combined, allows the cost of live microalgae culturing to be estimated at 112 US \$ kg^{-1} dry weight (Coutteau and Sorgeloos, 1992). Depending on the study examined (Laing and Millican, 1992; Coutteau and Sorgeloos, 1992), the cost of dried microalgae is 20 to 50% lower than that of fresh microalgae. The actual cost incurred by the use of dried microalgae, which depends on the species cultured, its type of production (autotrophy or heterotrophy) and the drying procedure (oven drying, thin layer drum drying, spray drying or freeze drying), therefore remains to be accurately determined.

The low numbers of commercialised dried microalgae and the mediocre nutritional quality of most of them as live microalgae are, in our opinion, the main inconveniences at the present time. Indeed, to our knowledge, only four marine microalgae (*Nannochloris atomus, Tetraselmis suecica, Dunaliella salina, Cyclotella cryptica*) and the freshwater *Spongiococcum excentricum* have been industrially produced and used as food for molluscs (Laing *et al.*, 1990; Numaguchi and Nell, 1991; Laing and Millican, 1992; Knauer and Southgate, 1996). The two major species in the literature which have been used in dried form, i.e. *Nannochloris atomus* and *Tetraselmis suecica* are known to be respectively of poor or mediocre food value for larvae and spat of several bivalves when used alone as live microalgae (Loosanooff and Davis, 1963; Walne, 1974; Enright *et al.*, 1986; His and Robert, 1987; Laing *et al.*, 1990; O' Connor *et al.*, 1992; Robert *et al.*, 1996*a*). The lack or low levels of *n-3* HUFA in these microalgae (Brown *et al.*, 1989), their low ingestion and/or digestion by bivalve larvae (Walne, 1974; Loosanooff and Davis, 1963; Robert, 1996 unpublished data) may explain their poor quality. Moreover, drying can result in loss due to oxidation of highly unsaturated fatty acids (Dunstan *et al.*, 1992) which are essential components for larval growth (Brown *et al.*, 1989).

MICROALGAL PASTES

During the 1970s, mollusc larvae began to be fed concentrated axenic microalgae (Ukeles, 1975). This technique was adopted several years later by commercial hatcheries (Watson, 1986; Donaldson, 1991). This paste is obtained by the continuous centrifugation of cultures of one or more microalgal species. It can subsequently be stored for several days in the dark at 10°C according to Ukeles (1975), for several months at 4°C according to Watson (1986) or even up to one year (Donaldson, 1991). Although the production of this paste appears relatively simple, very few experimental studies have been carried out to determine its nutritive value (Table 1) and most of the data available, which originate from commercial hatcheries, remain elusive or confidential.

Watson (1986) obtained variable results (no data are given) which depended on the microalgal species and which were particularly variable for the naked flagellates. More reproducible results would seem to be obtained with diatoms. To explain this phenomenon, this last author suggested that the centrifugation process destroys a portion of the cellular material thus affecting the nutrient potential of the microalgae. This would help explain the poor results usually observed for naked flagellates and, conversely, the good growth rates generally recorded with diatoms, such as *Chaetoceros calcitrans* and *Thalassiosira pseudonana*. Coutteau *et al.* (1994*b*), however, revealed that it is not possible to generalise these results, as a better *Ruditapes philippinarum* juvenile growth rate was obtained with a *Chaetoceros neogracile* fresh culture diet than with an equivalent microalgal paste diet. To our knowledge, the only nutritional studies on larvae to use concentrated microalgae are those of Nell and O'Connor (1991) and our own (Robert *et al.*, 1996*a*). The former authors revealed that a paste made up of *Pavlova lutheri* and *Chaetoceros calcitrans* and stored at 4°C for 1 to 2 weeks can bring about a larval growth rate in the oyster, *Saccostrea commercialis*, which is greater to that obtained using fresh microalgae. A paste of *Pavlova lutheri* alone, however, generated poor growth results while concentrated and stored *Chaetoceros calcitrans* gave similar growth as the fresh control. In *Pecten maximus*, low larval growth rates have also been recorded with concentrated *Pavlova lutheri* and this despite the microalgal treatment (culture age, dilution or not of the paste: Robert *et al.*, 1996*a*; Fig. 2*a*). Conversely, promising (Fig. 2*b*) although non reproducible larval growth rates have been obtained with pastes made from *Isochrysis* aff. *galbana*.

Relatively good performances as a complement food source have been reported for these pastes but no data are given (Coutteau and Sorgeloos, 1992). These authors reported however that, in the Coast Seafood Company Hatchery, genitors and spat are fed a diet composed of 75% microalgal paste and 25% fresh microalgae.

At the present time, due to their hardiness to centrifugation process, diatoms such as *Chaetoceros calcitrans*, *Chaetoceros neogracile*, *Skeletonema costatum*, and *Thalassiosira pseudonana* as well as Prasinophyceae of the genus *Tetraselmis* are good candidates for the production of microalgal pastes but their nutritional quality deteriorates quite rapidly (Watson, 1986; Montaini *et al.*, 1996). The production of an microal-

gal paste is a delicate and fairly expensive process. The main difficulties involve, firstly, mastering centrifugation for the more fragile but nutritionally advantageous species (mainly the naked flagellates) and, secondly, determining the best storage conditions for pastes. The utilisation of microalgal pastes to feed mollusc larvae and postlarvae presents a certain number of advantages such as the elimination of ectocrines and nutrients as well as the noticeable decrease in bacterial density (O'Connor and Nell, 1992). This technique is only attractive, however, if the paste can be kept for a least

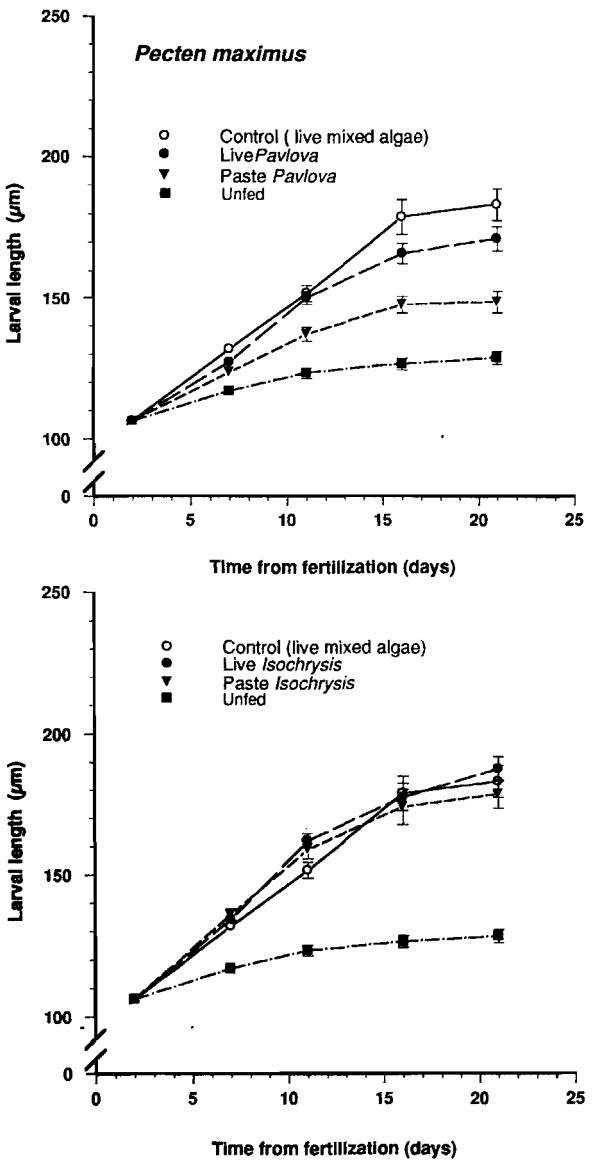

Figure 2. – Growth of *Pecten maximus* larvae fed with fresh diets or preserved microalgae (*Pavlova lutheri* or *Isochrysis* aff. *galbana*) obtained by centrifugation and kept at 4°C with different dilution treatments (concentrated or diluted), the cultures being collected at various ages, young or old (after Robert *et al.*, 1996*a*).

two months, this being the time required for genitor conditioning and/or postlarval development. Several authors have observed its rapid deterioration at 4°C, however, (Molina Grima *et al.*, 1994; Brown, 1995; Montaini *et al.*, 1995) and the addition of antibiotics and preservatives seem to be of little benefit (Watson, 1986). It is interesting to note that the polyunsaturated fatty acid profiles of these concentrated microalgae remain unchanged at 4°C for a month (Molina Grima *et al.*, 1994; Montaini *et al.*, 1995) and consequently, the lower larval growth results recorded for *Pecten maximus* fed these pastes are not linked to a deficiency in essential fatty acids (Robert *et al.*, 1996*a*).

At the present time, despite the limited nutritional quality of these concentrates a commercial application of this procedure to feed postlarvae exists with remote setting (Jones and Jones, 1988).

MICROCAPSULES

The potential of microcapsules in marine aquaculture was described during the 1970s (Jones *et al.*, 1974) and the first experiments carried out on molluscs were performed by Gabbot *et al.* (1976). To feed bivalve larvae and postlarvae, the size of the food particles, more or less oval in shape, must be smaller than 10 µm in diameter (Robert and Trintignac, 1996). In addition, they must not be too porous so as to limit the diffusion of the trapped nutrient compounds but must be digestible. The other characteristics of an "ideal" microcapsule are linked to their preparation, biochemical content and performance in aqueous suspension (Southgate *et al.*, 1992). Several types of microcapsules have been tested over the last twenty years of which four have been the object of more extensive studies (Fig. 3). Several studies have investigated the use of microcapsules for bivalve larvae, juveniles and adults. They have been reviewed in detail by Southgate *et al.* (1992) and only the main results are reported here (Table 1).

Nylon protein and protein-walled microcapsules

These microcapsules allow the encapsulation of water soluble compounds, essentially proteins and sugars. The fabrication protocol was defined by Chang *et al.* (1966): a water soluble food made up of haemoglobin and diaminohexane is emulsified in an organic solution of Sebacoylchloride. This last compound reacts with the diaminohexane to form nylon 610 and with the free amino acid groups of the haemoglobin to produce chains of nylon-protein. In order to limit the formation of aggregates, Jones *et al.* (1974) suggested the addition of 4 4' - diamino-2 2'biphenyldisulphonic acid.

Very few studies on molluscs have been carried out using this type of microcapsule. Chu *et al.* (1982) reported its ingestion and digestion by *Crassostrea virginica* larvae while similar growth and survival to

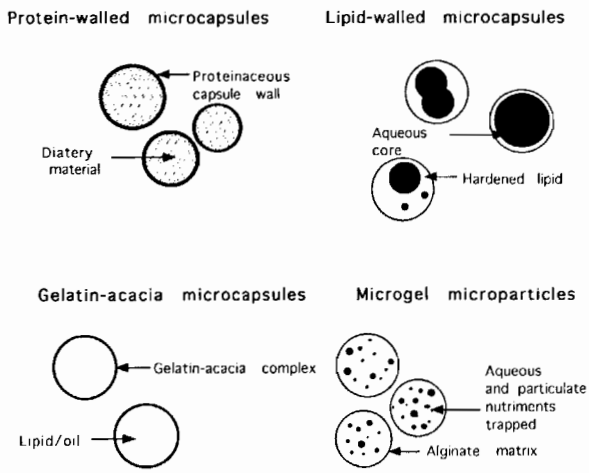

Figure 3. – Main types of microcapsule used in mollusc feeding studies (from Southgate *et al.*, 1992).

those fed the algae *Chorella* have been shown on *Chlamys nobilis* larvae which, however, died prior to settlement (Teshima *et al.*, 1982).

Later studies used a modification of this method which eliminated the "nylon" component, suspected to have a low level of digestibility (Chu *et al.*, 1982). This modified technique leading to the production of protein-walled microcapsules (Fig. 3*a*), detailed by Langdon and Debevoise (1989), allowed the development of clam (*Ruditapes philippinarum* and *Mercenaria mercenaria*) and oyster (*Crassostrea gigas, C. virginica and Ostrea edulis)* juveniles (Laing, 1987). These animals, however, possessed a growth rate which was substantially lower than that observed with a fresh microalgal diet.

It has subsequently been shown that 10-day-old *Saccostrea commercialis* larvae fed protein-walled microcapsules had growth rates in shell length of 80% that of algal-fed larvae and did not differ significantly in ash-free dry weight (Southgate *et al.*, 1992*b*). Moreover, mixed with gelatin-acacia microcapsules, they supported good larval growth (90% that of algal fed controls) and development through metamorphosis of *Tridacna gigas* larvae (Southgate *et al.*, 1992*a*).

This illustrated clearly that these protein-walled microcapsules, used alone or as supplementation, may be of high nutritional value for bivalves. They can encapsulated complete diet which contain both aqueous nutrients and lipid components. Moreover, its size (6 ± 2 µm; Langdon, 1989) advocates its utilisation in larval rearing and are accordingly very promising in hatchery.

Lipid-walled microcapsules

These microcapsules allow the incorporation of hydrophilic compounds and, as a result, contain both aqueous nutrients and lipids. Made up of a double emulsion, their role is to introduce water soluble compounds with a lesser risk of diffusion than is possible with nylon-protein microcapsules: a vitamin mixture is dissolved in a water soluble acacia gum and calcium chloride solution. This mixture is then emulsified with fish oil which is often combined with ethyl cellulose thus rendering the capsule less permeable (Langdon *et al.*, 1985; Fig. 3*b*).

Langdon and Bolton (1984) and Langdon and Siegfried (1984) first tested these microcapsules (mixed artificial diet) with *Crassostrea virginica* juveniles and growth rates of 41% to 73% that of algae fed controls were achieved. Subsequently, *C. virginica* larvae were grown to metamorphosis on this type of microcapsules thus revealing that this species does not feed exclusively on microalgae (Chu *et al.*, 1987). These same authors, however, note a substantially lower growth rate compared to that obtained with a microalgae diet. However, its role as a food supplement is worth considering. Indeed, the growth of *C. virginica* juveniles previously provided a nutritionally deficient food was restored after addition of vitamin rich lipid microcapsules (Urban and Langdon, 1984).

The production and storage of these microcapsules is problematic due to their instability (Chu *et al.*, 1987). Morever, "complete" diet can be encapsulated but these microcapsules have a very high lipid content which results in nutritional imbalance (Southgate, 1997 pers. com.). They lead to unsatisfactory development and their potential application seems to be limited in hatchery and nursery.

Gelatin-acacia microcapsules

These particles allow the encapsulation of hydrophobic compounds, essentially lipids. Microparticles are made up of a gelatin-acacia wall incorporating lipids (Fig. 3*c*).

In order to determine the fatty acid requirements of *Crassostrea gigas* juveniles, microcapsules of 2 to 3 µm in diameter have been used (Langdon and Waldock, 1981). These microcapsules allowed a resumption of growth in oysters previously fed only *Dunaliella tertiolecta* which lack n-3 highly unsaturated fatty acids. They have also been used as a food source for larvae of *C. virginica* and *Saccostrea commercialis* although lower growth rates were recorded in comparison with an microalgal diet (Chu *et al.*, 1987; Numaguchi and Nell, 1991; Fig. 4). However, mixed to protein-walled microcapsules, they supported *Tridacna gigas* larval development through metamorphosis (Southgate *et al.*, 1992*a*).

Gelatin-acacia microcapsules are interesting from several points of view. They are stable and can be autoclaved in view of long-term storage (Chu *et al.*, 1987). Sterilisation, however, will affect fatty acid incorporation and modifications in pH may cause microcapsule aggregates to be formed or random break-up of these aggregates (Rodriguez *et al.*, 1992). They retain hydro-

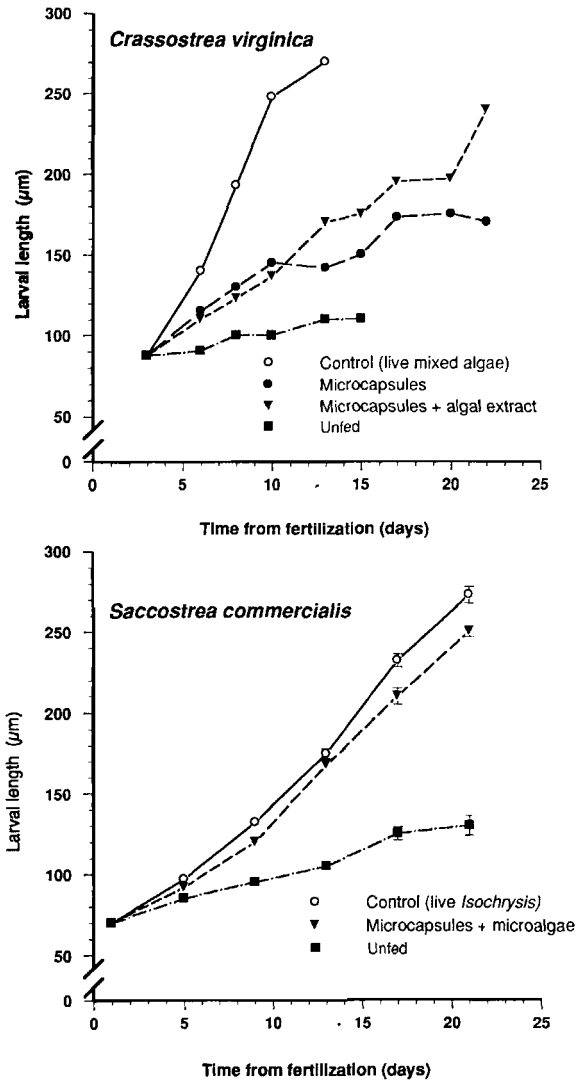

Figure 4. – Growth of *Crassostrea virginica* (**a**) and *Saccostrea commercialis* (**b**) larvae fed with fresh mixed diets and/or microcapsules (from Chu *et al.*, 1987 and Numaguchi and Nell, 1991).

phobic compounds well and allow the encapsulation of fatty acids, hormones and certain vitamins (Delaunay, 1992). The main disadvantage of this particle type is that it is incomplete due to its inability to encapsulate hydrophilic nutrients. These microcapsules can, therefore be used as a lipid food supplement in hatchery and nursery and their high assimilation efficiencies, proved on *Crassostrea gigas* spat (57%) supported that potentiality (Knauer and Southgate, 1997).

Microgel microcapsules/ Microbound diets

These microcapsules are often used to encapsulate high molecular weight soluble nutrients such as certain proteins and/or starch, but complete diets, containing both aqueous and lipids components, can be used (Teshima *et al.*, 1982). Several microgel types have been tested such as alginate microcapsules (Fig. 3*d*).

Chlamys nobilis larvae fed mixed carrageenan micro-bound diet and *Chlorella* (1/1 ratio) showed higher growth and higher survival than with algal diet alone (Teshima *et al.*, 1982). Used as a supplement with microalgae (1/1 ratio), excellent larval development has also been obtained in *Ruditapes philippinarum* (better than the algal control) whereas poorer results have been recorded for *Crassostrea gigas* (Kean-Howie *et al.*, 1994).

Microgel microcapsules (mixed artificial diet) have also been used successfully to feed *Crassostrea virginica* juveniles, the best results being obtained with microcapsules containing albumin (Langdon and Siegfried, 1984; Langdon and Bolton, 1984) leading to growth rates up to 73% of those obtained with an exclusively microalgal diet.

However, microgel microcapsules present two major handicaps. They sediment rather easily which can be detrimental to the nutrition of mollusc larvae (Kean-Howie *et al.*, 1994). It is also difficult using present day methods to produce microcapsules smaller than 20 µm in diameter and, as a result, their use as a larval feed is limited. At present their utilisation in hatchery and nursery is limited accordingly.

LIPID MICROSPHERES AND LIPID EMULSIONS

More recently, lipids have been delivered to bivalves in the form of lipid microspheres (Robinson, 1992*a*, *b*) or lipid emulsions (Coutteau *et al.*, 1994*c*): Table 1. They appeared to be more convenient because they are easily produced, have suitable buoyancy characteristics, do not release any toxic substances, are lightly polluting the water and are easily ingested and digested by adult oysters, *e.g. Crassostrea gigas* (Robinson, 1992*a*, *b*) and *Ostrea edulis* (Heras *et al.*, 1994). In addition, the incorporation of the lipids present in emulsions has been validated in larvae of *Ostrea edulis* and juveniles of *Placopecten magellanicus* (Coutteau *et al.*, 1996).

The importance of such lipid emulsion as supplementation to algae, has been reported by Coutteau *et al.* (1994*c*) on *Mercenaria mercenaria* larvae with better growth and survival throughout metamorphosis than that of controls fed only microalgae.

In terms of genitor conditioning, Robinson (1992*a*) observed gonadal development in *Crassostrea gigas* Kuamoto which was similar to that seen with an microalgal diet while a positive effect on spawning and size of eggs was reported on *Argopecten purpuratus* (Coutteau *et al.*, 1996).

Despite these initial promising results, the biological effects of such feed supplements remains to be precised for all stages of mollusc development.

DISCUSSION AND CONCLUSION

Efforts to develop a food substitute for live microalgae have been conducted along three major research avenues (Table 1). The first consists of replacing microalgae by other microorganisms such as bacteria and yeast. This necessitates a sound understanding of the microorganisms' physical and chemical characteristics and necessitates that all the incompatible aspects (size, toxicity, nutritional quality) be defined with respect to the bivalve and/or the stage of development involved.

The risk of pathogenicity when using bacteria is too high in mollusc hatcheries and their nutritional input too low (< 10%) to foresee a future for this type of feed. We believe that the possible role of selected bacteria (positive or neutral) in hatcheries is one of prophylaxis. Their addition to larval and/or microalgal cultures could result in the limiting of bacterial pathogen proliferation which would allow a decrease in the curative and even preventive use of antibiotics (Robert *et al.*, 1996*b*).

As for yeast, the production methods used recently seem promising (chemical treatment and enrichment with ω3 fatty acids). This last food supplement should be of use in nurseries and the Veneridae in particular, appear to be good candidates for this new food source.

The second approach involves the transformation of raw material, microalgae in this case, into a secondary product which is easier to use. Regardless of the transformation method used, however, be it by concentration, freezing or lyophilisation, the original nutritional potential of the microalgae is generally decreased. All of the above transformation methods directly or indirectly affect the physical and chemical characteristics of the microalgae. As has been demonstrated by a number of authors, microalgal pastes (Nell and O'Connor, 1991; Robert *et al.*, 1996*a*) and dried microalgae (Laing and Millican, 1991; Curatolo *et al.*, 1993) do not produce growth rates in bivalves that can be compared to those obtained with a fresh microalgal diet, although the simplicity in usage and storage has been increased. To concentrate microalgae into slurry, alternative harvesting technologies (*e.g.* membrane filtration, flocculation), which are potentially less damaging, have to be developed. The low number of dried microalgae produced on an industrial scale is to date a limiting factor. There is a need to identify more nutritionally valuable dried microalgae and such investigations are in progress (Barclay and Zeller, 1996; Knauer and Southgate, 1996). Both types of food, algal paste and dried microalgae, should be of use as supplement diet in hatcheries (conditioning genitors) and nurseries. The Veneridae appear again to be good candidates for which Curatolo *et al.* (1993) have proposed a feeding spat strategy based on live/ dried *Tetraselmis suecica*.

The third approach involves the production of artificial food particles. Inert artificial foods have been successful in conditioning oysters (*see* review Nell, 1993) but they often promote bacterial contamination. Microencapsulation greatly reduces leaching and the risk associated with high numbers of bacteria, and microcapsules are therefore suitable for larvae and spat. The main goal of these types of feed, as opposed to other substitute products, is to produce a food source whose nutrition potential is greater than that of microalgae and which can be modified in relation to certain parameters (species, stage of development). Another interesting characteristic involves the ability to add nutritional stimulants and medicines. Finally, there are other advantages such as the simplicity of usage and storage (Coutteau *et al.*, 1996) as well as the low production cost (Southgate *et al.*, 1992*a*). Despite the undeniable progress that has been made in terms of animal growth and survival (Southgate *et al.*, 1992*a*, *b*) the results obtained to date are not yet fully convincing for bivalve rearing but two types of microcapsules are very promising as an alternative to microalgal food for mollusc: protein-walled and gelatin-acacia microcapsules.

However, it must be remember that live heterotrophically grown algae is another low-cost alternative to live phototrophically grown algae (Nell, 1993). At present only a few species (*see* above in dried algae section) can be grown by this technology, but if strains capable of heterotrophic growth with high nutritional value for aquaculture could be used, this might obviate one of the major reasons for seeking an artificial diet (*i.e.* cost).

Nevertheless, as pointed out by Southgate *et al.* (1992*a*) development of benchmark diets, such as microcapsules, provides a reliable tool for bivalve nutritional requirements (*e.g.* Kreeger and Langdon, 1993 and 1994; Knauer and Southgate, 1997) and in turn will lead to further development of non living microalgae food. Further research in artificial diet is undoubtedly necessary to allow a more precise determination of the nutrient requirement of molluscs.

It is of interest to note that the results obtained by researchers testing food substitutes are often in disagreement with the observations reported by the professional shell-fish farmers (Coutteau and Sorgeloos, 1992). These authors suggested that these discrepancies are due to differences in scale and/or rearing practices. A generally poor understanding of the molluscs' basic nutritional needs in conjunction with the paucity of experiments (Table 1) using these new food types is, in our opinion, also responsible for this situation. Indeed the nutrition of bivalve larvae and spat has not received the same research attention as that of crustaceans and fish larvae for which non living food diets are now available commercially. For bivalves, these products have long been considered to be substitute

feed as opposed to food supplements and, as a result, attempts to use them have often failed. The budding understanding that to date fresh microalgae are necessary in proportions which will vary with the mollusc species to be reared and its stage of development should allow future experiments to be properly oriented and will thus contribute to the expansion of these new products. A number of non algal live diets are available or their development in progress. Their use will greatly simplify hatchery-nursery procedures and lead to more consistent and economic hatchery-nursery production of bivalve spat.

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Book notices

Biodiversity Dynamics and Conservation: The Freshwater Fish of Tropical Africa. Edited by Christian Lévêque. 1997, Cambridge University Press, 438 p., ISBN 0 521 57033 6 (hardback) £55.00 (US$84.95).

In order for biodiversity to be conserved, it is important to know how and where diverse assemblages of plants and animals exist, to understand the effects of human impacts on them and to find the means by which these impacts can be lessened and even reversed. While tropical systems are know to be amongst the most diverse and most threatened globally, tropical freshwater systems have been neglected, and the tremendous variety of fish, amphibians, invertebrates and plants that live in them is poorly known yet seriously threatened. This comprehensive book brings together a wealth of information on the fish of tropical African systems, and discusses how these systems evolved, what holds them together and what is tearing them appart. If will be an important reference work not only for those interested in fish, but for all those concerned with biodiversity conservation anywhere.

Poissons de Guyane. Guide écologique de l'Approuague et de la réserve des Nouragues, par T. Boujard, M. Pascal, F. Meunier, P.-Y. Le Bail, J. Gallé. 1997, 219 p., ISBN 2 7380 0719 8. 300 FF. INRA Editions, Route de St-Cyr, 78026 Versailles cedex, France. Fax : +33.01.30.83.34.49.

Cet ouvrage propose une synthèse des informations scientifiques disponibles sur les peuplements piscicoles d'eau douce de Guyane française : bio-géographie, répartition spatiale, cycles saisonniers, biologie. Il fait par ailleurs une description exhaustive d'une centaine de poissons peuplant la rivière Approuague et la réserve naturelle des Nouragues. La détermination des espèces est rendue particulièrement aisée grâce à une excellente iconographie inédite (24 planches en couleurs, 6 planches en noir et blanc), faisant clairement apparaître les caractères d'identification. Des clés de détermination, fondées sur l'utilisation de caractères discriminants accessibles pour le néophyte, sont également proposées.

Baseline Studies of Biodiversity: The Fish Resources of Western Indonesia. Edited by D. Pauly and P. Martosubroto. 1996, ICLARM, 312 p., ISBN 971 8709 48 7. US$31.50 air mail.

The entire set of data analysed here is available in digitized form, *i.e.*, NAN-SIS file for the trawl data, and on the FishBase CD-ROM for the biological data on fish. Contact ICLARM for details (International Center for Living Aquatic Resources Management, MCPO Box 2631, 0718 Makati City, Philippines. Fax +63-2 816-3183, iclarm@cgnet.com). This book presents and analyses data on fish resources collected in Western Indonesia – off Sumatra, Java, Bali, Southern Kalimantan (Borneo) and South of the Lesser Sunda Islands (Nusa Tenggara) – during bottom trawl surveys conducted from 1974 to 1981 by a variety of research vessels. Then gathered with the aim of fostering the development of bottom trawl fisheries; these data are shown here to identify fish communities that subsequent fishing has much altered and whose diversity can be expected to gradually decline upon exploitation. These community descriptions, by an international cast of authors, are complemented by contributions on the historical background and on the physical and conceptual contexts of the original surveys, reinterpreted as baseline studies of fish biodiversity.

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