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# Early weaning of marine fish larvae onto microdiets : constraints and perspectives

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**Abstract** — Weaning small sized marine fish larvae at first feeding directly onto compound pellets is still difficult, while good results can be obtained when used in combination with live prey or when a prefeeding period on live prey is provided up to a size of about 2-3 mg. Microdiets are generally well ingested as their acceptability and visual detection may be improved using feeding activators and increasing good perception contrast. In theory, food may be processed to have a correct nutritional balance, selection of high digestible components is very important as larval digestive system is not fully functional during the first 2 or 3 weeks of life. Specific larvae requirement are partly unknown, common nutritional indices of dietary value are not usefull, and any nutritional deficiency may cause disorders, skeletal abnormalities and/or more generally growth retardation. MD use, microcapsules or microparticles, is now developing fast in Europe and Japan. The different feeding strategies usable are discussed from an economic point of view.

## INTRODUCTION

It is generally admitted that for all marine fish species with small sized larvae, live food utilization is necessary for a short time after hatching to ensure high survival and growth rates. This feeding strategy based on live prey is rather expensive, it requires manpower and expensive equipment, while the nutritional and sanitary values of prey are difficult to control. It would be economically advantageous either to use live prey substitutes directly at first feeding, or at least to minimize the duration of this period in the absence of a suitable artificial diet usable directly at first feeding.

During the last two decades, many attempts have been carried out at a laboratory scale on the early weaning of most marine fish larvae concerned by aquaculture programs, with relative by poor success in comparison with fresh water fish species such as Coregonids (Gatesoupe et al., 1977; Kanazawa et al., 1982; Dabrowski, 1984; Zitzow et Millard, 1988). Now, the interest of microcapsules and microparticles in



marine fish farming is growing up in Europe and Japan and rapid progresses are expected as in Penaeid crustaceans (Kanazawa, 1981).

The purpose of this paper is (1) to draw the attention to the fact that larvae with an uncompletely developed metabolic system are not as easy to wean as juveniles, (2) to sum up the main requirements of a suitable microdiet (MD) with respect to larval feeding behaviour and nutritional requirements, (3) to compare different types of MD potentially suitable for young stages, and (4) to discuss the main feeding strategies usable in marine fish early weaning, predator species being exclusively concerned.

## PECULARITIES OF DIGESTIVE PHYSIOLOGY IN LARVAE

The most important changes in the digestive tract during ontogenesis are summed up for a typical marine fish larva lacking a morphological and functional stomach until juvenile stage (Fontaine, 1981; Govoni et al., 1986; Cousin et al., 1987).

### Morphological aspects

At hatching, the digestive tract is a straight tube closed at the mouth and histologically undifferentiated along its length (Fig. 1). It remains quite unchanged from mouth opening until the completion of yolk absorption, then becomes segmented into a buccopharynx, foregut, midgut and hindgut. The larval period ends with the development of a stomach with gastric glands and pyloric caeca. The liver and the pancreas are formed at hatching and are functional at first feeding.

### Digestive enzymes

At first feeding, the digestive system appears not fully functional with an amylolytic and a proteolytic activity : amylase, trypsin, chymotrypsin and aminopeptidases should provide a good starch and protein digestion. Lipid digestion during embryonic and larval stages still remain unclear : in most species, a lipase activity is detected quite late, while esterases are present at first feeding. Alkaline and acid phosphatases are observed very early; if their role is not well known, they are probably involved in yolk lipid and oil globule utilization. A pepsin activity (gastric gland secretion) is detected very late (generally not before 1 month posthatching).

Enzyme activities are observed to be generally low at first feeding and each enzyme develops independently during ontogenesis. Larvae can partly control their enzymes production (trypsin at least) in response to feeding (Hjelmeland et al., 1988). Exogenous enzymes of live preys may play an important role in initiating larvae digestion, but apparently they are destroyed soon after.

### Digestive mechanisms

In larvae, digestion and absorption mechanisms are concentrated in the mid-gut and hind-gut but they are not well known. Lipids are digested

to fatty acids and monoglycerid in the mid-gut lumen, then absorbed in the mid-gut epithelium and after an intracellular resynthesis to lipids they are deposited in large lipid droplets for a short time. Proteins are digested in the epithelial cells of the hind-gut after a predominant pinocytotic absorption of macromolecules along the intramicrovillous plasma membrane. To our knowledge, carbohydrate absorption has not yet been studied in marine fish larvae.

In juvenile stages, when the stomach becomes functional, secretions of gastric glands facilitate the complete hydrolysis of proteins, in the mid and hind-gut, into peptides and amino acids through the action of pancreatic and intestinal enzymes : trypsin, chymotrypsin and aminopeptidases.

In larvae, digestion efficiency seems relatively low as the digestive system is not fully functional and that the digestive tract is short and transit rapid compared to juveniles : in sea bass larvae, live prey pass through the fore-gut within seconds, through the anterior midgut within minutes and remain in the posterior mid-gut and hind-gut for a few hours, so defaecation begins less than one hour after first live prey has been eaten.

## FEEDING BEHAVIOUR

Some problems in early weaning may be due to a bad knowledge with respect of feeding and/or social behaviour of larvae. Feeding behaviour may be divided in an appetite phase : detection-identification of the potential prey (alert), then location (search) and a consummatory phase subdivided into a bite, test and ingestion phase. According to Mackie and Mitchell considerations (in Cowey *et al.*, 1985), smell plays an important role in the alert phase, then larvae approach the food guided by chemical and/or visual stimuli or sounds. Close to the food an incitant invokes initiation of feeding. Taste (taste buds in the mouth) nearly always plays a role in the test phase. If food feels right (size, texture, roughness), it is ingested and continuation of feeding is promoted by a stimulant. There are different ways to improve the detection, perception and palatability of microdiets.

### Feeding activators

The attractiveness of food made from fresh materials such as squid, molluscs or fish flesh is generally sufficient. When powdered ingredients are exclusively used, incorporation of appetizers is recommended to stimulate feeding. Previously natural appetizers were used with respect to the feeding habits of wild fish under natural conditions. Now chemical substances identified as feeding activators for a lot of marine fish are in current use in larvae food formulation (Mackie and Mitchell, in Cowey *et al.*, 1985). In juveniles they belong to a fairly small group of chemical substances : L amino acids, glycine betaine, inosine or inosine 5' monophosphate. Glycine betaine and inosine are the most efficient feeding activators in sole juveniles, while it is inosine in turbot (Person-Le Ruyet *et al.*, 1985). Practical levels of specific feeding activators are 1 % of the

diet in turbot juveniles during 5 to 10 d., 5% or more in sole during all weaning period and 2% in sea bass (complete mixture of L amino acids) for a few time, but they are probably overestimated.

Feeding activators have not been studied in larvae, but as their role is potentially more important than in juveniles, it is advisable to use at least juvenile optimum levels known. If no specific information is available, Mackie and Mitchell's complete mixture (L amino acids plus inosine and glycine betaine) may be used first.

#### Food location by sight

Most larvae locate food mainly visually, mechanical disturbance of the ear or lateral line by vibrations seem to play a minor role with inert food. Vision in marine fish larvae has been studied by Blaxter (1980) and Neave (1984). They are not very efficient hunters at first feeding, their ability to catch prey is progressively enhanced during ontogenesis once eyes developed. In turbot (*Scophthalmus maximus*) larvae, visual behavioural acuity is very low at hatching but increases from 6-7° at first feeding (d.3) up to 11° at early metamorphosis (d.15). In most species, past first feeding, light threshold for feeding averages about 10-1 lux, equivalent for late dusk or early dawn (0.4  $\mu\text{W}/\text{cm}^2$  at the surface).

Whether or not a potential prey is visible most of all depends on the contrast between the prey and its background. Contrast perception may be easily manipulated by changing prey and tank walls colour and illumination conditions. Feeding efficiency may be improved in staining MD red, using canthaxantine, to match *Artemia* nauplii, colour, then particles selection by colour is avoided. On the other hand, additional artificial light after dusk or before dawn to prolong the feeding day has beneficial effects on growth and generally on survival but not necessarily on food conversion. Scarce is the data concerning optimum light intensity, colour and distribution for larvae, while it is advisable to use either a continuous soft illumination or preferably, as regards to physiological rhythms, long daylength (18 L/6 D).

#### Physical characteristics and food availability

It may be partly possible to copy the motility of live prey both by controlling the density of MD and their sedimental speed. Good results are generally obtained using slightly hydroscopic particles which float at the surface for a few minutes before sinking slowly in the tank to be definitely lost. Some help may also be found in a correct control of water supply, water direction and intensity, and in good food distribution. Small quantities of dry MD may be easily supplied each 10 or 15 minutes using automatic feeders. It is an advantageous way to control food availability, to reduce larvae fasting and avoid or limit social disturbances, cannibalism for example.

Little is known on larvae food texture preference. Sole (*Solea solea*) and turbot larvae are relatively independent of food palatability compared to juveniles which prefer soft texture diets. The use of particles as round

as possible (as microcapsules) is recommended, with MBD a good selection of components of adapted size and/or the elimination of any kind of detrital materials such as chitin by a proper sewage is necessary.

## NUTRITIONAL REQUIREMENT

The unsuitability of MD may be partly linked to the incomplete knowledge of larvae nutritional requirement. MD formulation is based both on the nutritional requirements of juveniles of the same species or group and on the composition of larvae natural foods, zooplankton. Some practical recommendations for MD formulation are made here, detailed informations on fish nutrition could be found in Fontaine (1981) and Cowey *et al.*'s (1985) books.

### Main sources of energy

Some data are available on the best balance between the main energy sources : proteins, lipids and carbohydrates, as on the basal energy requirement of larvae. Dietary protein levels as high as 55-60 % are most often used for several reasons : since larvae grow fast, they are supposed to have a high protein demand, high quality proteins are observed to promote growth in juveniles and larvae natural diets are rich in proteins. Lipids are the most energy rich class of nutrients in addition to supplying the essential fatty acids. As in young juveniles, limits above which growth rate decreases are relatively high (around 12 % in sea bass, 9 % in gilthead sea bream (*Sparus aurata*) and 10 % in red sea bream (*Chrysophrys major*), in MD, lipid levels are generally increased to 15-20 %. In spite of the fact that most marine fish are said to have a limited ability to digest carbohydrates, some digestible forms tend to be more and more included in MD at levels ranging from 10 to 20 % of the diet.

### Amino acid requirement

There are 10 essential amino acids (EAA) : arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophane; cystine and tyrosine being considered as semi-EAA. There is a general similarity in quantitative AA requirements between species, and a good correlation between the AA requirement pattern and the muscle and/or eggs AA pattern (Cowey and Luquet, 1983; Wilson and Poe, 1985); so the EAA profile of fish eggs may serve as a reference in formulating diets.

To overcome any EAA deficiency, natural protein sources are commonly selected according to their AA composition (AA availability values and digestibility coefficients of different proteins may be found in NRC tables) and deficient proteins may be supplemented with synthetic AA.

In practise, as long as high value protein diet such as fish meal (AA composition close to the fish one) are used, the EAA requirement is more



or less covered when protein requirement is met (dietary protein levels are most often overestimated). Whereas it is advisable to pay attention to methionine levels, for the european sea bass, methionine requirement is 1 % of the diet (Alliot et al., 1985).

### Essential fatty acid requirement (EFA)

Due to a limited ability to chain-elongate and desaturate linolenic acid, marine fish larvae have a general dietary requirement for 20 :5 n-3 and 22 :6 n-3 (EFA), C18 : n-3 being much less efficient. Excess of n-6 series FA may have detrimental effects. Whereas in some species, as in turbot, 20 :4 n-6 PUFA seems to be required in high quantities (Bell et al., 1985).

In practise, the n-3 EFA requirement of most marine fish larvae (dietary levels range from 0.5 to 1 % of 20 :5 n-3 plus 22 :6 n-3) are met when diets provide a high lipid level of marine origin. As fish are susceptible to peroxidative problems, attention must be paid to oxydation risks of fish oils unless stabilized by synthetic or natural antioxydants.

On the other hand, to be as near as possible to the PUFA pattern of wild zooplankton, high levels of phospholipids are recommended. They are more easily emulsified than triglycerides in larvae gut and facilitate the absorption of FA and cholesterol. A positive effect of dietary phospholipids (supplied by 1-2 % lecithin sources) has been observed on red sea bream and ayu (*Plecoglossus altivelis*) culture by Kanazawa et al., 1983.

### Vitamin and mineral requirement

Vitamin dietary levels given in classical tables for Salmonids, are in theory usable for larvae provided that losses during processing, storage and sea water leaching are not excessive. In the best storage conditions (cold and dry conditions), a good preservation of vitamins is only guaranteed for 6 months except for vitamin C. In practise, to overcome any vitamin shortage or degradation, MD dietary vitamin supply is increased in comparison with juveniles, more specially for vitamins C and E. The tendency is either to use dietary vitamin C levels as high as 2 to 3 % (as 20-80 % losses are recorded during food processing) or when possible to supplement MD just before use. To limit the risks of peroxydation, MD are also overfortified in vitamin E, minimum dietary levels used : 30 to 50 IU per Kg dry diet.

In marine fish the major part of minerals are absorbed from sea water. Whereas compound diets may require a mineral supplementation even if they contain a high proportion of fish meal, a relatively good source of dietary mineral. The most important point to consider is probably phosphorus supplementation, with soluble salts such as mono or disodium phosphate, as it is found in insufficient quantities in sea water. The dietary requirement of available phosphorus is about 0.6 % in red sea bream. Little is known about calcium and iron potential demand, while trace metals have been shown to be beneficial in larvae by Robin et al., 1987.

## DIFFERENT TYPES OF MICROPARTICLES

As aforesaid to be suitable for larvae, MD should have a high acceptability, a correct stability in sea water, and preferably they should be easily graded to an adapted size (under 150-200  $\mu\text{m}$  for rotifer substitutes and from 150 to 250  $\mu\text{m}$  for artemia substitutes) and usable in automatic feeders. Consequently, wet pastes or gels and moist pellets (40-60 % and 20-40 % water content respectively) may not be considered as realistic foods. Dry MD are commonly classified in 3 or 4 types : flakes and rehydratable diets obtained by pressure cooking methods, microbound diets (MBD) obtained by binding powdered diets or a mash, microencapsulated diets (MED) obtained by encapsulating a complete diet with a membrane, and microcoated diets (MCD) obtained by coating powdered diets by specific materials.

### Flakes and expanded diets

Both types are obtained through pressure cooking methods with compaction at high temperature (130-190°C) on a rotative drum for flakes or with extrusion cooking under high temperature and pressure followed by an abrupt depression. Their stability in sea water may be high and preparation of both diets requires a special machine. Extrusion cooking is a high cost technique presenting many advantages : improvement of digestibility of some components, possible destruction of antinutritional or toxic factors (avidine of hen eggs). Most of all, this MD type has a good aptitude to flottability and a very high ability to rehydration or absorption of various solutions allowing some modifications of texture and attractiveness just before use and compensation of losses due to heating during food processing (Melcion *et al.*, 1983).

### Microbound diets (MBD)

Different MBD types are classified according to the binder used : most often agar, carragheen and alginate, sometimes sodium polyacrylate and gelatin. Mixed to the diet ingredients, agar and kappa carraghen needed to be heated up to 100 or 85°C until they coagulate completely. Sodium alginate which is soluble in cold water is precipitated by calcium ions with, in theory, restricted losses of thermolabile substances. Raw fresh ingredients or dry powders are usable and no special equipment is required. MBD processing is quite simple and sea water stability of particles may be very high.

### Microencapsulated and microcoated diets (MED and MCD)

Two main methods are used to prepare microparticles more or less well protected by a water proof wall which will be in theory easily broken down just after ingestion by enzymes, bacteria or pH variation in the gut : microencapsulation by coacervation which consists in precipitating a polymer in liquid phase around the microparticles and microcoating where



the wall is obtained by evaporation of the external phase (Teshima et al., 1982).

With microencapsulation losses of soluble substances may be important and it is difficult to obtain a complete food but easy to have well graded small size particles of high stability in sea water. Capsules can be made of natural (gelatin, zein) or synthetic (nylon, polystyrene) polymers. This technique is usable at a laboratory scale and is developing at a commercial scale.

For coating, zein is most often used, sometimes a cholesterol-lecithin mixture. The coating solution may be evaporated in an oven and very small sized particles are easily obtained by atomisation. Soluble substances are spared and coating solution can contain feeding activators.

## DIFFERENT FEEDING STRATEGIES

To suppress or reduce live prey utilization, different feeding strategies can be used : direct weaning at first feeding in large sized larvae, direct weaning prior to metamorphosis or progressive weaning as soon as possible for small sized larvae.

Direct weaning possibilities at first feeding : sole

Plaice (*Pleuronectes platessa*) and sole (*Solea solea*) are said to be relatively easy to wean very early. First successes, reported by Adron et al., (1973) and Gatesoupe et al., (1977) using either conventional particles or microcapsules were therefore associated with low survivals and poor growth. When weaning sole at first feeding onto MD is singly used, survival at metamorphosis is low, about 30 %, and time required to complete metamorphosis is increased by about 50 %. In contrast, when a 10 cl *Artemia* prefeeding is used, similar growth rate and survival may be obtained at metamorphosis with MD and live preys, while a growth retardation is always observed post metamorphosis : at d.70 juvenile weight represents from 50 % to 80 % of the control and *Artemia* saving is about 90 % from hatching (Appelbaun et al., 1985; Gatesoupe, 1983).

These weaning success differences related to larvae age can be partly explained by the fact that completely different stages are concerned : in 10 days old larvae, yolk sac and oil droplet are completely resorbed and they are approaching 3mg weight, in contrast, at first feeding (d.2), larvae are 0.3-0.4 mg weight and their digestive system is still primitive (fig.2).

Direct early weaning of old larval stages : sea bass at day 20.

As sea bass are said difficult to wean at first feeding, the strategy chosen over the past three years at IFREMER was to develop a MD suitable for 20 days old larvae for 2 main reasons : at this age the chances of success are high as larvae are about 3 mg weight (as 10 days old sole) and up to this age, live prey demand is rather limited but starts to increase sharply, so important *Artemia* sparing is expected (Fig. 3 and 4). Results obtained are reported in Table 1 and 2.

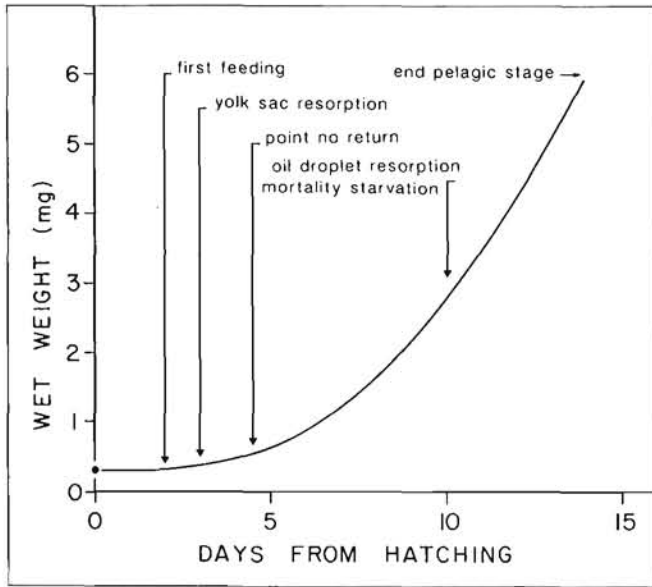


Fig. 2. — Sole larvae development and growth at 19°C.

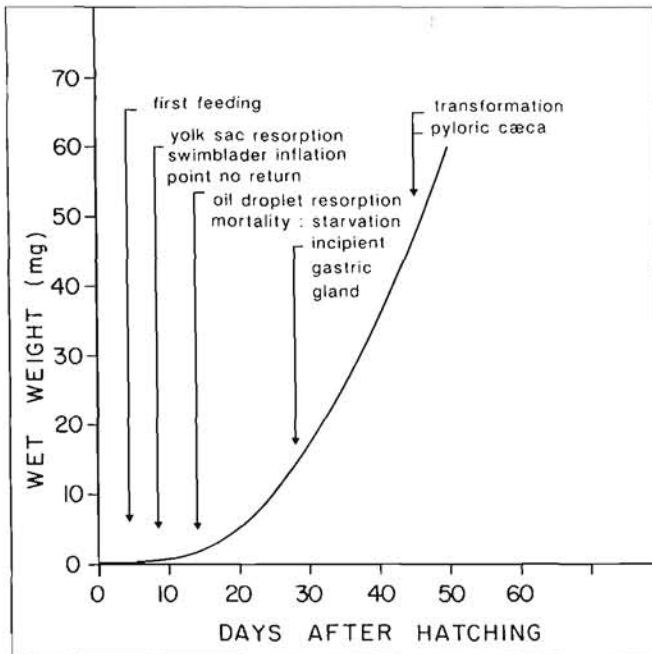


Fig. 3. — Seabass larvae development and growth at 19°C.

**Tab. 1.** — Composition and proximate analyses of experimental diets expressed in % of dry matter

DIET	I	II	III	IV	V
Expensed basal diet <sup>(1)</sup>	—	—	89.90	—	—
Fish autolysate	—	20.50	—	—	—
Casein	—	22.70	—	—	—
Whole squid	44.00	—	—	—	35.70
Squid mantle	—	—	—	45.20	—
Whole shrimp	20.68	—	—	—	—
Pollack filet	—	—	—	27.18	20.00
Pollack eggs	—	—	—	—	8.00
Salmon liver	—	—	—	—	12.00
Fresh hen eggs	15.00	—	—	15.00	—
Deshydrated hen eggs	—	15.00	—	—	—
Fresh yolk eggs	—	—	—	—	5.00
Soya lecithin	—	3.00	1.00	3.00	—
Cod liver oil	4.00	7.00	3.60	2.00	2.00
Precooked starch	—	10.00	—	3.00	3.00
Glucose	—	5.00	—	1.00	1.00
Sucrose	—	—	—	1.00	1.00
Sodium alginate	10.00	—	—	3.00	6.00
Carrageen	—	5.00	—	—	—
Carrophyll red R <sup>(2)</sup>	0.30	0.30	—	0.30	0.30
Vitamin premix <sup>(3)</sup>	2.00	5.00	3.48	2.00	2.00
Ascorbic acid	2.00	—	—	2.00	2.00
Cholic acid	0.02	0.02	—	0.02	—
BHT	—	—	0.01	—	—
Mineral premix <sup>(4)</sup>	2.00	4.00	—	2.00	—
Mineral premix <sup>(5)</sup>	—	—	—	—	2.00
Attractants <sup>(6)</sup>	—	2.50	2.00	—	—
Dry matter	93.8	94.5	93.7	94.8	
Protein	56.7	50.2	57.8	64.32	
Lipid	18.9	16.7	9.5	13.2	
Ash	13.6	10.3	7.5	8.8	
Ascorbic acid <sup>(7)</sup>	1260.0	—	1770.0	4880.0	

(1) Metailler *et al.* (1983).

(2) Containing 10 % canthaxantin.

(3) IFREMER vitamin premix 69 % (per kg of premix : vit.A acetate - 1 000 000 IU; vit.D<sub>3</sub> - 100 000 IU; alfa tocopherol acetate - 4 000 mg - vit.K<sub>3</sub> - 100 mg; Thiamin - 1 000 mg; Riboflavin - 2 500 mg; D Ca pantothenate - 5 000 mg; pyridoxin - 1 000 mg; Cyanocobalamin - 6 mg; Niacin - 10 000 mg; folic acid - 500 mg; Biotine - 100 mg; Meso-inositol - 100 000 mg). choline chloride 21 % - ascorbic acid 10 %.

(4) In % : Na<sub>2</sub>HPO<sub>4</sub> - 90 and FeSO<sub>4</sub>·7H<sub>2</sub>O - 10.

(5) Luquet (1971) mineral premix, (Na<sub>2</sub>SeO<sub>3</sub>) 0.5 ppm, (NiCl<sub>2</sub>) 2 ppm, Cr(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O 2 ppm and (Na<sub>2</sub>SeO<sub>3</sub>) 1 ppm.

(6) In % : L-Proline 50.3; glycine 30.6; L-alanine 9.3; L-threonine 1.5; L-serine 1.1; L-valine 1.2; DL-methionine 1.0; L-isoleucine 1.0; L-leucine 1.8; L-tyrosine 0.75 ; L-phenylalanine 1.0.

(7) mg/kg of dry matter.

From the 3 types of MD tested first (experiment 1) : alginate MBD, carrageen MBD and expanded MD, the first one appeared the most efficient : at day 40, average juveniles weight represented 50 % of the control and survival 70 %, while the level of skeletal abnormalities (like scoliosis and lordosis) was excessive. As the acceptability of alginate MBD

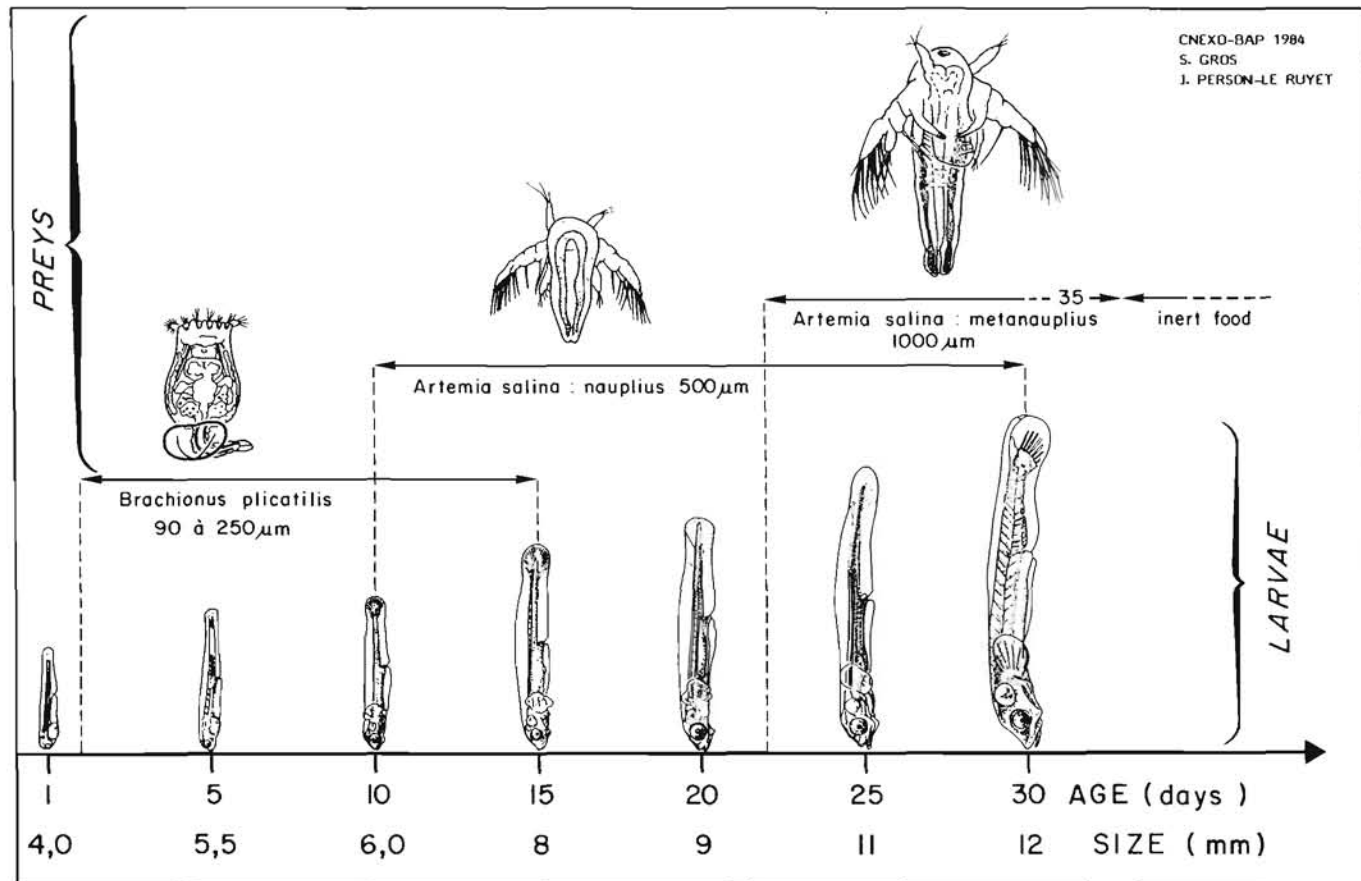


Fig. 4. — Seabass larvae development and feeding scheme at 19°C.

was very high in comparison with the other 2 diets, it was selected as a basis for the next experiments. First modifications made to diet I formulation concerned a reduction of lipid content and undigestible components, chitin and alginate, supposed to be excessive. General weaning results were highly improved, more particularly in experiment 3. Differences were observed between diets I and 4 until day 40, while at day 50, 10 days after MD were changed for a classical weaning diet (diet 3 formulation), a significant growth improvement was obtained with diet 4. The percentage of skeletal abnormalities was with both diets very low : nought with the control as previously, it was significantly improved with diet 4. As long as the initial weight range was over 3-4 mg, weaning success seemed more dependent on larval healthy state (expressed by specific growth rate from hatching and susceptibility to swimbladder stress syndrome) than on weaning age. Variations of trypsin and amylase specific activities controlled in experiment 4, during development in relation with diets and weaning age show that larvae may adapt quite well to food ingested.

Tab. 2. — General weaning conditions and results obtained at IFREMER in seabass rearing at 19°C

Exp	Initial conditions				Weaning results			
	Diet	Age	Weight	Nb lots	Age	Weight	Survival	Abnormality
1	I	23(1)	4.4(2)	2	40(1)	50(3)	66(3)	62(4)
	II		3.5	2		30	21	—
	III		3.7	2		29	83	45
2	I	23	3.4	2	40	100	72	18
	IV		3.4	2		81	92	7
3	I	21	4.4	2	50	72	100	6
	IV	21	4.1	2		72	100	0
4	I	23	4.4	2	50	73	100	30
	I	29	10.8	2		76	100	25
5	I	20	2.5	4	40	52	82	66
	V	20	2.5	4		6041	47	

(1) day post hatching.

(2) mg.

(3) as % of control.

(4) D.50 as % of control

Seabass larvae can be successfully weaned as early as day 20-23 (3-4 mg weight) onto alginate MBD which acceptability and stability in sea water are very high. As tank design is correct no signs of water pollution has ever been observed. Whereas, with MD, a growth retardation is always observed, or less soon (either after a 20 days MD period or 10 days later. In comparison with *Artemia*, with MD, at 50 days juvenile weight loss is about 30 %, while survival may be similar, up to 60-80 % or more from day 20 to day 50 post hatching. From an economic point of view, when larvae are weaned at D.23 instead of D.40, the feeding cost of a 50 days juvenile is significantly reduced, mainly by decreasing by 4 or 5 the *Artemia* biomass used (Fig. 5).

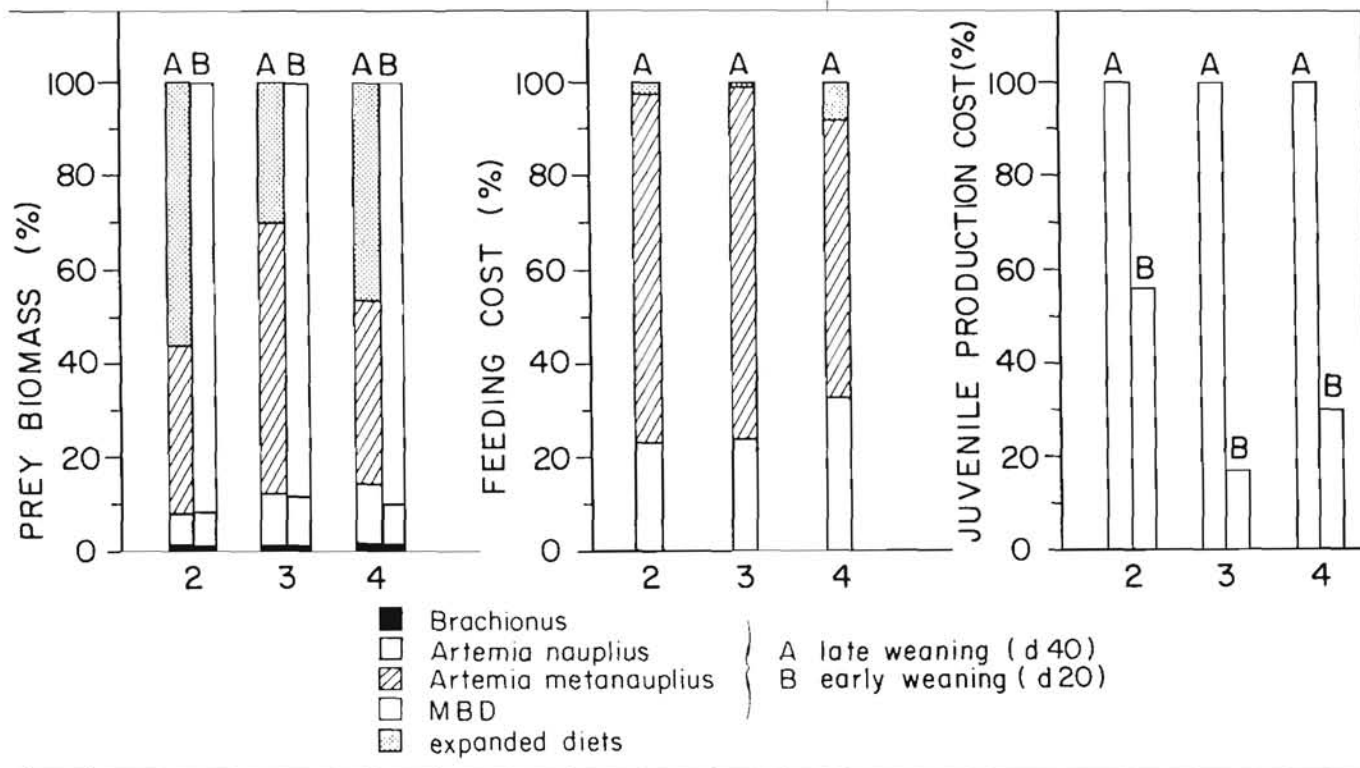


Fig. 5. — Preys requirement and relative feeding cost of a 50 d. juvenile according to weaning age.



Both the lower nutritional value of MD compared to live prey and the occurrence of skeletal abnormalities in some experiments, cannot be clearly explained through all the analyses and other controls performed on diets and larvae. Food intake is high and near a maximum 2 days post hatching as more than 80 % of larvae are observed to feed correctly on diet I. No sign of nutritional deficiency is evidenced and some possible causes of skeletal abnormalities may be rejected : (1) the EFA requirements are fulfilled with all diets which lipid content range is 13-19 %, and the dietary PUFA pattern on body pattern seems correctly balanced (an excess of n-6 FA series in diet 4 is well controlled by larvae); (2) a reinforcement in dietary phospholipids (by a better selection of dietary components) has not had any clear effect on juvenile quality; (3) there is a good correlation between the EAA pattern of diets I and 4 and eggs or larvae pattern, and apparently no shortage in methionine and tryptophane; (4) ascorbic acid levels are correct in diets and larvae, larvae contents are similar in MD batches and in controls, and in abnormal and normal larvae (Table 3).

Tab. 3. — Ascorbic acid content (in mg of wet matter) of seabass larvae in relation with age and diets (Table 1 legends)

Experiment	Diet	Ascorbic Acid (mg/WM)				Abnormality (%) d. 50
		d. 0	d. 24	d. 40	d. 50	
2	Control	—	90	57	68	0
	I	—	—	55	78(2)	18
	IV	—	—	65	74(3)	7
3	Control	—	—	62	53	0
	I	—	—	70	47	6
	IV	—	—	76	71	0
4	Control	—	—	—	48	0
	I	—	—	—	70	30
	I(1)	—	—	—	59	25
5	Control	32	—	46	—	0
	IV	—	—	55	—	66
	V	—	—	52	—	47

(1) Weaning age is d.30 instead of d.20 - 23 in all other experiments.

(2) Average level, in normal and abnormal fish, 76 and 85 respectively.

(3) Average level, in normal and abnormal fish, 80 and 54 respectively.

In contrast, some signs of disturbance in digestive physiology are pointed out : (1) after a 2 days use, evacuation rate is twice than longer with diet I that with *Artemia*, 7 hours instead of 3.30 hours at 19°C; (2) when larvae are fed on diet I, basal specific activity of trypsin increases slowly, that is generally interpreted as a mechanism by which an inadequate diet (of poor digestibility here), is compensated (Hofer, in Cowey et al., 1985); (3) MD food intake tends to decrease with time, as a nutritional unbalance possible consequence.

On the other hand, the relative unreliability in quality juveniles with diets I and 4, specially in experiment 5 suggests that with MD made mainly from fresh materials, part of problems may be due to antinutritional factors

or toxin accumulation in foods (no thiaminase in fish fillet used and eggwhite avidine destroyed by heating). As the sanitary quality of alginate MD made of fresh materials is difficult to control before and during food processing, it seems advisable to change for powdered components before developing this food technology at a production scale.

Progressive early weaning with additional live preys.

MD may be used in combination with live prey more or less early according to the adaptation age of each species to be weaned. This strategy has been applied for a long time in Japan in several species : red sea bream, japanese flounder (*Paralichthys olivaceus*), striped knifejaw (*Oplegnathus fasciatus*), while MD technology has been improved progressively. Now MCD efficiency, specially zein-MCD, is so high that no significant differences in growth and survival are observed in comparison with live foods as reported by Kanazawa et al. (1987). Commercial MD (Kyowa Co) are tested in Japan at a production level. According to the feeding models supplier, MD are used for sea bream after a 5 days rotifer prefeeding and in combination with rotifer and *Artemia* up to day 14, while japanese flounder are fed MD after a 25 days rotifer period and in combination with live prey up to day 40, at that age the stomach is functional (it starts to develop at day 20-25).

## CONCLUSIONS

Weaning marine fish larvae at first feeding directly on compound pellets is still difficult, while good results can be obtained when used in combination with live preys or when a prefeeding period on live prey is provided up to a size of about 2-3mg. This weight range is equivalent to fresh water species such as carp (*Cyprinus carpio*) and vendace (*Coregonus albula*) at hatching, which are known to be relatively easily weaned at first feeding (Dabrowski, 1984). Most marine fish larvae are active feeders, and MD may be well accepted quite immediately. There are many ways to increase the acceptability of MD and also their visual detection. Feeding behaviour may also be affected by larvae appetite which may be partly controlled by environmental conditions such as temperature, water quality and tank design. In theory, inert food may be processed to have a correct nutritional balance. Selection of high digestible components is very important particularly because the larvae digestive system is not completely functional the first 2 or 3 weeks of life. Since growth is rapid any nutritional deficiency may cause disorders, malformation of axial cord and/or more generally growth retardation. Specific requirements of larvae are partly unknown and common nutritional indices of dietary value are not useful at that age. When known the best reference is probably the composition of wild zooplankton which differs from most artificial foods in many points : soft texture and high digestibility (no larvae overloading), large amounts of soluble proteins of high nutritive value, digestive enzymes that may activate fish zymogens, and perhaps different unknown nutrients that may be essential or that may promote larvae digestion directly or not.

As yeast and mollusc flesh or meal may contain « unknown growth factors », they are often used in food formulation in combination with preferably powdered natural components and predigested protein sources. The reasons why natural foods are more efficient than compound foods are still questions to be solved to progress surely in microdiet formulation.

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