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MEDITERRANEAN REGIONAL AQUACULTURE PROJECT

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MEDITERRANEAN REGIONAL AQUACULTURE PROJECT

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Training Session

NUTRITION IN MARINE AQUACULTURE

LISBON - 20-30 October 1986

edited by
Amamaria BRUNO
MEDRAP

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Acknowledgements

On behalf of MEDRAP, I should like to express my sincere thanks:

- to all the people who participated at the Session,
- to the lecturers and trainees for the collaboration,
- to the I.N.I.P. for their excellent organization and warm welcome,
- to Mr. METAILLER and GABAUDAN, with whom I had the pleasure of working for the organization of the Session.

GENERAL PRESENTATION

The 13th training session organized by MEDRAP (GCP/REM/049/ITA) in collaboration with I.N.I.P (Istituto Nacional des Investigacoa das Pescas) on the theme "Nutrition in Marine Aquaculture" took place in LISBOA, Portugal, from the 20 to 30 October 1986 (See Annex I).

This seminary took place at I.N.I.P, who furnished all the material (reunion room, projector, etc...) along with all the help necessary for the success of the session.

DEVELOPMENT OF THE SESSION

a) The participants (See Annex II)

This session regrouped 17 participants from member countries of MEDRAP.

All the participants have worked in the aquaculture sector for many years, either at government level on in private enterprises.

b) The lecturers (See Annex III)

Fourteen lecturers took part at the session and the following points were taken up:

- Theoretical aspects of fish and shrimp feeding,
- Nutritional requirement of fish and shrimp reared in the Mediterranean,
- Feeding strategies,
- Use of raw-materials and by-products in aquaculture,
- Food manufacture,
- Practical experiments in feeding,

c) Development of the lectures

Each lecturer had two hours at disposal (including translation) to present his subject and answer any immediate questions.

At the end of each afternoon, a round table regrouped both participants and lecturers, which allowed more than simple questions-answers, but the confrontation of experiments in the different Mediterranean countries.

INDEX

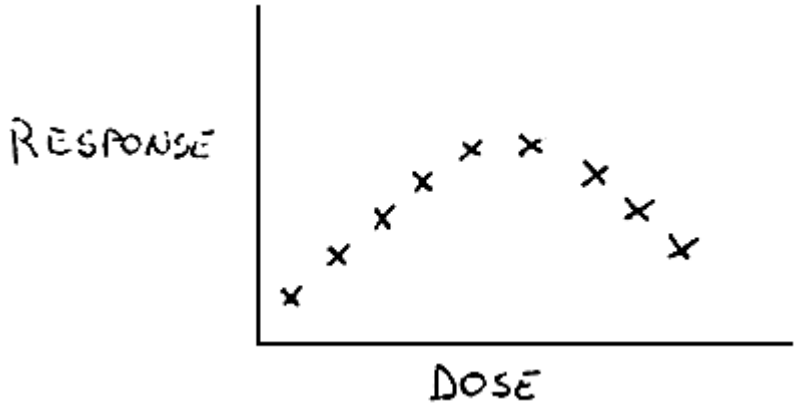
CONFERENCES

PROTEINS

C.B. COWEY

The method most commonly employed to determine protein requirements is that of dose-response curve.

This method consists in including in a basal diet all essential nutrients with the exception of the one under investigation. This nutrient is then added in stepwise amounts to other portions of the basal diet to enable a graded intake be given. The response measured is some index of growth (weight gain, length increase, etc...). generally a dose-response curve for a nutrient, which the animal cannot synthesize itself from ordinarily available materials, will slowly increase up to a plateau stage and then remain at that level or even decrease when the requirement level has been reached.



When this happens, at the intersection of the two lines, the apparent requirement for the nutrient will appear.

With protein the test diet must include all the nutrients with the exception of the one under investigation. If we increase the level of protein, we also increase the content of energy. But, when we are doing a dose-response curve, we have to ensure that the diet with low protein content has the same energy level as the diet with high protein content. One of the most common methods employed to solve this problem is to substitute carbohydrates for proteins. This assumes that the energy furnished by carbohydrates is the same that of protein. But normally a completely digested protein has an energy value of perhaps 4.5 Kcal/gr. while for a well digested carbohydrate it has an energy value of 4 Kcal/gr. The problem arising is that if we increase the carbohydrate content of the diet we lower the extent to which it is utilized. Another important question is that protein requirement may change during the life of the fish. Evidence was presented to illustrate this. The protein requirements of some fish, which were determined essentially at the juveniles stage are shown below. In fact in a laboratory, people normally use fry or juveniles for experiments and requirements will decrease in size. All the values shown are high with the exception of that for channel catfish. A possible explanation is that if fish or any animals are given the choice of substrates for use as energy source (either proteins or carbohydrates), the animal will preferentially use the protein as energy source rather than carbohydrates. So that in doing a dose-response curve with a higher level of protein intake, you tend to increase the use of protein as a source of energy.

PROTEIN REQUIREMENTS OF DIFFERENT SPECIES OF FISH

SPECIES	g/kg	Ref.
Rainbow trout	400-460	SATIA, 1974; TIEWS et al., 1976
Common carp	380	OGINO and SAITO, 1970
Chinook salmon	400	DE LONG et al., 1958
Japanese eel	445	NOSE and ARAI, 1972
Gilthead sea-bream	400	SABAUT et LUQUET, 1973
Grass carp	410-430	DABROWSKI, 1977
Channel catfish	220-320	GARLING and WILSON, 1976
Tilapia zilli	350-400	TESHIMA et al., 1978

Fig. 1 shows another experiment on channel catfish. There are four groups of fish of 2 different diets, one with 250 g. of protein/kg and the other with 350 g/kg. At the end of the experiment, there was a significant difference in the weight of the smaller fish fed on 2 diets while no significant difference was noted for larger fish. This seems a reasonable demonstration that smaller fish need more protein than larger ones.

Another claim that was made was that the water temperature affected the requirement for protein. With chinook salmon, the dose-response curve has been interpreted as showing a difference in protein requirements between 8° and 15° C, but the curve can be interpreted in other ways.

Concerning salinity an experiment was carried out with rainbow trout at two different levels of salinity : 10 ppt and 20 ppt. 10 ppt corresponds to about 1/3 of the sea water salinity and it is probably isotonic with the tissue of fish. With 20 ppt fish have to osmoregulate to some extent to get rid of the excess salt. The protein requirement is around 40 % at 10 ppt while at 20 ppt it is 45 %. A possible conclusion is that salinity can affect the protein requirement of the fish capable of living at these salinities.

AMINO-ACID REQUIREMENT

The essential amino-acids (EAA) are those that fish cannot synthetize by themselves from ordinarily available materials. They number ten in all.

The main way of determining these nutrients is by the dose-response curve. Preparation of test diets presents some problems. For example, if we have to carry out a dose-response curve for tryptophan and the fish require 40 % protein, we can give this amount by putting 20 % protein in the diet, this will fix the minimum level of tryptophan to make up the protein content of the diet. Then we prepare diets containing gradually increasing levels of tryptophan. The use of this type of diet entails an important assumption : the free amino-acids are used equally as well as proteins. This means that a free amino-acid mixture which has the same composition as a protein gives the same rate of growth. This assumption as we shall see later on, is questionable.

Another important point is that deficiencies in some nutrients, like vitamins cause high mortality rates, in fish. Often amino-acid deficiencies show very few pathological signs and mortality rates are low. Growth is very poor. One of the few amino-acids which does whow pathological symptoms in deficiency is tryptophan. In rainbow trout deficiency of tryptiphan causes scoliosis, lordosis and renal calcinosis.

Up to the present, we have concentrated on weight gain as the response that we can measure and obviously we will be more confident in our results if we have more than one way of determining the requirements. Another method is to inject fish with 14C

tryptophan, put the fish into a chamber and mesure radioactivity in the expired carbon dioxide. What we can see is that at low level of tryptophan intake a small amount of the radioactivity in the tryptophan is expired in CO_2. In other words, at low level, tryptophan intake, all the tryptophan is used by the fish for maintenance and also for growth and thus, above these levels, we will have excess tryptophan. One other method employed to find out how stepwise increases in dietary tryptophan will affect the animal is to examine the level of free tryptophan in tissues (such as liver) and in the plasma.

Fig. 3 shows the amino-acid requirements of fish measured by various authors. The requirements are expressed in g/kg dry diet. What this figure suggests, is that for certain amino-acids, we get enormous differences between different species. But, if we express these values as mg/kg body weight, these differences becomes almost non-existent (See fig. 4). The same applies if we express the results as g. required per kJ of metabolisable energy in the diet.

Fig. 5 shows the relative proportions of essential amino-acids in fish muscle and the relative proportions required by 4 species of fish. The maintenance requirements for fish are probably realtively low in comparison with those of warm-blooded animals; The latter must maintain their body temperature, particularly at colder temperqtures, they have special maintenance requirements for this purpose (production feathers or wool or hair). Fish do not need large amounts of energy to form non-toxic excretory products. Ammonia can be excreted into surrounding water.

There is a similarity between the proportion of the amino-acid in the muscle and the proportion required by the fish. It seems advisable when preparing diets for species of fish which have only recently been cultivated (e.g. marine fish) to examine the relative proportions of the amino-acids in the muscle and to try and match these proportions in the food.

Fig. 6 shows the results of an experiment on the growth of fish which were given diets containing high levels of protein or a mixture of free amino-acids and protein; this mixture of free amino-acids was identical in amino-acid composition with that of the whole protein. The growth of the fish fed on a complete protein is considerably better than that obtained with fish fed on a mixture of free amino-acids alone or with protein.

This probably means that when we define the amino-acid requirement in a laboratory, we are probably not obtaining maximal growth rates. The poorer utilisation of free amino-acids is also of importance in practice, especially when we use a cheap protein of inferior quality in a diet and need to improve its biological value by supplementation with free amino-acids.

Fig. 7 shows an interesting experiment concerning the utilization of arginine, free or protein bound, by catfish. The authors gave a mixture of casein and gelatin to channel catfish. On the first line all the arginine was furnished by casein (1.2) and the weight gain was 11.6 %. In adding some arginine (free arginine) to the diet, no improvement in growth occurred. But when the authors added arginine as a component of protein (gelatin) they obtained an increase in weight gain. These authors concluded that free amino-acids can not be used to supplement a deficient protein. Protein bound amino-acids were effective in this respect. But (See fig. 8), in a similar experiment with lysine, other authors reached the opposite conclusion: a supplementation with lysine of a protein deficient in lysine was effective in promoting growth.

From a practical point of view, the problem may be resolved by the high price of free amino-acids, the less expensive amino-acids are lysine and methionine which are

required by the animal feed industry, especially the poultry industry. Consequently, when using a protein which is deficient in some amino-acids (e.g. soyabean meal is deficient in methionine) a more practical solution is the addition of another protein of high quality (e.g. a good fish meal) to supplement this protein.

Some experiments demonstrated that when a fish is fed with a mixture of amino-acids, the amino-acid peak in the plasma is reached much more quickly than when they are fed with protein (12 h. for amino-acids, 24 h. for protein). Free amino-acid concentration in plasma is higher when free amino-acids are fed leading to more rapid catabolism and less use being made of amino-acids for protein synthesis than when protein is fed.

It has been claimed by some people that differences in temperature may affect digestibility. Experiments carried out by CHO and SLINGER at 2 different temperatures (9° C and 18° C) showed that the digestibility varied slightly. At low temperatures, all metabolic processes are reduced in unison so that reduced environmental temperature has little effect on the measured value of protein digestibility.

Processing of the raw material can damage the proteins. This will affect the availability of some of the essential amino-acids. The problem arising is that although amino-acids are chemically measurable in the food protein they are not biologically available.

Figure 1 - PROTEIN REQUIREMENTS OF CHANNEL CATFISH OF DIFFERENT SIZE

DIETARY PROTEIN	DIGESTIBLE ENERGY	INITIAL WEIGHT	FINAL WEIGHT	INITIAL WEIGHT	FINAL WEIGHT
g/Kg	MJ/Kg	g	g	g	g
2 50	9.7	14	97	114	526
3 50	10.7	14	126	114	497

Page & Andrews (1973) J; Nutr. 103 1339

Figure 2 - EFFECT OF DIETARY PROTEIN LEVEL ON GROWTH OF RAINBOW TROUT AT DIFFERENT SALINITIES

% protein in diet	% increases initial weight	
	10ppt	20 ppt
30	127	111.5
35	165.3	136.1
40	206.7	187.9
45	187.1	221.9
50	204.0	213.8
55	195.4	220.8

data of ZIEIOUN et al. (1973)

Figure 3 - AMINOACID REQUIREMENTS OF FISH (various authors) g/Kg dry diet

	Arg.	Lysine	Histidine	Trypt.
Chinook salmon	24	20	7	2
Eel	17	20	8	4
Carp	16	22	8	3
Rainbow trout	18-27	28.7	-	2.5
Channel catfish	10.3-17	15	3.7	1.2
	Threonine	Leucine	Isoleucine	Valina
Chinook Salmon	9	16	9	13
Eel	15	20	15	15
Carp	15	13	9	14
Rainbow trout	-	-	-	-
Channel catfish	5.3	8.4	6.2	7.1
	Methionine	Met + Cyst	Phenilal.	Phe + Tyr
Chinook salmon	16	6 + 10	20	17 + 4
Eel	12	9 + 10	22	12 + 20
Carp	12	8 + 20	25	13 + 10
Rainbow trout	10	5 + 20	-	-
Channel- catfish	5.6	-	4.7	-

Figure 4 - REQUIREMENTS OF TROUT AND CATFISH FOR CERTAIN AMINOACIDS

	Percent diet		mg/100g B. W./day	
	Trout	Catfish	Trout	Catfish
Tryptophan	0.24	0.12	4.7	3.6
Methionine	1.00	0.56	20	20
Lysine	2.18	1.50	43	45
Arginine	1.80	1.03	28	31

	Percent diet		g/KJ.metab.energy	
	Trout	Catfish(1)	Trout	Catfish(1)
Tryptophan	0.24	0.12	0.14	0.11
Methionine	1.00	0.56	0.50	0.57
Lysine	2.18	1.50	1.3	1.1

(1) Wilson et al. (1978)

Figure 5 - RELATIVE PROPORTIONS (%) OF ESSENTIAL AMINOACIDS IN FISH MUSCLE AND RELATIVE PROPORTIONS REQUIRED BY 4 SPECIES OF FISH

	Muscle	C. salmon	C. catfish	J. eel	C. carp
Lys.	16.9	14.6	20.1	13.5	16.1
Hist.	5.8	5.1	4.9	5.4	5.8
Arg.	11.2	17.5	13.8	11.5	11.7
Tryp.	2.3	1.5	0.9	2.7	2.2
Thre.	8.9	6.6	7.1	10.1	10.9
Val.	9.3	9.5	9.5	10.1	10.2
Mer.	5.3				
		11.7	7.5	8.1	8.8
Cys/2	2.3				

Figure 6 - GROWTH OF FISH GIVEN DIETS CONTAINING EITHER PROTEIN OR A MIXTURE OF FREE AMINOACIDS (F.A.A.) AND PROTEIN

	% Increase in initial weight	
	Channel catfish	Trout
24 % whole EGG Protein	319	-
5.7 % Protein + 22.1 % F.A.A	189	-
50 % Casein		312
25 % Casein + 25 % F.A.A.		204

Figure 7 - UTILIZATION OF ARGININE, FREE OR PROTEIN BOUND BY CATFISH

Casein (%)	Gelatin (%)	ARG HCl (%)	Tot Arg. (%)
35	0	0	1.2
35	0	0.58	1.7
25	10	0	1.7
35	6.5	0	1.7

(ANDREWS et al., 1977)

Figure 8 - RESPONSE OF CATFISH TO DIETS SUPPLEMENTED WITH LYSINE

Diet	Available lysine	Initial weight	Weight gain
	%	g.	%
Basal	0.88	201	81.1
Basal + 0.33 Lys.	1.22	201	170.7
Basal + 0.65 Lys.	1.54	199	200.9
Basal + 0.68 % Lys	0.88	196	73.6

(ROBINSON et al, 1980)

LIPIDS AND FATTY ACIDS IN FISH NUTRITION

C. LEGER

PLAN

1. Introduction : Nutrition and lipids
2. Characteristics of lipids
3. Characteristics of fish lipids
4. Nutritional implications
   - Lipids origins and progression in fish
   - Consequences on nutritional requirements.

1. INTRODUCTION

Nutrition is the study of interactions between food and animals; therefore to study correctly nutrition, it is necessary to have a good knowledge of both the food and the animal, in this case, fish. It is necessary to know the metabolism of fish and, speaking about lipids, the metabolism of lipids.

Not alone is metabolism involved, but also the cell structures of the animals.

Lipids are not only a source of energy (fuel) but also important elements of cell structure and, sometimes, they can become essential as they are not completely synthetized by the organism.

2. CHARACTERISTICS OF LIPIDS

Lipids are schematically shown in figure 1.

Triglycerides are formed by a molecule of glycerol and by 3 fatty acids; their position on the molecule of glycerol is of importance and must not be neglected. Triglycerides are the primary energy deposits of animals.

Phospholipids are also formed by a molecule of glycerol, by 2 fatty acids in position 1 and 2 and a phosphate having 4 possible bases: Choline, Serine, Ethanolamine and Inositol.

Sphyngophospholipids, glycophospholipids and waxes also exist. The latter are a very important source of nutrients for certain marine fish because they are present in copepods. We will see later on that waxes are molecules with the same functions as triglycerides. They are also present in mullet eggs, while absent in the adult.

Triglycerides are forms of energy storage, consequently they are present in adipose tissue, when it exists and, in the case of fish, this tissue is not very frequently found. Lipids are then stored in the muscles and the liver.

Phospholipids are the main lipid constituents of cellular membranes and their functions are very important.

Figure 2 shows the de novo (bio) synthesis of fatty acids and the origin of CoA acetyl. The end product is palmitic acid which is a saturated fatty acid with 16 carbon atoms, which can be elongated into stearic acid with 18 atoms of carbon.

The animal and fish synthetize both palmitic and stearic acid. The latter can be converted into oleic acid (figure 3).

Oleic acid has 18 atoms of carbon and one double bound in position 9 (n-9, the 9th carbon from the methyl end).

This bioconversion (desaturation) is possible in all animals.

Linoleic acid is a fatty acid with 18 atoms of carbon and 2 double bounds in position n-9 and n-6; but this form is not synthetized by animals. Generally speaking, for animals, it is impossible to insert double bounds in position n-6 and n-3. This impossibility makes these fatty acids essential.

Animals have 3 series of polyunsaturated fatty acids (P.U.F.A): the n-9 series (oleic series), the n-6 series (linoleic series), and the n-3 (linolenic series); only the oleic series is synthetizable by animals (figure 4).

There are two sorts of enzymes of bioconversion: elongases and desaturases (figure 5). Desaturases prefer the more unsaturated forms, this notion of competition is very important. Consequently 9, 6, and 5 desaturases may be regulated physiologically by the food, in relation to the type of lipids found present in the latter. For example, this means that if we have a food with both n-3 and n-6 fatty acids, the 6 desaturase action is decreased along with the whole de novo biosynthesis which is also repressed when greats amounts of P.U.F.A are found present in the food (figure 6).

Glycerol may be derived through the degradation of glucose, or the hydrolysis of triglycerides. Triglycerides are hydrolized in monoglycerides which can synthetize other triglycerides or phospholipids.

The bases which are present in phospholipids may be derived from other phospholipids or may be synthetized from glycine (figure 7). A second way for the Choline synthesis requires the presence of both ethanolamine and methionine which are furnished by food alone.

Figure 8 shows the role of lipids.

Fatty acids are important sources of energy and they can also have a structural role in phospholipids.

Phospholipids have an important role in membrane structure.

P.U.F.A. are also precursors of hormone like substances. This must be taken into account especially during the reproduction period of fish (figure 9).

3. CHARACTERISTICS OF FISH LIPIDS

3.1. Lipid body partition

Figure 10 gives a classification of fish depending on their lipid content. It can be remarked that there is a reverse relation between the lipid content in the liver and that in the muscle. When there is high lipid content in the muscle that of the liver is low (figure 11).

Figure 12 shows some characteristics of the fatty acid composition of fish. The content of n-3 fatty acid is very high in all fish. There is a slight difference between marine and freshwater fish, in n-6 fatty acid (higher in the latter). Exceptionnaly high contents of n-6 fatty acids exist in Tilapia/oreochromis.

There exists an important correlation between the percentages of total lipid content and triglycerides.

In figure 13, the graphic shows that when the total lipid content rises, the phospholipid content remains at a low level while the triglyceride content increases. In other words, high contents in total lipids are mainly represented by triglycerides; phospholipids which are structural elements remain quite at the same level. Thus, a fatty fish will mainly contain triglycerides.

Between marine fish, there are also some differences in the fatty acid composition. Some fish, such as anchovy, sardine, etc... are very rich in n-3 fatty acids while others (such as herrings, etc..) have lower content in n-3 fatty acids and increased amount of 20 : 1 and 22 : 1 (figure 14); the reason for this is still unknown.

Mugil cephalus (mullet) presents the peculiarity of having n-8 fatty acids (figure 15), perhaps the origine comes from food, but it has not been clearly defined as yet.

Natural food plays an important role in fatty acid composition; an importnat difference exists in capelan in Summer and Winter (figure 16). This does not apply exactly for other fish (figures 17, 18, 19). In fact, there are differences in eicosapentaenoic and docosahexaenoic acids but no relevant differences in the total n-3 fatty acids are remarked. In the same way, it is natural that fatty acid composition also depends on the geographic origin (figure 18)

There also exist differences in the fatty acid composition of phospholipids and triglycerides. Phospholipids are more unsaturated than triglycerides (figure 20). Thus as fish oils are principally sources of triglycerides, they are relatively less unsaturated than fish meals.

3.2. Lipid structure and metabolism

Triglycerides

The structure of triglycerides is the partition of fatty acids between the 3 positions of glycero-alcohol functions. In general, mammals have triglycerides containing saturated fatty acids in position 1.3 and unsaturated fatty acids in position 2 of glycerol. The triglyceride structure is similar in trout. It can be obtained even if the food triglycerides have the inverse structure. Accordingly, there is a complete hydrolysis and re-synthesis of triglycerides.

It is very important to stress that fish do not conserve the fatty acid in position 2 of triglycerides. This is in complete contrast to all that has been assumed up to present. In terms of metabolism; there are two possible pathways for the passage of triglycerides : the monoglyceride pathway and the L glycerophosphate pathway. Fish do not conserve the fatty acid in position 2 and this means that the L glycerophosphate pathway is more important in fish enterocyte.

Wax esters (mainly present in the eggs of mullet) present :

partition between saturated and unsaturated chains which are very similar in acid and alcohol moieties and large amounts of odd chains.

Alkyl diacylglycerol and 1 alkenil 2, 3 DAG

- C – R_1	- O - CH = R_2
- O - CO - R'	- O – CO - R'
- O - CO - R''	- C – CO - R''

They are present in very large amounts in the liver and the muscles of Squalus acanthias and they are minor components of the liver and muscle in fish, respectively,

Phospholipids, as in other animals, teh fatty acid composition depends on: - Phospholipid class (ex. phosphatidlethanolamine is more unsaturated than phosphatidcholine)

- Type of membrane
- Tissue
- Species
- Food (This is very important. The influence of food on the composition of triglycerides is well known, but food can also have an influence on the fatty acid composition of phospholipids, thus consequently, on the membrane structure and the physiological activities of the membrane. The unsaturation rate of phospholipids has an effect on the fluidity of the membrane and consequently on the biological activity of the membrane proteins).
- Environmental conditions, in particular, temperature and salinity. Low temperatures increase the degree of unsaturation (with n-3 and/or n-6 which increase). High salinity increase the rate of 22 : 6 n-3 (figures 21, 22).

But when very specialized membranes are observed, no particular differences between animals exist (ex. rod cell of the retine). In the case of the brush border membrane of enterocyte, very important differences exist. In very specialized membranes differences or no differences in composition could exist between species (figures 23, 24).

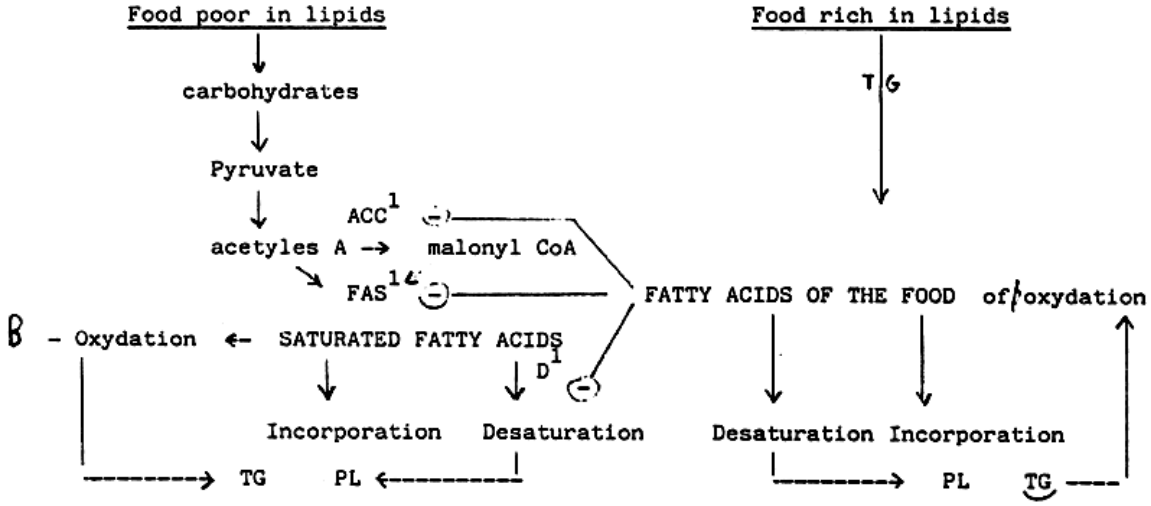
4. NUTRITIONAL IMPLICATION

An essential element is an element which is physiologically indispensable and which is not completely synthetized (de novo synthesis) by the organism.

WHERE ARE THE FATTY ACIDS DERIVED FROM WHICH

WHAT FATTY ACIDS

ARE INCORPORATED IN THE CELL ?



Principal neosynthetized fatty acids which are incorporated : 16 :0, 18 :0, 18 : 1 n-9	Principal fatty acids of the food which are incorporated : 16 : 0, 18 : 0, 18 : 1 n-9, 18 : 2 n-6, 18 : 3 n-3, 20 : 4 n-6, 20 : 5 n-3, 22 : 6 n-3.

1. ACC = Acetyl CoA carboxylase
   FAS = Fatty Acid Synthase
   D = Δ 9 desaturase

Figures 25, 26, 27 show schematically the progression of ingested lipids and fatty acids from food to tissues.

Triglycerides enter into special structures, called chylomicrons, which are lipoproteic structures, then a very complex process for the transport of fatty acids in the blood stream starts.

Figure 28 shows the pathway of phospholipids synthesis. The most important stage is that of phosphatidic acid, which is derived from glucose and L glycerophosphate. There are some differences for the synthesis of the different phospholipids. It must be underlined that food influences the fatty acid composition of both phospholipids and triglycerides.

Figures 29, 30, 31 show some examples of this influence and stress the concept that the bioconversion of fatty acids which are incorporated into the phospholipids is very important.

Lipids are very important for both energetic and structural purposes. But :

1. Lipids -A concentrated form of energy supply- could be replaced by other forms of energy (although not recommended).
2. Some lipid components of the cell structures can be <u>de novo</u> synthetized from non lipid components.
3. Some other lipid components of the cell structures cannot be : THESE COMPONENTS ARE ESSENTIAL.

Some examples of lipid components belonging to point 2 are: glycerol, serine, ethanolamine, saturated fatty acids, monounsaturated fatty acids. Examples of point 3 are : polyunsaturated fatty acids from the n-3 or n-6 series (They are also essential for the synthesis of some hormone like substances), probably inositol and choline.

Figure 32 shows the Essential Fatty Acid (E.F.A.) requirements in trout.

The presence in the food of a mixture of P.U.F.A has a favourable effect. All fish cannot be compared with trout (figure 33). The main difference is for the unfavourable effect of linoleic acid. For some marine fish, such as the red sea-bream and turbot, the presence of the precursor of the n-3 series is not efficient because the bioconversion in these fish is impossible, or possible at very low rates The efficiency of the bioconversion is around 1 - 5 %.

A particular case is seen with Tilapia, which need only n-6 fatty acids. This is very important from an economic view point, because the vegetable oils are cheaper and more available than those derived from fish which are necessary for the other fish.

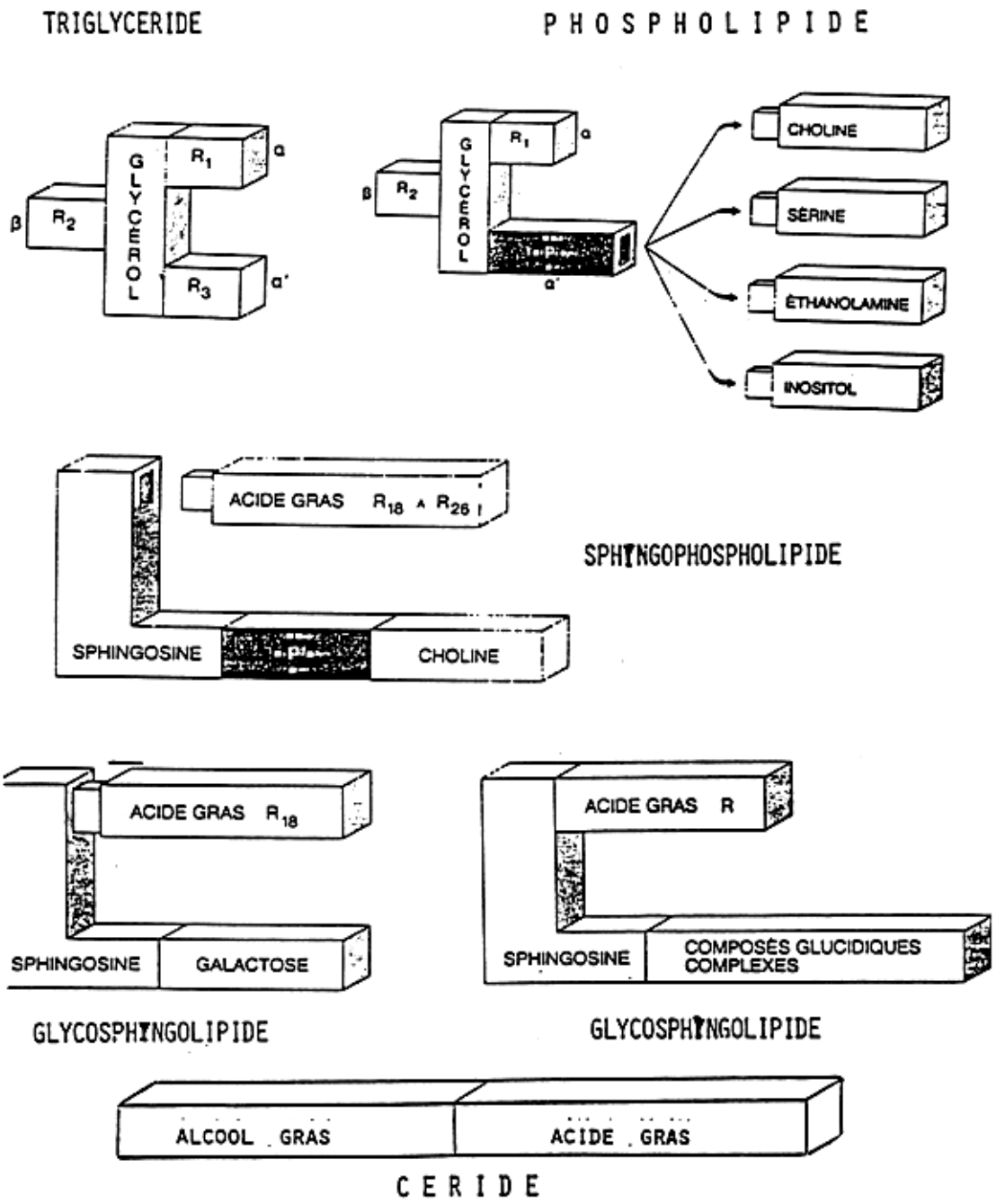
Figure 34 shows some requirements of "semi-essential" components. It is possible that the efficiency of the conversion of serine and ethanolamine in choline is very low in fish and crustaceans.

A good diet formulation concerning the required E.F.A. supply by food is very important particularly in :

- vitellogentic females
- at early stages of fish development.

A deficient diet produces important disorders in embryo (figures 35, 36) and can result in a decreased hatching ratio and larva abnormalities.

FIGURE 1


TRIGLYCERIDE
PHOSPHOLIPIDE
GLYCEROL
R1
R2
R3
α
α'
β
CHOLINE
SÉRINE
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SPHINGOSINE
COMPOSÉS GLUCIDIQUES COMPLEXES
GLYCOSPHINGOLIPIDE
ALCOOL GRAS
ACIDE GRAS
CERIDE

FIGURE 2

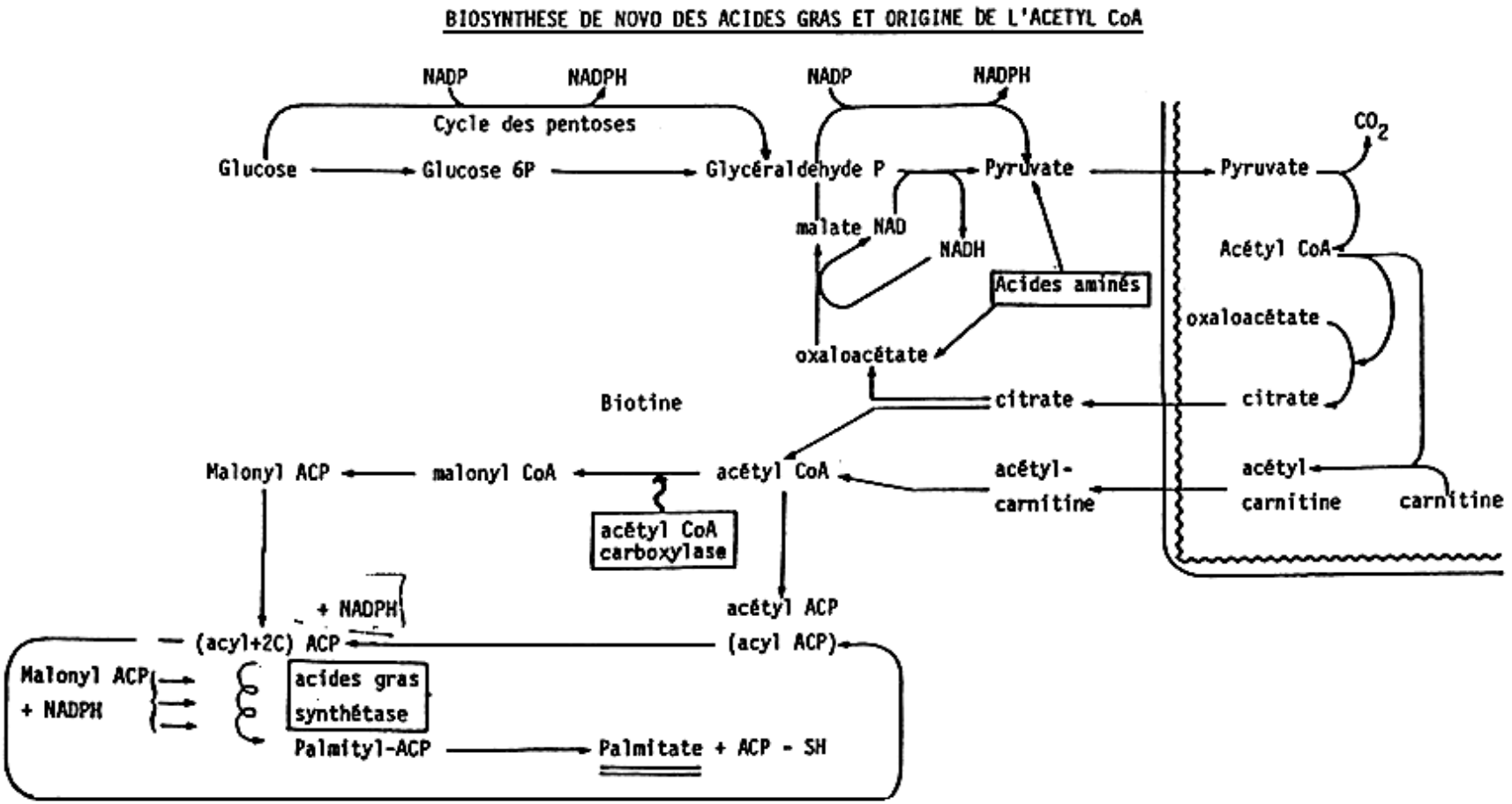

BIOSYNTHESE DE NOVO DES ACIDES GRAS ET ORIGINE DE L'ACETYL CoA
NADP
NADPH
Cycle des pentoses
Glucose
Glucose 6P
Glycéraldehyde P
NADP
NADPH
Pyruvate
malate NAD
NADH
Acides aminés
oxaloacétate
Biotine
citrate
Malonyl ACP
malonyl CoA
acétyl CoA
acétyl CoA carboxylase
acétyl-carnitine
+ NADPH
acétyl ACP
(acyl+2C) ACP
(acyl ACP)
Malonyl ACP + NADPH
acides gras synthétase
Palmityl-ACP
Palmitate + ACP - SH
CO_2
Pyruvate
Acétyl CoA
oxaloacétate
citrate
acétyl carnitine
carnitine

FIGURE 3

PRINCIPAL FATTY ACIDS OF ANIMAL ORIGIN

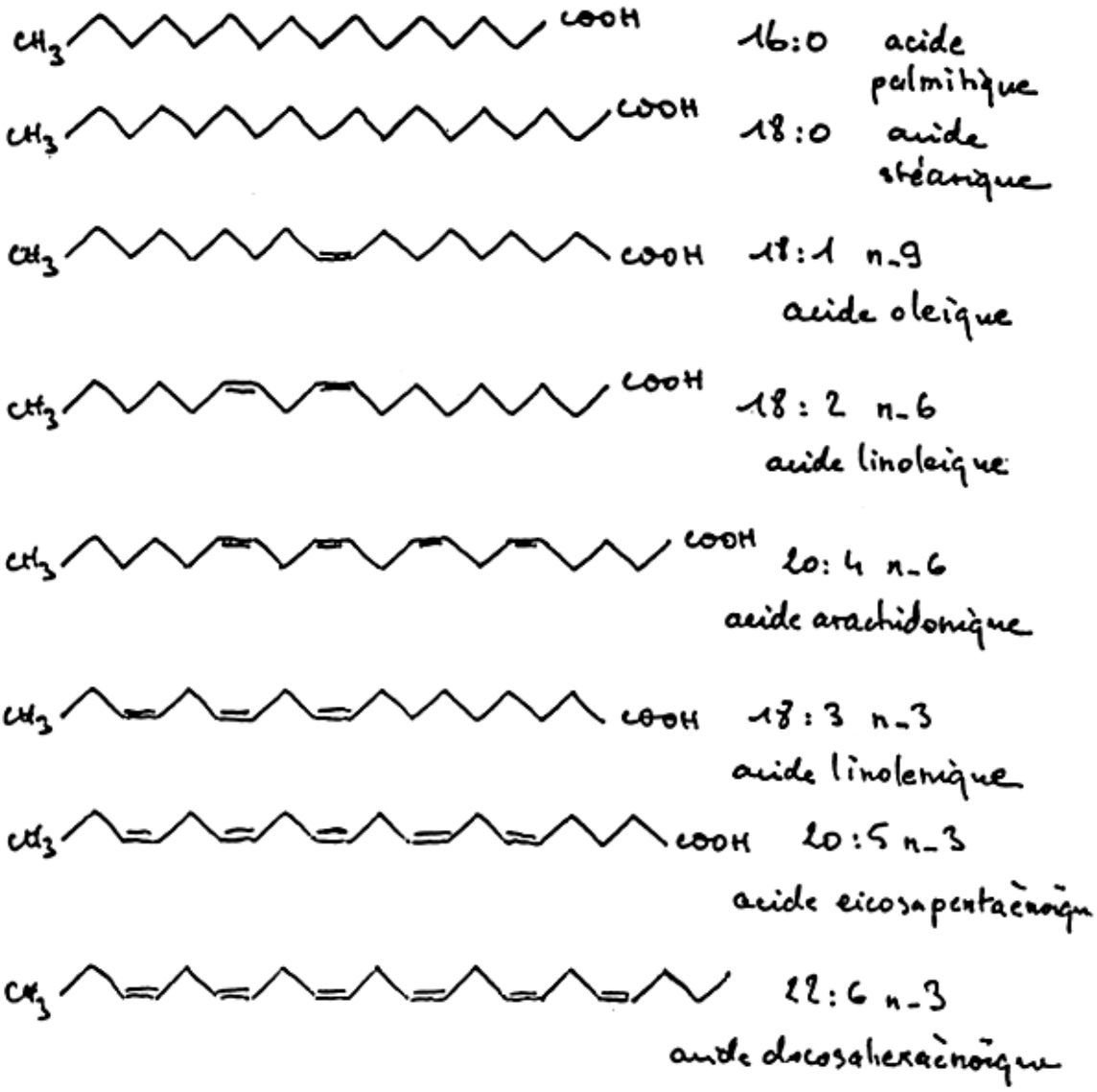


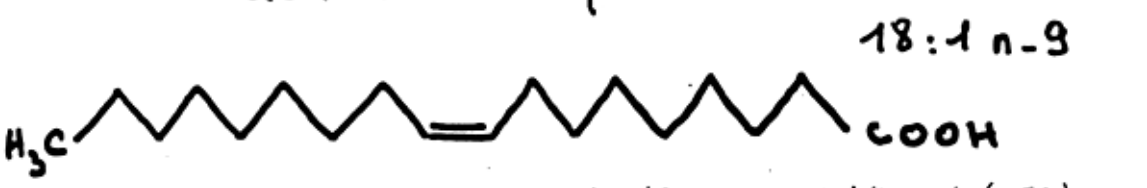
FIGURE 4

Polyunsaturated fatty acids (P.U.F.A.)

- Les acides gras (poly)insaturés (AGPI)

De-novo synthesis is possible : family of oleic acid

- synthèse de novo possible : famille de l'acide oleique



De novo synthesis is impossible : essential fatty acids (E.F.A.) -

- synthèse de novo impossible : acides gras essentiels (a.g.e.) des familles de l'acide linoleique et de l'acide linolénique

family of linoleic and linolenic acid

acide linoléique 18:2 n-6
linoleic acid

acide linolénique 18:3 n-3
linolenic acid

FIGURE 5

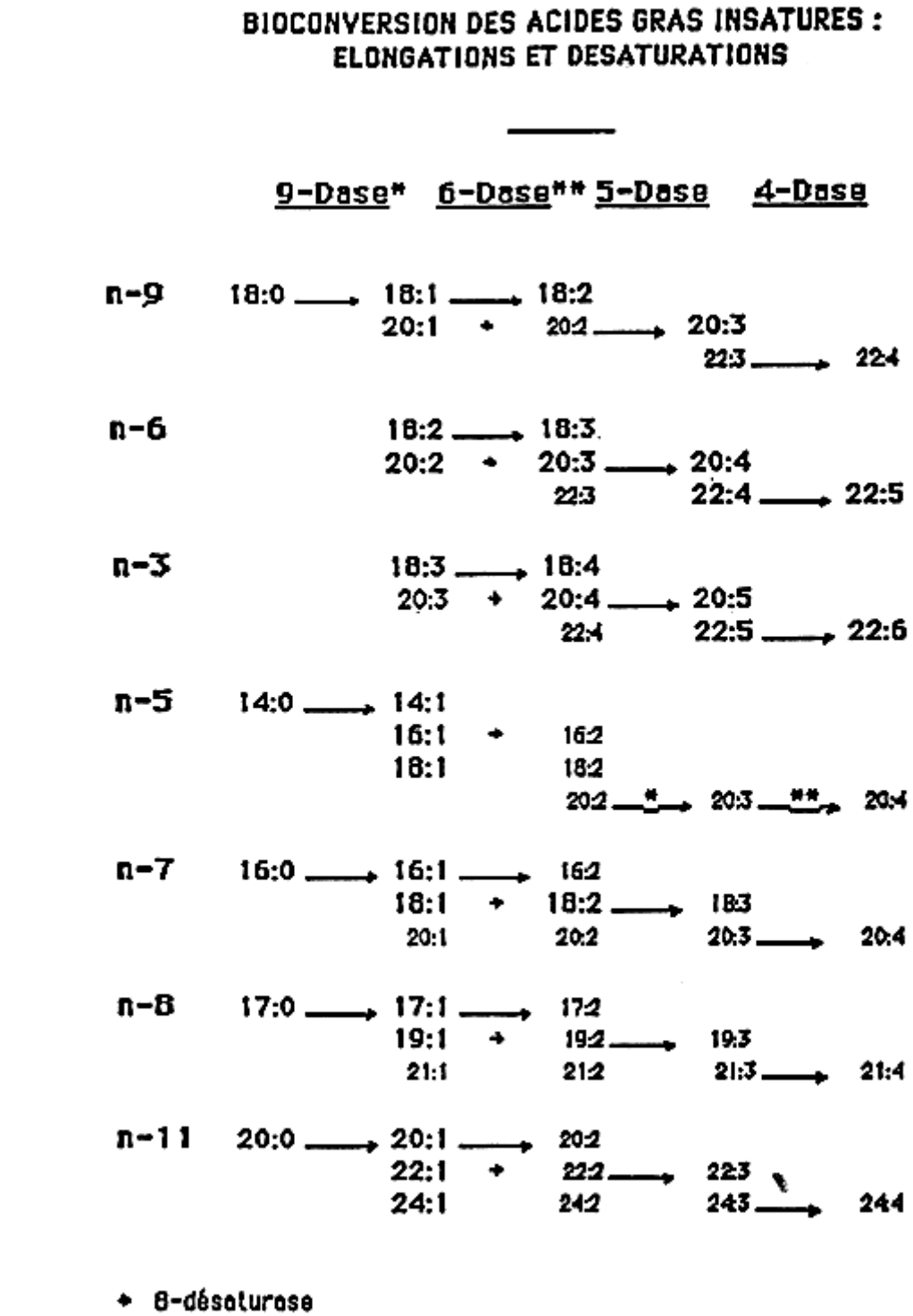
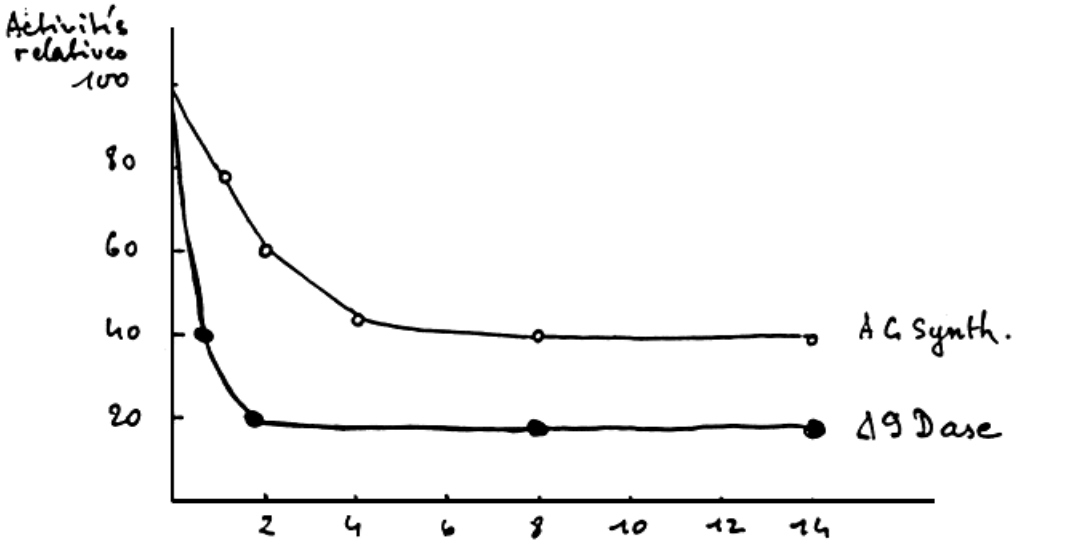

BIOCONVERSION DES ACIDES GRAS INSATURES :
ELONGATIONS ET DESATURATIONS
9-Dase*
6-Dase**
5-Dase
4-Dase
n-9
18:0
18:1
18:2
20:1
20:2
20:3
22:3
22:4
n-6
18:2
18:3
20:2
20:3
20:4
22:3
22:4
22:5
n-3
18:3
18:4
20:3
20:4
20:5
22:4
22:5
22:6
n-5
14:0
14:1
16:1
16:2
18:1
18:2
20:2
*
20:3
**
20:4
n-7
16:0
16:1
16:2
18:1
18:2
18:3
20:1
20:2
20:3
20:4
n-8
17:0
17:1
17:2
19:1
19:2
19:3
21:1
21:2
21:3
21:4
n-11
20:0
20:1
20:2
22:1
22:2
22:3
24:1
24:2
24:3
24:4
* 8-désaturase
9-désaturase, 6-désaturase ou 3-désaturase
déplacement de haut en bas dans chaque famille : élongations

FIGURE 6 –

-REGULATIONS DES Δ9-, Δ6-, Δ5 Dases

ENZYMES	DEPRESSEURS	ACTIVATEURS
Δ 9 Dase	A.G.E. Lipides alim^{ts}	Rég. riche en glucides et pauvre en lipides carence en A.G.E.
Δ 6 Dase	A.G.E. glucose (diabète) carence protique âge	Carence en A.G.E protéines insuline
Δ 5 Dase	carence en A.G.E. glucose	A.G.E.



Jeffcoat, James, FEBS Lett. 85, 114 : 1978

FIGURE 7 –

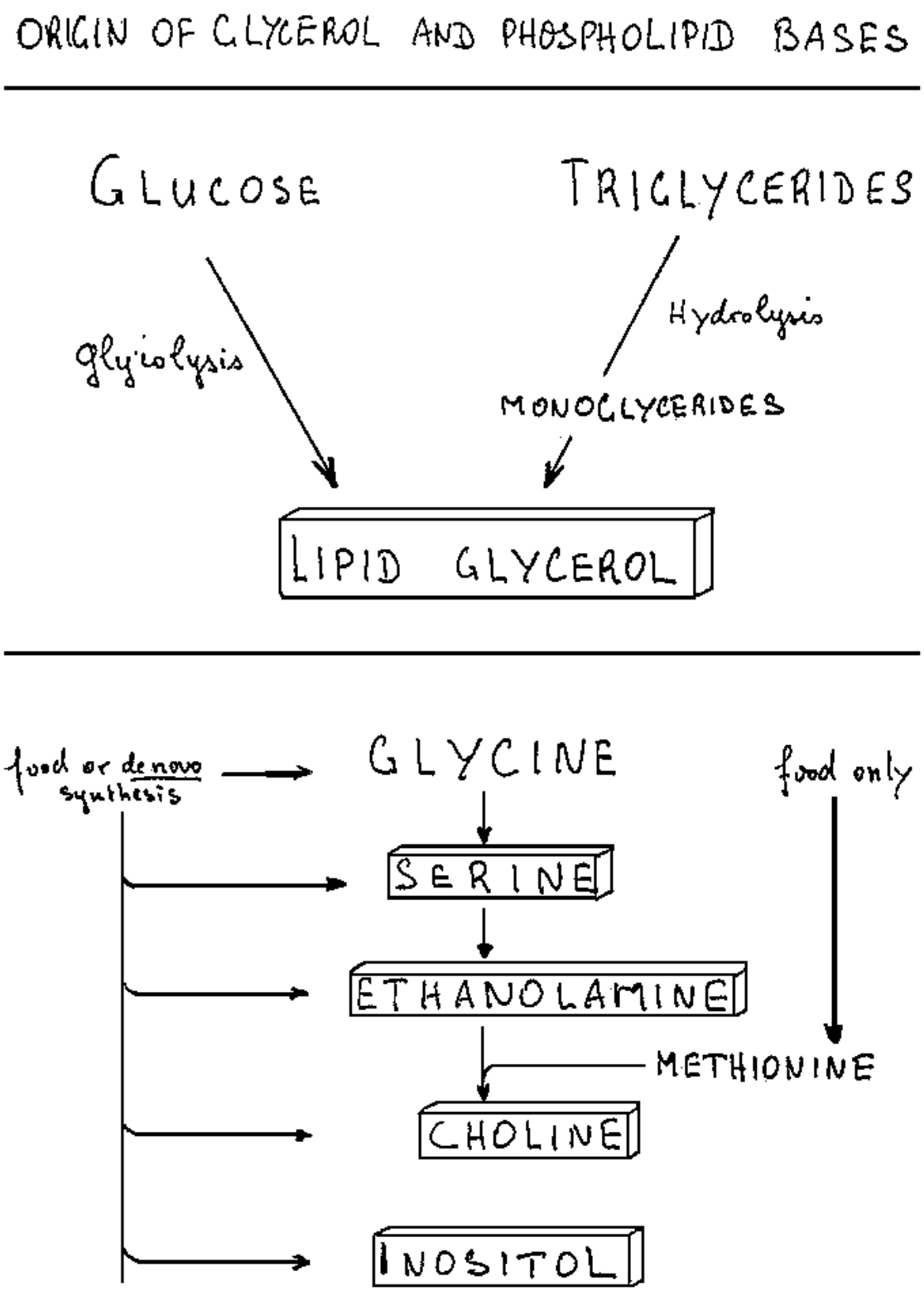

ORIGIN OF GLYCEROL AND PHOSPHOLIPID BASES
GLUCOSE
TRIGLYCERIDES
glycolysis
Hydrolysis
MONOGLYCERIDES
LIPID GLYCEROL
food or de novo synthesis
GLYCINE
food only
SERINE
ETHANOLAMINE
METHIONINE
CHOLINE
INOSITOL

FIGURE 8

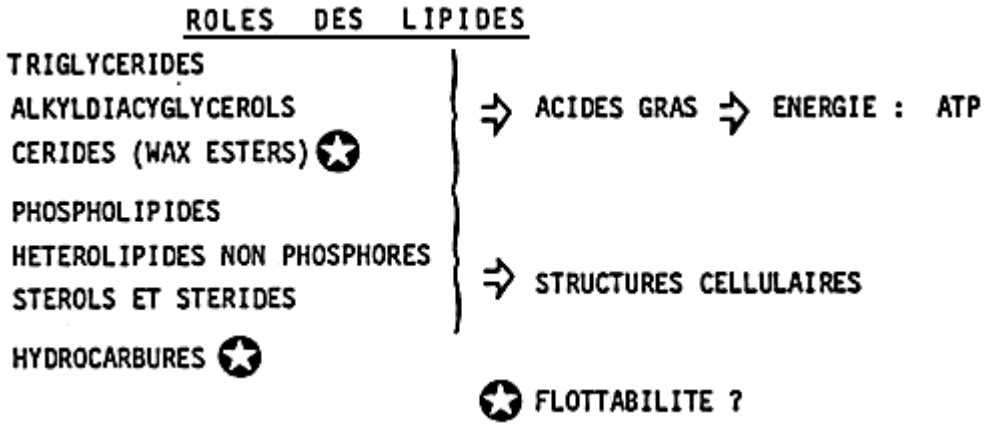

ROLES DES LIPIDES
TRIGLYCERIDES
ALKYLDIACYGLYCEROLS
CERIDES (WAX ESTERS)
PHOSPHOLIPIDES
HETEROLIPIDES NON PHOSPHORES
STEROLS ET STERIDES
HYDROCARBURES
ACIDES GRAS
ENERGIE : ATP
STRUCTURES CELLULAIRES
FLOTTABILITE ?

FIGURE 9 : ROLE DES ACIDES GRAS

	De 16 : 0	à 22 : 6 n - 3
COMBUSTIBLE	0,50 à 0,52	Moles ATP/G AG
	(130 à 169 utilisant :	Moles ATP/ mole AG)
	88 à 90 moles O_2/ G. AG	
	(23 à 29 moles O_2/ mole AG)	
STRUCTURAL	Tous, mais AGE non synthetisés	
PRECURSEUR	De dérivés oxygénés (PG, TX, PGI)	
	(hydroxy et hyperoxy-lineaires) Lemotriènes)	
	à activité quasi hormonale	
	Les AGPI (AGE + AGPI n - 9 uniquement	

FIGURE 10 : POISSONS CLASSES D'APRES LEUR RICHESSE EN LIPIDES TOTAUX (TG + PL)

CLASSE	% de lipides dans le muscle	POISSONS
I	5 %	Morue, Colin, Mulet, Merlu, Bar Lieu jaune, Eglefin, Flétan, Daurade
II	5 - 15 %	Maquereau, Hareng, Sardine, Menhaden, Capelan, Chinchard, Pilchard, Anchois
III	15 %	Les poissons de la Classe II passent quelquefois en Classe III

Tableau inspiré de STANSBY (1969)

I	Cod, Saithe (for Cod steaks), Hake, Mullet, Sea-bass, Pollack, Haddock, Halibut, Sea-bream
II	Mackerel, Herring, Sardine, Menhaden, Capelin, Horse mackerel, Pilchard, Anchovy
III	Sometimes class II fish become class III fish.

FIGURE 11

RELATION INVERSE ENTRE TENEURS EN LIPIDES DU MUSCLE ET DU FOIE

Poissons	Lipides du muscle (%)	Lipides du foie (%)
Morue	0,4[a]	50 - 75(b)
Eglefin	0,3[a]	50 - 75(b)
Bar[c]	1 - 5	7 - 20
Hareng[d]	11	2
Maquereau[d]	13	8
Saumon (Atlantique)[d]	15	7

[a] D'après STANSBY (1953)
[b] D'après BRODY (1965)
[c] D'après STEFAN et METAILLER
[d] D'après BRAEKKAN (1959)

FIGURE 12

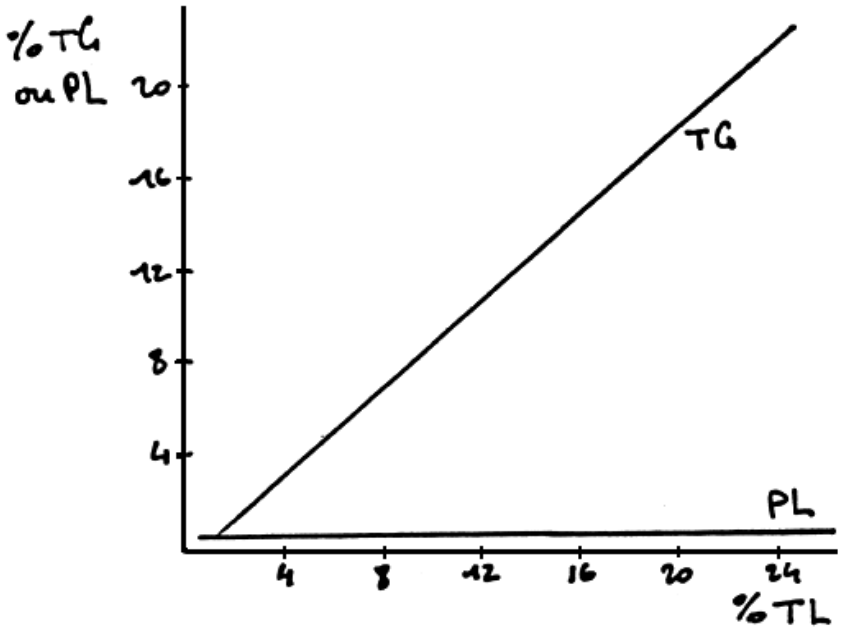
COMPOSITION CARACTERISTIQUE DES POISSONS *

Acides gras	Poisson de mer	Poisson d'eau douca
16:0	10-30	10-20
16:1	2-11	7-11
18:0	2-6	3-4
18:1	12-28	18-28
18:2	1-3	4-6
18:3	0.5-1.2	3-5
19:0	0.5-2	-
20:1	1-10	1-3
20:4	0.5-4	2-4
20:5	5-14	5-7
22:1	1.5-3	0.5-3
22:5	0.6-3	2,5-4
22:6	8-20	8-20
∑ ω5	1-6	5-10
∑ ω3	15-35	18-35

* D'après STANSBY (1969)

FIGURE 13

CORRELATION ENTRE % LT ET LES % DE PL ET DE TG DANS DIFFERENTS TISSUS ET CHEZ DIFFERENTES ESPECES DE POISSONS (d'après OPSTVEDT)



CORRELATION BETWEEN PERCENTAGES OF TOTAL LIPIDS, ON THE ONE HAND, AND PHOSPHOLIPIDS AND TRIGLYCERIDES, ON THE OTHER, IN DIFFERENT TISSUES AND SPECIES (from OPSTVEDT)

FIGURE 14

FAMILLES DE POISSONS CLASSEES D'APRES LEUR RICHESSES RELATIVES EN 20:1 ET 22:1, D'UNE PART, ET EN A.C. n-3 D'AUTRE PART

Familles	n-3	20:1	22:1
Anchois, Pilchard Sardine, Chinchard Menhaden	25-40%	0-4%	0-4%
Hareng (atl.+pac.) Capelan, Maquereau Morue (foie)	20-30%	10-15%	10-15%

FIGURE 15

PRINCIPAUX ACIDES GRAS INHABITUELS CHEZ LE MULET MUGIL CEPHALUS*

Acides gras	5 des A.G.T.	Autres acides gras présents (<15)		
15:0	11,2	15:1ω6	15:2ω6	15:3ω3
		15:1ω8	15:2ω3	
16:3ω4	1,2	16:2ω7		
		16:2ω4		
17:0	1,2	17:2ω8		
		17:4ω2		
17:1ω8	4,6			
17:2ω5	2,5			
17:3ω5	1,2			
18:1ω7	2,1			
19:4ω5	1,7	19:1ω8	19:2ω7	
		19:2ω8	19:2ω5	

D'après SEN et SCHLENK (1964)

FIGURE 16

INFLUENCE DE LA SAISON DE PECHE SUR LA COMPOSITION EN A.G. DE L'HUILE DE CAPELAN

A.G.	ÉTÉ	HIVER
18:1 n-9	12.5	20.2
20:1 n-9	15.0	19.8
22:1 n-11	16.4	16.7
n-6	2.6	1.8
n-3	21.8	13.8

d'après Ackman et al, 1982

FIGURE 17

INFLUENCE DE LA SAISON DE PECHE SUR LA COMPOSITION EN A.G. D'HUILES DE POISSONS*

A.G. :	Janvier	Avril / mai
14:0	8	12
16:0	16	12
16:1	10	12
18:1	10	8
20:1	3	2
22:1	2	1
20:5 n-3	21	31
22:6 n-3	14	6

* mélange Sardine + Pilchard originaire d'Afrique du Sud
Données rapportées par ACKMAN, 1982, in Nutritional Evaluation of Long-Chain Fatty acids in Fish oil. Ed par BARLOW et STANSBY ; Ac. Press.

FIGURE 18

INFLUENCE DE LA LOCALISATION GEOGRAPHIQUE DE LA PECHE SUR LA COMPOSITION EN A.G. D'HUILE DE POISSONS

A.G.	Anchois mexicains	Anchois chilien
14:0	8	10
16:0	20	17
16:1	7	11
18:1	10	7
20:1 n-9	3	5
22:1 n-11	3	3
20:5 n-3	14	19
22:6 n-3	12	4
n-3	32	31

d'après ACKMAN, 1980, Advances in fish science and technology.

FIGURE 19

COMPOSITION EN ACIDES GRAS DU BROCHET*

Acides gras	Testicules		Foie		
	TG	PL	EC	TG	PL
16:0	8	21	2	11	24
16:1	7	9	3	9	2
18:0	1	5	-	4	5
18:1	6	11	3	16	7
18:2ω6	3	3	-	8	3
18:3ω3	2	3	-	4	1
20:4ω6	2	17	-	5	12
20:5ω3	1	14	-	5	6
22:5ω6	-	2	-	1	3
22:5ω3	-	2	-	3	2
22:6ω3	2	13	-	9	32
$P_1\ C_{19}H_{32}O_3$	3	-	-	1	-
$P_2\ C_{20}H_{34}O_3$	6	-	-	3	-
$P_3\ C_{21}H_{36}O_3$	2	-	3	ND	-
$P_4\ C_{21}H_{36}O_3$	15	-	18	6	-
$P_5\ C_{22}H_{38}O_3$	12	-	3	1	-
$P_6\ C_{23}H_{40}O_3$	30	-	69	11	-

*D'après GLASS et al. (1974)

FIGURE 20 : FEATURES OF TRIGLYCERIDES - COMPOSITION EN A. G. n - 6 et n - 3 DES PHOSPHOLIPIDES (PL) ET DES TRIGLYCERIDES (TG) DE DIFFERENTS POISSONS

ESPECES	n - 6		n - 3	
	TG	PL	TG	PL
MENHADEN [a]	2.2	3.9	25	32
CHINCHARD[b]	4.5	5.0	25	50
HARENG[c]	1.4	2.5	12	46
CAPELAN[d]	2.6	3.1	14	44

a = ACKMAN et al, 1976, J.Food Sci. Agric. b = TOYOMIZU et al, 1976. Bull. Japan. Soc. Sci. Fishes. c = ADDISON et al, 1969, J. Fish Res. Bd Canada. d = ACKMAN et al., 1969. J. Fish. Res. Bd. Canada.

COMPOSITION EN A.G. "SIMPLIFIEE" CHEZ LE CAPELAN

A.G	Capelan entier	Farine	Huile
16 : 0	11	16	9
16 : 1 n – 7	8	8	9
18 : 1 n - 9	17	16	17
20 : 1 n - 9	20	10	25
22 : 1 n - 11	15	7	20
n - 6	2	2	1
n - 3	15	32	6

URDAHL et NUGARD, 1970, non publié

FIGURE 21

INFLUENCE DU MILIEU SUR LA COMPOSITION EN A.C. DES PL DE LA BB DE L'ENTEROCYTE CHEZ LA TRUITE

	PC		PE	
	Eau douce	Eau de mer (1 jour)	Eau douce	Eau de mer (1 jour)
16:0	29	31	25	24
18:0	22	12	14	16
18:1 n-9	9	7	7	6
20:4 n-6	5	4	3	4
22:5 n-6	1	-	2	2
22:5 n-3	-	1	1	-
22:6 n-3	11	28	12	14

Ces modifications ne s'accompagnent d'aucun changement de la repartition des classes de PL et Ch/PL = cste

Mais s'accompagnent d'une diminution de $\frac{PL}{Prot}$

La "fluidité" augmente à 1 jour d'acclimatation à l'eau de mer.

FIGURE 22 : SALINITY DEPENDENT FATTY ACID COMPOSITION IN CARP*

		U/S	n - 3/ n - 6
Total PL	10° C	2.2	0.8
	26° C	1.8	1.4
PC	10° C	2.2	1.2
	26° C	1.6	2.8
PE	10° C	1.7	0.75
	26° C	1.4	1.65

* NODTKE E, 1978, Biochim. Biophys. Beta, 529, 280 – 291

FIGURE 23 COMPOSITION EN A.C DES 2 PRINCIPALES CLASSES DE PL DU SEGEMENT EXTERNE DE LA CELLULE EN BATONNET DE LA RETINE (In the enter segment of retina rod cell)

	Homme	Rat	Poisson rouge
PHOSPHATIDYL ETHANOLAMINE			
16 : 0	9.7	5.5	6.4
18 : 0	36.2	28.1	13.4
18 : 1 n - 9	6.2	3.2	8.1
18 : 2 n - 6	0.3	0.6	0.9
18 : 3 n - 3	-	-	-
20 : 4 n - 6	3.5	2.9	2.4
22 : 6 n - 3	34.2	54.8	58.0
PHOSPHATIDYL CHOLINE			
16 : 0	32.5	29.5	29.7
18 : 0	16.8	11.7	14.6
18 : 1 n - 9	14.9	8.3	17.7
18 : 2 n - 6	1.1	0.8	1.2
18 : 3 n - 3	-	-	-
20 : 4 n - 6	4.7	3.4	1.2
22 : 6 n - 3	19.5	38.4	24.4

FRIESLER and ANDERSON, 1983, Progr. Lipid. Res 22, 79 - 131

FIGURE 24 : FATTY ACID COMPOSITION OF TWO MAIN PHOSPHOLIPIDS OF INTESTINAL CELL MEMBRANE
(Brush border membrane of enterocytes)

	Trout	Pig	Guinea pig
PHOSPHATIDYL ETHANOLAMINE			
16 : 0 + 18 : 0	55	36	33
18 : 1 n - 9	1	12	5
18 : 2 n - 6	2	12	12
20 : 4 n - 6	-	9	2
22 : 6 n - 3	31	5	-
PHOSPHATIDYL CHOLINE			
16 : 0 + 18 : 0	55	35	42
18 : 1 n - 9	14	17	7
18 : 2 n - 6	1	18	18
20 : 4 n - 6	-	5	2
22 : 6 n - 3	23	2	1

FIGURE 25

PROGRESSION OF INGESTED LIPIDS AND FATTY ACIDS FROM FOOD TO TISSUES

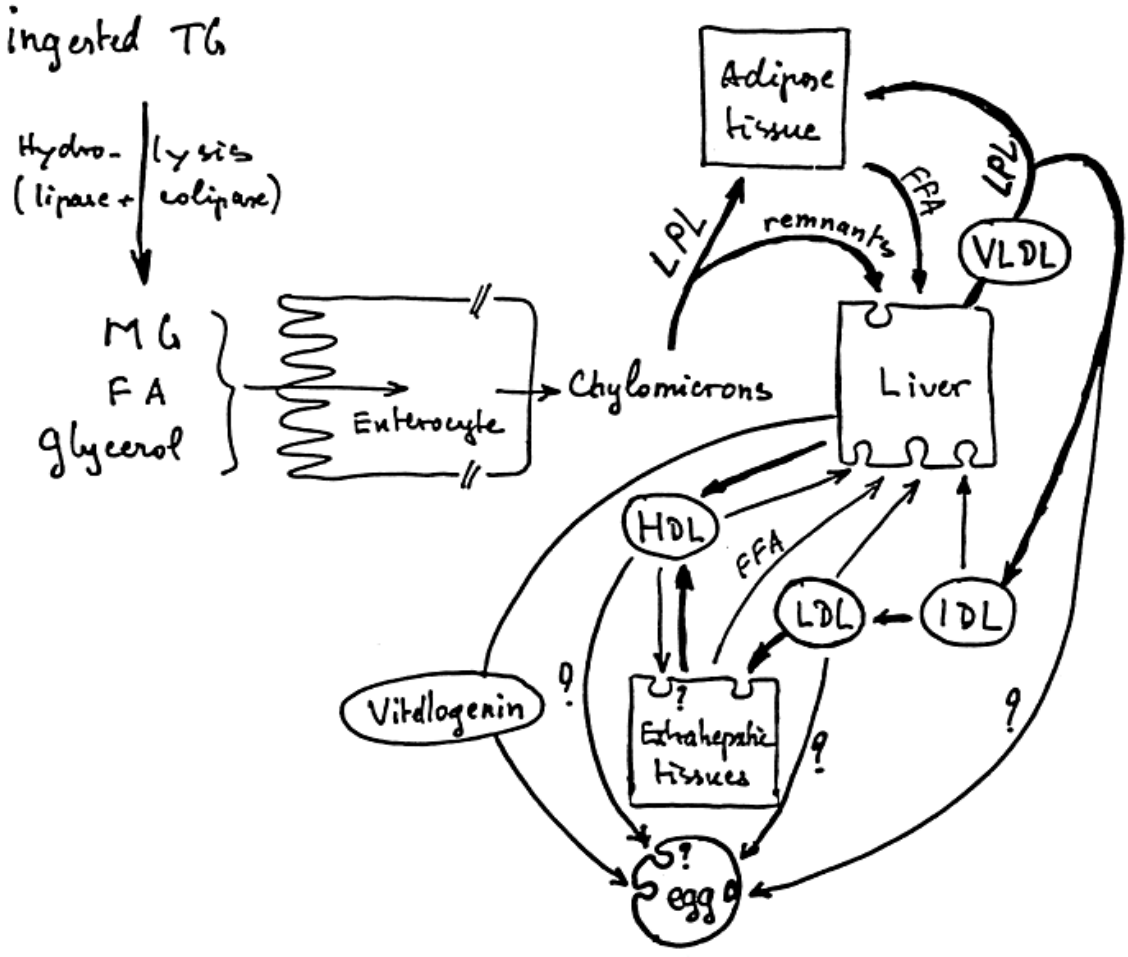


FIGURE 26

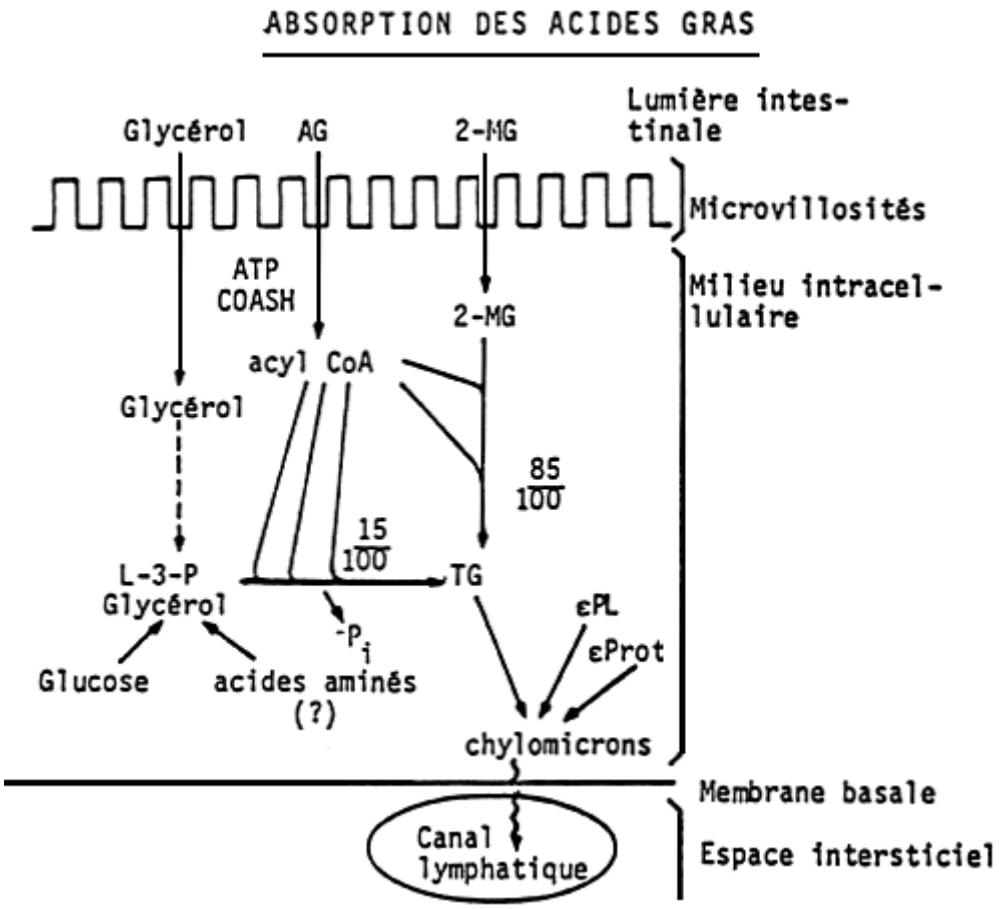

ABSORPTION DES ACIDES GRAS
Glycérol
AG
2-MG
Lumière intestinale
Microvillosités
ATP
COASH
2-MG
Milieu intracellulaire
acyl CoA
Glycérol
85/100
15/100
L-3-P Glycérol
TG
-Pi
εPL
εProt
Glucose
acides aminés (?)
chylomicrons
Membrane basale
Canal lymphatique
Espace intersticiel

FIGURE 27

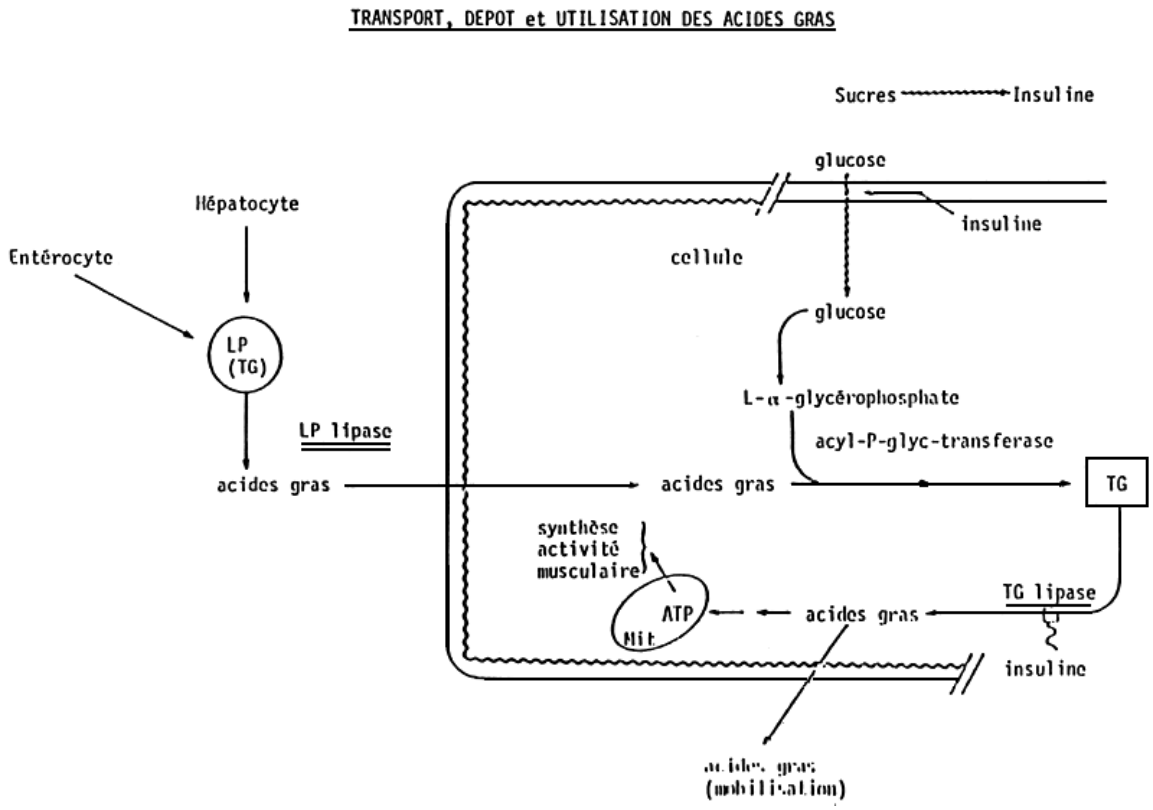

TRANSPORT, DEPOT et UTILISATION DES ACIDES GRAS
Sucres
Insuline
glucose
Hépatocyte
Entérocyte
insuline
cellule
LP
(TG)
glucose
L-α-glycérophosphate
LP lipase
acyl-P-glyc-transferase
acides gras
acides gras
TG
synthèse
activité
musculaire
ATP
Mit
TG lipase
acides gras
insuline
(mobilisation)

FIGURE 28

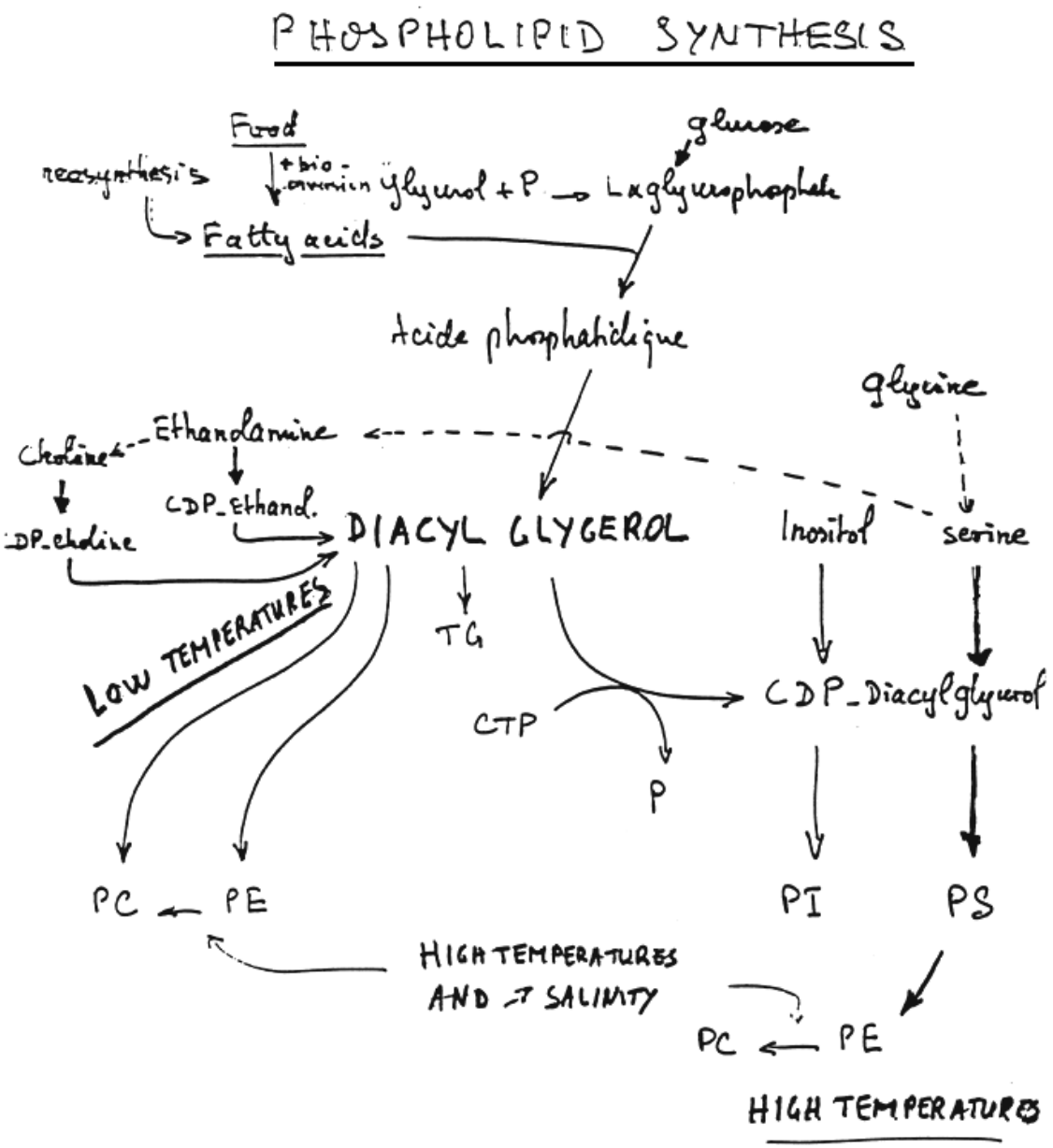

PHOSPHOLIPID SYNTHESIS
Food
glucose
resynthesis
+bio-conversion
glycerol + P
L-α-glycerophosphate
Fatty acids
Acide phosphatidique
glycine
Choline
Ethanolamine
CDP-choline
CDP-Ethanol.
DIACYL GLYCEROL
Inositol
serine
TG
LOW TEMPERATURES
CTP
CDP-Diacylglycerol
P
PC
PE
PI
PS
HIGH TEMPERATURES AND ↑ SALINITY
PC
PE
HIGH TEMPERATURES

FIGURE 29

TRUITE : INFLUENCE DU REGIME SUR LA COMPOSITION EN "ACIDES GRAS DU TISSU ADIPEUX PERIGASTRIQUE

	Aliment "standard"	Tissu adipeux	Aliment "saindoux"	Tissu adipeux
16:0	17	16	24	21
18:0	5	4	12	6
16:1ω7	5	6	3	10
18:1ω7	2	3	-	-
18:1ω9	20	26	52	49
20:1ω9	3	4	2	2
22:1ω11	3	2	-	-
18:2ω6	26	21	6	7
20:2ω6	-	1	-	-
22:4ω6	-	3	-	-
18:3ω3	3	2	1	2
18:4ω3	1	-	-	-
22:5ω3	2	-	-	-
22:6ω3	6	5	-	-

FIGURE 30

BAR : INFLUENCE DE L'ALIMENTATION SUR LES PRINCIPAUX ACIDES GRAS DE L'ANIMAL SAUVAGE OU NOURRI

	Alimentation "sauvage"	Alimentation "rationnelle" % lipides=17,5	
	Tissu adipeux	Aliment	Tissu adipeux
18:1ω9	26	23	26
20:1ω9	1	3	4
18:2ω6	-	38	34
18:3ω3	-	1	2
18:4ω3	-	-	-
20:5ω3	7	3	2
22:5ω3	3	-	-
22:6ω3	9	4	3

FIGURE 31

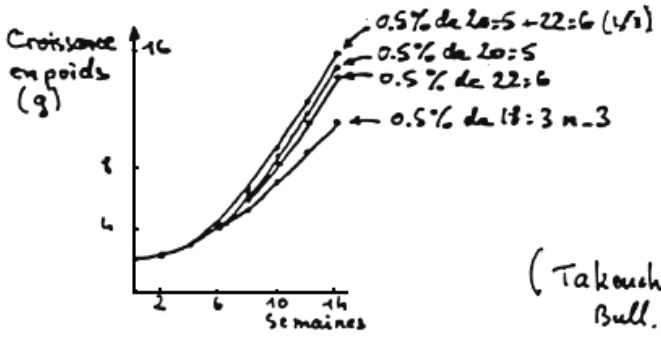
PHYTOPLANCTON →		ZOOPLANCTON →	POISSON (Guppy)
CHAETOCEROS SIMPLEX (aliment pour A. salina)		ARTEMIA SALINA (aliment pour L. reticulatus)	LEBISTES RETICULATUS
14:0	13	5	2
16:0	18	12	23
16:1	48	45	16
18:0	-	2	8
18:1	9	18	18
20:5	-	12	5
22:5	-	-	6
22:6	-	-	17

FIGURE 32

CHEZ LA TRUITE, LES A.G.E. SONT LES AG n-3

AG	Besoins	Effet défavorable	Remarque
18:3 n-3	1 à 2 %	⩾ 3 %	18:3 n-3 ↓
18:2 n-6	0	⩾ 1,5 %	
20:5 n-3	0.5 à 1 %		20:5 n-3 ↓
22:6 n-3	0.5 à 1 %		22:6 n-3
20:5 n-3 + 22:6 n-3	0.5 à 1 %	⩾ 2 %	

IL EXISTE UN EFFET DE COMPLEMENTARITE ENTRE 20:5 n-3 ET 22:6 n-3



SYNENGISTIC EFFECT

(Takeuchi et Watanabe, 1977, Bull. Jap. Soc. Sci. Fish. 43, 947-953)

CLASSEMENT DE L'EFFICACITE ACE

$$18:3\,n\text{-}3 < 20:5\,n\text{-}3 = 22:6\,n\text{-}3 < \left\{\begin{matrix} 20:5\,n\text{-}3 \\ + \\ 22:6\,n\text{-}3 \end{matrix}\right\}$$

FIGURE 33

BESOINS EN A.G.E. COMPARES DE LA TRUITE ARC-EN-CIEL, LE SAUMON COHO ET LE SAUMON CHUM

EFA REQUIREMENTS IN TROUT COMPARED TO SALMON

	Besoins en 18:3n-3	Besoins en 20:5n-3 +22:6n-3	Effet du 18:2n-6
Truite	2%	1%	défavorable si ≥ 25%
S. Coho	2%	>2%	défavorable
S. Chum	1% avec 1% de 18:2n-6	1%	favorable

PRATIQUEMENT....

L'aliment pour S. Coho doit contenir (p. ex.):

< 10% h. de poissons

L'aliment pour S. Chum doit contenir (p. ex):

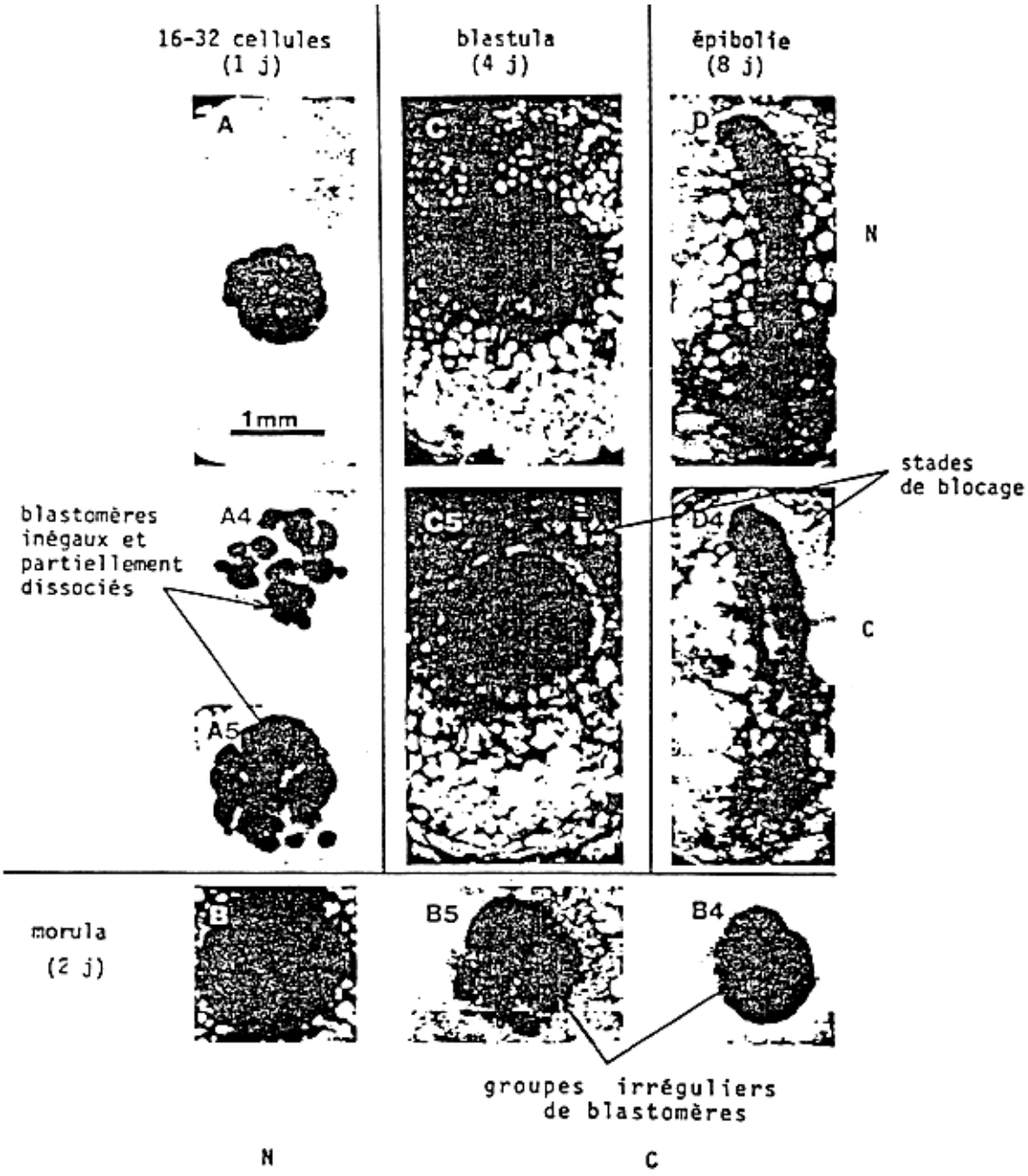
- soit 5% H. de soja ou de colza +5% H. de poissons
- soit 10% H. de soja ou colza
- soit 5 à 10% H. de poissons.

FIGURE 34

REQUIREMENTS IN SOME "SEMI-ESSENTIAL" COMPONENTS

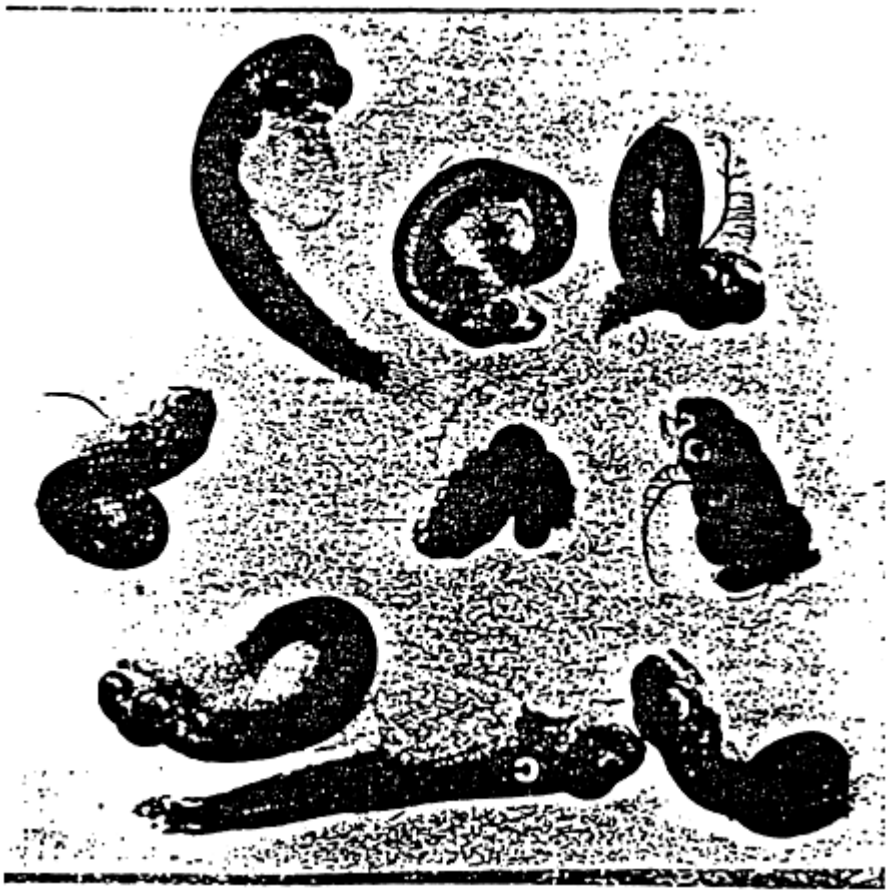
mg./Kg dry diet	Rainbow Trout	Salmon	Carp
Choline	Req.	600-800	1500-2000
Inositol	200-300	300-400	200-300

FIGURE 35



d'après LERAY et al., R.N.D., 25, 567 : 1985

FIGURE 36



CARBOHYDRATES

C. COWEY

CARBOHYDRATES

Generally speaking native carbohydrates are not very well utilized by fish. In a natural environment fish are located at the top of the food pyramid, consequently most fish are carnivorous; they are adapted to eating diets high in protein and their carnivorous diet contains very few carbohydrates.

Some Japanese authors have carried out experiments, utilizing purified diets where they substituted dextrin for protein. Their conclusion were that optimum dextrin level for carp, which is perhaps an omnivorous fish, is 30 to 40 %, for marine carnivorous fish, such as red sea-bream, it is 10 % to 20 % and for yellowtail it is about 10 %.

FURUICHI and YONE, 1980

Dextrin substituted for protein on a weigh basis in diets.

Optimal dietary levels :

Carp	30-40 %
Red sea-bream	20 %
Yellowtail	10 %

For all 3 species, liver glycogen concentration was elevated.

The experimentation carried out by FURUICHI and YONE is shown below.
FURUICHI and YONE, 1981

Oral glucose loads of 167 mg/100 g body weight to yellowtail, carp and red sea-bream previously fed diets with 0.10 or 40 % dextrin for 30 days.

Glucose tolerance was lowest in yellowtail, followed by red sea-bream then carp.

Plasma insulin, initially 20 uU/100 ml rose to 70 uU (carp), 53 uU (red sea-bream) and 51 uU (yellowtail).

The levels of plasma insulin indicate that fish have little response to glucose in comparison to what is found in omnivorous animals.

It has been repeatedly shown reasonably high levels of carbohydrates given in the diet of fish result in swollen liver (hepatomegaly).

Levels of amylase activity in yellowtail and carp are shown below. Amylase is, of course, the enzyme primarily responsible for attacking starch in the digestible tract of fish; other enzymes will be involved in breaking down the smaller carbohydrate fragments to glucose which is presumably absorbed.

SHIMENO, HOSOKAWA, HIRATA and TAKEDA, 1977

Amylase, pepsin and trypsin activity in the digestive tract of carp and yellowtail

(Umoles liberated/min/g tissue)

	Amylase	Pepsin	Trypsin
Yellowtail	5,6	73.4	23.2
Carp	350	-	21.6

The amylase activity of yellowtail, in the digestible tract, is very low, if compared to that of carp.

The apparent digestibility or raw starch by yellowtail decreases as the level of starch in the diet increases (see below), in all cases, it is low.

SHIMENO, HOSOKAWA, HIRATA and TAKEDA, 1977

Apparent digestibility of starch by yellowtail

Level of component in the diet (%)	Apparent digestibility
Starch 8.9	57.2
Starch 17.2	56.4
Starch 40.5	39.2

The same applies for rainbow trout. The relatively old data by SINGH and NOSE illustrates this point very nicely.

SINGH and NOSE, 1967

Apparent digestibility of starch for rainbow trout

% in diet	20	30	40	50	60
Dextrin	77.2	74.8	60.0	50.1	45.5
Potato - starch	69.2	65.3	52.7	38.2	26.1

If we recall again the problem of substitution of dextrin for protein (in studies on protein requirement) to maintain the energetic level of the diet constant it can be argued that isoenergetic levels are maintained only for substitutions of small quantities protein.

Treatment of raw starch may improve the digestibility of it.

CHO and SLINGER, 1978

Apparent digestibility of starch by rainbow trout

Material	g/kg diet	% digested
Dextrin, white	807	100
Wheat middlings, raw	541	0
Weat middlings, autoclaved	391	62
Soyabean meal	75	54
Corn gluten meal	168	62

Autoclaving of wheat middlings increases its digestibility from 0 to 62 %. These methods (heat treatments, autoclaved, etc...) which change the physical status of starch making it more acceptable. Pre-gelatinized starch is now known to be well utilized by salmonids and other fish.

The following table refers to Channel catfish which utilize starch better than salmonids, but here again, it must be stressed that cooking starches before putting them in the diet appreciably increases their digestibility (from 26,1 % to 58.5 %).

Percent digestibility of gross energy for channel catfish in certain feedstuff.

Raw corn	26.1
Cooked corn	58.5
Wheat	60
Wheat bran	56.2

Liver glycogen is very slowly utilized by fish. Japanese workers carried out the experiment shown below some years ago.

Glycogen utilization from fish liver is very low

Carp starved 22 days, glycogen in liver was 10.65 %

Initially it had been 8.51 %

Even after 100 days starvation 1.55 % glycogen remains.

This contrasts very markedly with, for example, the omnivorous rat, whose liver glycogen disappears after 24 h of starvation.

Another example taken from a natural environment confirms the low utilization of liver glycogen. Salmonids during spawning migration. Canadian workers took measurements of liver glycogen in salmonids during their up river migration and they showed that for this long period of starvation during which fish form their gonads, the level of liver glycogen was almost constant and that the glycogen utilized was on occasion exceeded by gluconeogenesis.

We have already seen that activities of amylotic enzymes in fish, especially in marine carnivorous fish, enzymes that break down complicated carbohydrates to glucose, are low in activity. This is one explanation of the low utilization of carbohydrates by fish.

Another factor concerns glucose phospharylation. Nothing will happen metabolically to glucose within animals (fish) until it is phosphorylated.

GLUCOSE PHOSPHORYLATIQN

$$\text{ATP} + \text{GLUCOSE} \xrightarrow[\text{GLUCOKINASE}]{\text{HEXOKINASE}} \text{GLUCOSE-6-P} + \text{ADP} \qquad (1)$$

$$\text{GLUCOSE-6-P} + H_2O \xrightarrow{\text{GLUCOSE 6 PHOSPHATASE}} \text{GLUCOSE} + \text{P} \qquad (2)$$

ATP ------ ADP + P (Sum of equations (1) and (2))

Another carbohydrates which fish may meet in quite high quantities, in natural environments, is chitin. Chitin is a homogenous unbranched polymer of N-acetyl glucosamine. Chitinase is found in fish tissues but apparent digestibility studies ($Cr_2 O_3$) showed no significant digestion of chitin at 10 % or 30 % in the diet of rainbow trout.

Amino sugars are however respired (utilized) by trout (14 C substrate; I.P. Injection; 14 CO_2 out).

ANNEX I

Glucose utilization in fish measured isotopically

Kelp bass (BEVER et al., 1977)

Coho salmon (LIN et al., 1978)

Fish given tracer dose (6-3H) glucose replacement rate from indwelling arterial cannula at zero time; serial blood samples taken at intervals thereafter.

(1) It is then possible to determine glucose replacement rate R 3 (Production of glucose in post-absorptive animal G 6 P ----G) from 6-H glucose.

(2) Apparent glucose replacement rate R_a from (6- 14 C) glucose

(3) % glucose recycling

(4) Minimal transit time (average sojourn of a glucose molecule)

(5) Minimal glucose mass

(6) Glucose space

ASSUMPTION

Tritium (3H) in C-6 glucose is irreversibly lost to body waters in certain gluconeogenic/glycolytic actions.

14C glucose under-estimates glucose replacement rate because of recycling of labelled C into newly synthetized glucose.

RESULTS

	Kelp bass	Coho salmon	Rat
Body weight (g)	146-355	242	250-275
Glucose replacement (mg/min/100 g)			
R (6-3H) glucose	0.029	0.043	0.630
R_a (6-14C) glucose	0.030	0.035	-
Glucose recycling	zero	19	28
Glucose transit time (Min.)			
(6-3H) glucose	209	377	-
(6-14C) glucose	217	377	-
Glucose body mass (mg/l00g)			
(6-3H) glucose	5.6	16.2	24.5
Glucose space (ml/100 g)	17	-	-

VITAMIN AND MINERAL REQUIREMENTS WITH FISH

J. KOENIG

PLAN

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II. VITAMINS

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    2. Where do vitamins take effect
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        2.2. Mucous membranes
        2.3. Erythrocytes
        2.4. Cartilagenous tissues

b) Principal symptoms of deficiency with fish
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c) Securing the requirement
    1. Upkeep requirements
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    3. Special requirements
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III. Minerals

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b. Magnesium
c. Sodium, Potassium, Chlore
d. Sulphur
e. Iron
f. Iodine
g. Copper
h. Manganese

I. INTRODUCTION

At the beginning of the century, following the discovery of vitamins, there was a time when deficiency and a specific symptom were associated. For a certain number of vitamins, those which were discovered in the beginning, one appelation was kept: Axerophtol or vitamin A, Aneurin or vitamin B_1. The specific avitaminosis-symptom is the first approach to this problem.

However, a more discrete deficiency will lead to non specific disorders: anorexia, apathy, lack of growth. But these disorders must not be neglected, as, in rearing, they are of such importance and consequences that they can lead to catastrophies in profitability and be the forerunners of more severe physiological disorders.

We know that vitamins have an active and specific participation in all the metabolisms, which allow an organism to live and build itself up, by transforming the elements, nutriments that it absorbs, this is anabolism ; and by destroying and eliminating another part, this is catabolism.

Vitamins are prosthetic groups -in other words, biologically active parts-enzymes. Each vitamin can enter into a more or less great number of enzymes where it will have a specific function. Some vitamins have an unique biological function as is the case of vitamin E.

The vitamins of groupe B have been studied and their roles in the metabolism are rather well known. Their constitution is very specific and any slight modification of structure can block the reaction that they are meant to catalyse. These neighbouring compounds of vitamins are antivitamins. Certain medicaments and raw fish flesh contain antivitamins.

If a great number of reactions, where the vitamins of groupe B intervene, is known, there still remains a few points which are difficult to understand; in particular, for vitamin A whose exact role is still unknown, while yet very important in the synthesis of proteins.

We have seen that there are two action limits for vitamins, the inferior limit - expressed generally in mg per Kg of ration- below which appear specific symptoms; and the minimum superior limit for growth and normal behaviour. There is another superior level where therapeutic actions can appear within the frame of vitaminotherapy.

By this action, minerals logically fit in along side with vitamins. Some, for example macroelements participate in the structure of the organism. Microelements on the contrary, have a catalytic role and often they join in with vitamins in their action.

We must not forget to speak also of the technological questions when vitamins are incorporated into compound food. Questions linked with their more or less great fragility. Indeed, only vitamin C causes a practical problem.

As. this report concerns fish let us ask ourselves the question: do the requirements in vitamins and minerals for fish differ from those living in an atmospheric environment ? If, on general, the biological functions are the same, the specific symptoms are different for fish, with regard to their anatomic and physiological particularities. Gills instead of lungs for example ; which means that abnormalities of the gill organs in relation to certain deficiencies may be remarked.

II. VITAMINS

a) General functions of vitamins

1. Role played in the intermediary metabolisms

Table 1 - Synopsis of the vitamins of their active forms
and of their metabolic functions
W.F. KORNER and J. VOLLM (1976) - Doc. ROCHE

Vitamins	Active forms	Metabolic functions
A Retinol	Free "all trans-" retinol II - Cis retinene. (neoretinene b) Acid retinoïc	Proteic metabolism of the cells of all the organs of ectodermic origin, according to the history of their development (Skin, mucus). Formation of the visual purple (rhodopsin and iodopsin), a chromoproteïd with II-Cis retinene as prosthetic group. The stocking forms in the liver are esters of fatty acid and of retinol of which most are linked with proteins. Great partial vitaminic activity (growth, skin) is also attributed to retinoïc .
D Calciferol	1 25 - Dihydroxycholecalciferol 25 - Hydroxycholecalciferol	Regulation in the metabolism of calcium and of the phosphates, along with the that of citrates. Regulation of the calcium reabsorption and of the selection deposit of calcium in the organic bone matrix.
E Tocopherol	Free tocopherol	Has as physiological antioxydant a stabilizing action on the hormons, enzymes, other vitamins and lipids. Stabilization of the membrane and prevention of the formation of lipid peroxides. Participation in the intercellular respiration. Can be associated with selenium.
K_1 Phytomenadione	Free vitamin K1, plus probably menaquinone - 4 (Vitamin K 2)	Essential factor for the biosunthesis of prothrom bin in the liver. Necessary for the formation of the VII coagulation factor (proconvertin); In addition, it is implicated also in the biosynthesis of the IX factor (Christmas factor) and of the X factor (Stuart factor). Participation in the transport of elections in the respiratory chain.
B_1 Thiamin	Pyrophosphate of thiamin	As coenzyme of the decarboxylase-pyruvate and of the 2-Oxoglutarate dehydrogenases, determinant participation in the decarboxylation and in the oxidation of the 2 - oxo acids. In addition as coenzyme of the transcetolases, it is important for the transport of aldehyde groups in the pentosesphosphate cycle.
B_2 Riboflavin	Riboflavin 5 -Phosphate (FMN) FAD	As flavine - mononucleotide (FMN), but especially as flavine - adenine - dinucleotide (FAO), it formes the prosthetic group of the flavinic enzymes, which function as hydrogen conveyors, especially in the metabolism of amino acids and fatty acids (NADH - cytochrome c - reductase NADPH - cytochrome c - reductases; amino-oxydase acids; diaminoxydases; aldehydoxydases; fatty acids - CoA – deshydrogenases succinate of deshydrogenases - glutathione-reductase)
B_6 Pyridoxin group	Peridoxal 5' -phosphate	As coenzyme of decarboxylases - amino acids.

		amino transferases, hydrolases, phosphorylases and cystathionase, it is of essential importance for the proteic metabolism. In the metabolism of the brain, it participates in a determinant way in the preparation of biogen amines.
Nicotinamide	Nicotinamide - dinucleotide (NAD^+) Nicotinamide -dinucleotide (NADPH)	Coenzymes of dehydrogenases whose specifity is established by the type of each protein of the enzyme (apodehvdrogenous). Transport of hydrogen in the intermediary metabolism, during which the flavinic enzymes function especially as acceptors, for example oxydative phosphorylation. Preferably, NADPH serves as coenzyme.
Pantothenic acid	Coenzyme A	Functions in the enzymatic systems transporting the acyl groups, where the acceptors are especially amino-acids, amines, glucosamines and phosphatides. Transport of the acetyl remainder (acetyl CoA) coming from the oxydative decarboxylation of pyruvate (endoxydation in the metabolism of carbohydrates) to the oxalacetate in the formation of citric acid. Degradation of the fatty acids through B oxydation, in passing by the "active" fatty acids formed by liaison with CoA. Endogenic synthesis of the long chain fatty acids, phosphatides and steroïds.
Biotine	I' -N Carboxybiotine	Prosthetic group of carboxybiotine-enzymes (carbo-xylation enzymes such as acetyl - CoA - Carboxylase, pyruvate-carboxylase, propionyl-CoA – carboxylase and B methylcrotonyl - CoA - carboxylase, which intervene in the endogenic synthesis of fatty acids in the gluconeogenesis and in the degradation of amino-acids.
Folic acid (Pteroylglutamic acid	Acid 5 or 10 formyl - formimino - hydroxymethyl or substi tute methyl, 5,6 7, 8 tetrahydrofolic.	Constituant of folate-enzymes. Transport of "one carbon active units" when the methylation or hydroxymethyl reactions in the formation of active "formiate" or of "active hydroxymethyl". Transformation of glycine glutamate or into serine of cytosine into 5-hydroxymethyl - cytosine, of homocysteine into methionine, of deoxyuridylic acid, into thymidylic acid or of uracycle into thymidine (Synthesis of D.A.N)
B_{12} Cyanocoba-	Especially 5 -deoxy - adenosyl cobalamine (coen zyme B_{12})	Prosthetic group of methyl - malonyl - CoA - Isomerase and thus necessary for the transformation of propionic acid into succinic acid. Participation in the new formation of labile methyle groups), which are then transported by the process of transmethylation to the other acceptors of methyl groups.
C Ascorbic	Probably monodehydro – ascorbic acid	Redex compound capable of transporting reversible hydrogen or electrons. Participates in the process of hydroxylation. It is important for the biosynthesis of corticosteroids or catecholamines.
Choline	Has no function in the	- Precursor of acetylcholine

	coenzymes	- Conveyor of CH 3 - Constituent of phospholipids
Inositol	Has no function in the coenzymes	- Constituent of phospholipids

2. WHERE DO VITAMINS TAKE EFFECT ?

2.1. Plasmic membranes

Their role is essential in living beings: They constitute the interface between the organism and the environment, they maintain the constancy of the internal medium, they ensure the respiratory exchanges; the membranes actively defend the organism against agressions.

With fish the plasmic membranes in addition ensure:

- the constant internal osmotic pressure,
- the excretion of soluble substances.

The plasmic membranes consist in a double layer of phospholipids whose lipidic branches turn inwards and which have one hydrophilous molecule located on the outside of the membrane choline inositol. This double hydrophobous and hydrophilous character enables the membranes to have very particular properties : they constitute a barrier for exterior elements but can admit certain specific elements : thanks to the globular proteins found in the membrane. This is how in the gill membranes of the fish, so as to balance the internal osmotic pressure, organoids known as "the sodium pump" extract Na ions from the internal medium and cast them out; this against the osmotic gradient. We can clearly see that these plasmic membranes are "intelligent" organs.

Nevertheless, the constitution and functioning of the membranes depend on a certain number of vitamins :

INOSITOL and CHOLINE are constituents of phospholipids, and although structural elements of the membranes, they are considered as vitamins, as they can entail deficiencies.

The molecule of phospholipids comprehends 2 unsaturated fatty acids (UFA) which must have very specific constitutions permitting them to function correctly. These UFA are long chains of 22 to 24 carbon atoms. Some species such as the trout can use a C 18 precursor to synthetize, through elongation, the C 22 and C 24 UFA. This precursor is linolenic acid which is essential.

To do this, the trout will require catalysists supplied in the diet: Biotine, whose importance in the gill membrane is certified by the characteristic deformation in the case of deficiency.

Panthotenic acid, a prosthetic group of the coenzyme A, is also necessary in the synthesis of UFA. Its deficiency also provokes the extremities of the filaments to thicken.

In all cells of intensive metabolism, the free radicals can take a toxic effect. The membranes then play a protector role, either in capturing them or in destroying their product: peroxide.

One role played by vitamine E is the capture of the free radicals before they take effect at UFA level, in forming peroxides. A deficiency in vitamin E causes the destructuration of the muscular masses : muscular dystrophy.

Selenium, a compound of Glutation-peroxidase, take effect conjointly but after vitamin E, in destroying the peroxides which have formed vitamin E and Selenium are thus complementary ; Selenium ensure the safeguard of vitamin E.

2.2. Mucous membranes

These are fragile tissues which must stay humid continuously: ocular, intestinal mucous membranes, ...

VITAMIN A is necessary for the integrity of these mucous membranes. A deficiency leads to their keratinization, in other words, the formation of a corneal layer.

2.3. Erythrocytes

Their function, in the transport of oxygen, lead to intense metabolis where several vitamins intervene, more especially :

FOLIC ACID, is necessary for the formation of erythrocytes, a deficiency leads to megaloblastic anemia in salmonids, in other words cells which are abnormally enlarged with a segmented and strangled nucleus.

VITAMIN B_{12} is employed for the formation of red corpuscles. A deficiency creates a microcytic hypochrome anemia.

2.4. Cartilagenous tissues

VITAMIN C hydroxylates lysine and leucine, a stage which is necessary for the synthesis of collagen and of cartilages. A deficiency in vitamin C leads to the formation of a cartilage of poor quality and in fish to different bone structure deformations.

Therapeutic use of Vitamin C with fish is its capacity to activate the healing of wounds by stimulating the formation of collagen.

b) PRINCIPAL SYMPTOMS OF DEFICIENCY WITH FISH

1. Non-specific symptoms

SLOW GROWTH AND A LOW CONSUMPTION INDEX. These are the first signs which indicate vitamin deficiency, the other causes being eliminated (infection or unbalanced diet). The economic importance of these first symptoms must be emphasized.

ANOREXIA or lack of appetite, is another non specific symptom of vitamin deficiency.

EXOPHTALMIA - protruding eyes; which occur when the osmotic pressure is badly regulated; it can be provoked by different vitamin deficiencies, and also by certain diseases (especially V.H.S).

ABNORMAL COLOURING, moreover a dark colour. However, a decolourization can occur when there exists a Niacin deficiency.

DWARFISM - Remarked in salmoneids, can be related to different deficiencies Vitamin B_2, Vitamin C, Zinc.

2. Specific symptoms

The non-specific symptoms are, on general, the first to appear and these are then followed by the specific symptoms. There is a gradation in the specific symptoms; these are, when remarked in the beginning stages, reversible, which means that they can regress when the vitamin lacking is administered. But after a certain time, the

lesions become too important and can not be cured; the last stage is death. By this, we learn that we must act immediatly when the first symptoms appear.

It is possible to detect a deficiency in its early stages, in other words before the specific symptoms appear, by applying the appropriate biological tests. For example, the consumption of raw fish containing thiaminase causes a nervous disease in trout caused by the destruction of vitamin B_1. The quantity of transcetolases in the blood (an enzyme whose prostetic group is vitamin B_1) permits to detect the start of the deficiency before the specific symptoms appear. Biochemical tests are widely employed in the studies of vitamins.

The following tables give the symptoms of deficiencies per vitamin and for the principal types of fish reared. We shall describe the most characteristic symptoms one by one.

Table 1 : Major signs of vitamin deficiencies in fishes
(Hugh A. POSTON, 1985 - Doc. ROCHE)

Vitamin	Trout and Salmon	Channel catfish	Common Carp	Red sea bream	Japanese eel
Fat-soluble vitamins					
A	Impaired growth, Exophthalmia, eye lens displacement, Corneal thinning and expansion, Retinal degeneration, Edema, ascites, Depigmentation	Exophthalmia, Edema	Depigmentation, Exophthalmia, Twisted opercula, Fin and skin hemorrhages	-	-
D	Poor growth, Impaired calcium homeostasis, Tetany of white skeletal muscle	Low bone ash	-	-	-
Fat-soluble vitamins					
E	Reduced survival and growth, Anemia, Immature erythrocytes Variable sized erythrocytes, Fragile and fragmented erythrocytes, ascites, Nutritional muscular dystrophy, Lipid peroxidation Increased body water (i.e. exudative diathesis, Depigmentation	Poor growth, Mortality, Muscular dystrophy, Exudative diathesis, Depigmentation, Fatty livers	Poor growth, Exophthalmia, Lordosis, Muscular distrophy, Kidney degeneration, Pancreatic degeneration	-	-
K	Prolonged blood clotting, Anemia, Reduced hematocrit	Skin hemorrhages	-	-	-
Water-soluble vitamins					

Thiamin	Poor growth, Mortality, Anorexia, Hyperirritability, Convulsions, loss of equilibrium, Low transketolase activity in erythrocytes and kidney	Dark color, Mortality, Loss of equilibrium, Nervousness	Fin congestion, Nervousness Depigmentation, Subcutaneous hemorrhage	Poor growth Subcutaneous hemorrhage Congested fins	Trunk winding activity, Subcutaneou hemorrhage Congested fins
Riboflavin	Poor growth, Anorexia, Lens cataract, Adhesion of lens and cornea, Reduced activity of erythrocyte glutathione reductase, Dark pigmentation	Anorexia, Poor growth, Short, dwarf body	Anorexia, Emaciation, Mortality, Hemorrhagic heart muscle,	Poor growth,	Poor growth, Dermatitis, Photophobia, Fin hemorrhage, Abdominal hemorrhage
Pyridoxine	Poor growth, Mortality, Anorexia, Elep-form convulsions, Hyperirritability, Low resistance to handling, Erratic, Spiral swimming, Rapid breathing znf gasping, Flexing of opercula, Rapid onset of rigor mortis, Low erythrocyte and muscle amino-transferases	Nervous disorders, Tetany, Mortality, Greenish blue coloration	Nervous disorders, Skin disorders, Hemorrhage, Edema, Low hepato-pancreatic transferases	-	Poor growth, Anorexia, Eleptiform convulsions
Pantotheni c acid	Anorexia, Poor growth, Anemia, High mortality, Clubbed exudate-covered gills, Atrophied pancreatic acinar cells, Vacuoles and hialine bodies in kidney tubules	Anorexia, Emaciation, Clubbed gills, Anemia, high mortality, Eroded epidermis	Anorexia, Poor growth, Lethargy, Anemia, Exophthalmia	Poor growth, High mortality	Dermatitis, Congested skin, Hemorrhagic skin, Poor growth, Abnormal swimming

Biotin	Poor growth and feed conversion, Increased mortality, Degeneration of gill lamellae, skin lesions, Reduced liver acetyl CoA carboxylase and pyruvate carboxylase, Altered fatty acid synthesis, Lipid infiltration of liver, Degeneration of pancreatic acinar cells, Glycogen storage in kidney tubule	Depigmentation, Anemia, Reduced liver pyruvate carboxylase	Poor growth, Increased number of dermal mucous cells	-	Abnormal swimming
Niacin	Poor growth and feed conversion, Anorexia, Skin and fin lesions, Colon lesions, Anemia, photo-sensitivity	Poor growth, Skin and fin lesions, Skin hemorrhages, Exophthalmia, High mortality Anemia, Deformed jaws	Poor growth	-	Poor growth, Abnormal swimming, Incoordinatio Dark color, Skin lesions, Anemia
Folic acid	Slow growth, Anorexia, Poor feed conversion, Anemia, pale gills, Large, segmented erythrocytes	Lethargy	None detected	None detected	Anorexia, Poor growth, Dark coloration
B_{12}	Anemia, Small, fragmented erythrocytes	Reduced hematocrit	None detected	Poor growth	Poor growth, Anorexia

	Anorexia, Reduced growth, Lordosis, scoliosis, Lethargy, Hemorrhagic exophthalmia, Ascites, anemia , Intramuscular hemorrhage, Reduced concentrations of ascorbic acid in liver and anterior kidney, Abnormal histology of support cartilage in eye, gill and fin, Reduced serum thyroid hormone (T3), Elevated plasma cholesterol and tryglycerides	Lordosis, Scoliosis, Reduced bone collagen, Increased susceptibility to disease	Poor growth,	Poor growth,	Fin and dermal hemorrhages Lower jaw erosion
Choline	Poor growth, Fatty liver	Enlarged liver, Hemorrhagic kidney and intestine	Poor growth, Fatty liver,	Poor growth, High mortality	Anorexia, Poor growth, White-grey intestine
Inositol,	Anorexia, Poor growth, Poor feed conversion, Slow gastric emptying, Reduced cholinesterase activity, Reduced transaminase activity, Increased neutral lipids, cholesterol and tryglycerides in liver, Decreased phosphotidylcholine, phosphotidylethanolamine and phosphotidylinositol	None detected	Skin lesions, Reduced growth	Poor growth,	White-grey intestine

Vitamin A

Deficiency in vitamin A provokes characteristic lesions of the eye in salmoneids. As there exist several types of ocular lesions, linked with different deficiencies, we shall deal with this point alone.

Vitamin E

A deficiency is often provoked by the use of rancid oil in which toxic peroxides are found.

Lack of vitamin E leads to muscular distrophy which is the destructuration of the muscular masses, with a small enlargement, we can remark the alteration of the fibers and the proliferation of the conjunctive tissue.

The use of an antioxydant (Ethoxiquin) can prevent the formation of peroxide but can not replace vitamin E.

Vitamin B_1

In trout, the deficiency provokes the dizziness disease (loss of balance) seen in Danemark, when the basic food employed was raw fish. The distribution of 10 g/m^3 of vitamin B_1 in the water or 12 mg/kg of food will eliminate these symptoms.

Vitamin B_2

Deficiency in vitamin B_2 provokes the cataract in salmoneids. We shall examine the characteristics in the part dedicated to ocular disorders.

Vitamin B_6

Pyridoxal-phosphate being necessary for the synthesis of several neurotransmitters, the most typical symptoms of its deficiency will be nervous disorders: epileptic movements, hyperirritability, abnormal swimming or spinning, the rapid apparition of "rigor mortis". Among the characteristic symptoms, it has been remarked that the trout is unable to snap up its food as it is not capable of sensing distance.

Panthotenic acid

With trout and channel catfish, the lack of panthotenic acid provokes the swelling of the branchial scales of the filament extremities, known as clubbed gills.

Biotine

Lack of biotine also provokes the hyperplasy of the extremity of the branchial scales in trout but not in channel catfish. Dermatites called the "blue slime patch" where the skin breaks off in fragments has been confirmed when biotin is lacking.

Niacin

The most characteristic symptom of a deficiency in niacin in trout and channel catfish is the discolouring of the skin under the influence of the sun. Exposure to light (or UV rays) causes skin and fin lesions in carp also.

Folic acid

Folic acid being necessary for the formation of red corpuscles, its deficiency leads to anemia characterized by the destruction of erythrocytes and the presence of senile pre-erythrocytes (megaloblastic).

Vitamin B_{12}

Vitamin B_{12} has much the same function as folic acid and is necessary for the formation of red corpuscles. Deficiency leads to the reduction and fragmentation of red corpuscles. Vitamin B_{12} and folic acid used to be given in the form of fresh animal products in fishculture, and was considered, before industrial vitamins were made available, to be irreplacable by dry products.

Vitamin C

This vitamine intervenes in a large number of enzymatic reactions and in the most varied cases: synthesis of collagen, cartilages, synthesis of adrenalin from where it has an antistress effect, reproduction system, etc... with salmoneids. The most common and most spectacular manifestations are the bone deformations of the spine (scoliosis, lordosis, dwarfism, gibbosity) along with the shortening of the operculums.

Channel cat-fish show the same deficiencies as trout.

With eel, deficiency in vitamin C provokes haemorrhagies in the head region.

Cholin

It has a structural function in entering into the phospholipids, which form an integral part of the membrane. Nevertheless, cholin acts in the same way as does a vitamin; a deficiency leads to the liver turning yellow due to the infiltration of lipids.

Inositol

Like cholin, inositol enters into the composition of phospholipids. A deficiency does not lead to a real characteristic symptom, if not the scaling of the fins in carp.

c) SECURING THE REQUIREMENT

1. Upkeep requirements

The upkeep requirements have been defined, in starting with a synthetic diet lacking in the vitamin where deficiency is be studied. These are artificial conditions which make that in practice, a specific deficiency is very rarely observed. In varying the vitamin dose rates in ascending order starting at zero, we shall remark: A total deficiency provokes the stoppage of growth, specific symptoms and death. With a feeble dose, mortality is no longer remarked: if the dose is increased, the specific symptoms then disappear; finally, with the correct dose, growth becomes normal.

Certain biochemical parameters can be following on parallel, along with the transcetolase activity of red corpuscles for vitamin B 1. These parameters permit the detection of a deficiency from the beginning, before the specific symptoms appear. These biochemical indications are more reliable than certain specific symptoms which can cause confusion, such as the deformation of the gill filaments which are often difficult to define. It may indeed concern a deficiency in biotin, in panthotenic acid, in vitamin C, but also a toxicosis or a pathological state.

Certain vitamins interact with other elements, the requirements in vitamin E are proportional to the rate of unsaturated fatty acid. In addition, the needs in unsaturated fatty acids rise in Winter for salmoneids, when the temperature of the water drops. Thus a certain amount of vitamin E which is suitable in Summer may not be so in Winter.

Thus, we must be very prudent when using the tables of requirements and consider them more so as adjustable work hypothesis depending on the formulation and environmental conditions of the rearing.

Table 2 - Minimum requirements in vitamins per fish[1]

(cf. NCR, 1981; NCR, 1983) - POSTON, 1985

Vitamins	Trout and Salmon (per kg of food)	Channel catfish (per kg of food)	Common carp (per kg of food)	Red sea-bream (per kg of food)	Japanese eel (per kg of food)
Liposolubles					
A	2,500 U.I	2,000 U.I	10,000 U.I	-	-
D	2,400 U.I	1,000 U.I	-[2]	-	-
E	30 U.I	30 U.I	300 U.I	-	-
K	10 mg	R[3]	-	-	-

1 - Listed amounts do not allow for losses during processing and storage. Levels needed may vary under different environmental conditions.

2 - indicates requirement not tested or not known

3 - indicates dietary needs but in undefined quantities.

Table 2 Minimum dietary vitamin requirements for fishes (continued)

Vitamins	Trout and salmon (per kg diet)	Channel catfish (per kg diet)	Common carp (per kg diet)	Red sea-bream (per kg diet)	Japanese eel (per kg diet)
Water-soluble					
Ascorbic acid	100 mg	60 mg	--	R	R
Thiamin	10 mg	1 mg	--	R	R
Riboflavin	20 mg	9 mg	7 mg	R	R
Pyridoxine	10 mg	3 mg	6 mg	6 mg	R
Pantothenic acid	40 mg	20 mg	50 mg	R	R
Biotin	1 mg	R	1 mg	--	R
Niacin					
(Nicotinid acid)	150 mg	15 mg	30 mg	R	R
Folic acid (Folacin)	5 mg	--	--	--	R
B_{12}	0.02 mg	R	--	R	R
Choline	3,000 mg	R	4,000 mg	R	R
Inositol (Myoinositol)	400 mg	--	440 mg	900 mg	R

2. Therapeutic requirements

It has been remarked that certain vitamins, employed in greater amounts than in upkeep requirements, can have therapeutic effects. The therapeutic doses are usually 10 to 100 times more than the upkeep doses.

- As examples of therapeutic employments, we can mention:
- Vitamin C which is employed to speed up the healing of wounds.

Cholin which is commonly employed to reabsorb the lipidic excess of the liver caused by overfeeding. But the most interesting case of investigation is the immuno-stimulating effect of certain vitamins against bacterial agressions. We shall see this further down.

3. Special requirements

We call special requirements the supplementary requirements resulting from the use of certain nutriments or medicaments.

- The presence of a thiaminase, which destroys vitamin B_1, in the flesh of the fish has been pointed out. Certain vegetables (cabbage), empolium, have the same effect.

- The use of sulfaguanidin reinforces the symptoms of deficiency in Vitamin K.

- "High energy" food rich in UFA require the supplement of vitamin E.

4. Toxicity

Certain vitamins employed in excess can be toxic. Nevertheless, the toxicity threshold is generally extremely high, so that this sort of accident, except when a grave error is committed, is not to be feared.

An historical example is that of these polar explorers who had to eat bears'liver, so as to survive, and which is very rich in vitamin A. This caused a bone necrosis, which is characteristic of hypervitaminosis A.

A hypervitaminosis was provoked in trout in distributing 2.2 million of I.U/kilo of ration, which is 1.000 times the upkeep dose. This hypervitaminosis is manifested by a reduction in growth, anemia, and a necrosis of the caudal fin.

30.000 I.U of vitamin D_3 (normal dose x 50) provokes a significant decrease in growth in the channel catfish.

d. VITAMINS AND THEIR MAIN FUNCTIONS

1. Vitamin and reproduction

LIGNIERE pointed out in 1949, for trout fry, that a deficiency in vitamin E, provoked an atrophy of the ovarian chain and testicles; a supplement of vitamin E ensured normal sized organs.

With the Coho salmon, the breeders lacking vitamin C produce fry with a vitamin C deficiency, showing characteristic deformations. Another batch of breeders with a normal level of vitamin C produced fry exempt of deformation.

This shows the importance of food well provided in vitamin E and C for breeders, so as to obtain healthy offspring.

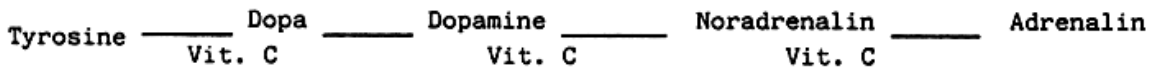
The case of caroteneids used in fishculture for pigmentation (canthaxanthin and soon astaxanthin) has not yet been defined. The report between the pigmentation of the eggs and their viability has been studied: There is a critical level of carotenoid (1 - 3 ug/g - egg) above which it is less than 50 %. This data is empirically confirmed by the aquaculturists who prefer coloured eggs to white eggs. Carotenoids act on the respiratory functions and as provitamin A.

2. Vitamins and defenses of the organism

Vitamin C plays an important part in the metabolism of tyrosin, especially in the synthesis of adrenalin and of noradrenalin - antistress hormones. These hormones,

called "of urgence" or catecholamines, permit the organism to face up to an agression in metabolizing an energetic cardiac and circulatory potential.

The catecholamines are synthetized from tyrosin in four stages, 3 of which mobilize vitamin C:



We can use vitamin C for fish each time a decrease in performance after treatment is remarked or after a stressing operation such as sorting out. Distribute 60 g per kg of live weight for 3 consecutive days. The method advised is the use of pellets having an oily covering.

Vitamins and immunity

During the past ten years, a number of studies revealed that certain vitamins have an immunostimulating effect. These effects were principally studied concerning vitamin C and more recently vitamin E.

Vitamin C

The resistance of Channel catfish to infection by means of an enterobacteria EDWARDSIELLA TARDA was studied. A decrease in mortality was remarked when these fish received 150 mg of vitamin C/kg of food. The test was carried out at 2 temperatures: 23° C and 33° C. At 33° C, vitamin C had less effect than at 23° C. This confirms the importance of the temperature in the mechanism of resistance to agression.

This test was followed by a more specialized experiment where a gradient of vit. C, of 0 to 3 000 mg/kg of food was given and different immunitary parameters were measured. There was total mortality at 0 and zero at 3 000. The production of antibodies was at its maximum at 3 000 mg along with the hemolytic activity of the complement. We can remark here that the immuno-stimulating effect is obtained with doses of 30 times more than upkeep doses.

Vitamin E

Recent studies, where the immuno-stimulating effect of vitamin E on calves was studied, proved that the distribution of vitamin E before and after infection, had a positive effect on the production of agglutinative antibodies and increased the % of success of the vaccination of calf infected by Brucella abortus.

With fish, there are few studies available, however a stimulating effect on the immunitary responses of trout was discovered with the use of vitamin E, after a period of 17 weeks of deficiency, no growth problem occurred. The Authors concluded that the deficiency in vitamin E would be responsible for certain immunodepressive states. This method of prevention against disease by reinforcing the natural defenses seems very promising, knowing the disappointing results obtained with the use of antibiotics, because of their ability of adaptation to infectious agents.

It was believed that this peculiar quality of vitamins could also apply for defense against viral diseases, as it was proven that vitamin C stimulated the production of interferon, a non specific immunitary response.

Practical tests carried out on these lines in the aim of fighting against the V.H.S of trout, proved disappointing, as the distribution of vitamin C increased the mortality. It was concluded that, in experimental conditions, the virus taking advantage of the cellular

metabolism also profited from the stimulation of this by the vitamin. As remarked, this method is not simple and can exert an inverse effect of that required thus the necessity to ensure precise research for application.

The immuno-stimulating effects with pyridoxin and pantothenic acid with Channel catfish has been pointed out.

3. Vitamins and the nervous system

Nervous symptoms are often the first to show up deficiencies. The nervous tissues have indeed an intense metabolism, which explains why they are the first to be affected.

VITAMIN B_1 - Its deficiency in trout, leads to hyperirritability, loss of balance (giddeness). Several biochemical relations have been found between Thiamin and the nervous system; Thiamin also participates at the synthesis of acetylcholin, a mediator at synopses level.

PYRIDOXIN - Pyridoxal phosphate is essential for the synthesis of neuroendocrine substances such as serotonin from tryptophan. For this reason, deficiency in vitamin B_6 is manifested by nervous disorders.

e) PARTICULAR PROBLEMS

1. Vitamins, amino-acids, oligo-elements in ocular diseases

Ocular disorders in fish are often of nutritional origin. However certain eye troubles can be of other origin: physical shock, anorexia, parasites, gassy oversaturation. When the ocular pathology is of nutritional origin, the lesions are generally bilateral.

VITAMIN B_2. Deficiency in riboflavin was the first to be described for ocular symptoms. This is a lesion which commences in the cristalline cortex by a cataract which progressively spreads and becomes opaque, then the cornea becomes affected, finally the ocular globe is liquefied. With the use of a synthetic vitamin, this type of problem has practically disappeared.

AMINO-ACIDS - This concerns another type of cataract completely different from the former one and linked with the degradation of the quality of the diet ingredients, following the rarefaction of the fish meal from Peru in 1973. The use of vegetable protein in excess led to a deficiency in methionin which caused a different type of cataract than that caused by a deficiency in vitamin B_2: The cristalline was affected, but the cornea remained undamaged. Then, liquefaction and necrosis occured, but the nucleus (Centre of the cristalline) was retained by the peripherical fibrous tissus. The addition of methionin to the diet prevented the development of this disorder.

ZINC - In 1973, the manufacturers also used white fish meal, coming from filleted fish meant for human consumption, a meal which was prepared on board. This meal, although relatively poor in proteins was of excellent quality but was excessively rich in mineral matter. The use of this type of meal caused a cataract which started in the perinuclear region in trout fry of less than 1 g and in the cortex region in older fry (more than 4 g). The evolution of the cataract continued with the liquefaction of the cristalline. It has be enpointed out that this cataract was caused by a deficiency in Zinc, although the meal contained 70 - 80 mg. The deficiency is, in fact, induced by a excess of calcium which blocks the Zn available. In adding either Zn (200 mg/kg of food, sulphate) or a chelating agent, this symptom disappears.

The deficiency was first defined in the U.S.A in 1978; the correction was applied to food for trout in France in the eigties. Since then, this type of cataract no longer causes any problem.

VITAMIN A - Deficiency in Vitamin A is the only one which implicates exophthalmia with the depalcement of the cornea. The cristalline becomes opaque and the retina degenerates. This deficiency was produced at laboratory level, but in practice, it is not a treat to fishculture. The generalized use of vitamin premix makes that the apparition of this cataract by means of avitaminosis is very unlikly to occur.

The same does not apply for deficiencies in amino-acids nor minerals, as already seen, they can be the consequence of formula modifications.

In a less spectacular way, bad sight in fish in rearing will lead to a bad perception of the food, which is also detrimental from an economic view-point. The fish must be carefully surveyed for this.

2. Problems caused by carotenoids

Carotenoids are employed for two reasons with fish.

- Vitamin A so as the cover the physiological requirements.

- Canthaxanthin and more recently astaxanthin for the pigmentation of salmon. Vitamin A, canthaxanthin and astaxanthin belong to the same biochemical family of carotenoids. The two latter molecules possess chetone functions.

At a certain period, the French Authorities were all against the use of canthaxanthin, which was pejoratively qualified as a colouring. So as the avoid confusion with the natural product, the commercial name "salmon trout" was prohibited the fish breeders then choose the name "pink trout". This name was then prohibited in the eighties and the only authorized one is "trout with natural reinforced colouring", a complicated and vaguely pejorative expression.

This side issue in the reglementation calls for some precision: Although manufactured by synthesis, canthaxanthin is prevalent in nature, especially in the skirret or chanterelle mushroom from where it takes its name, as for astaxanthin, it colours Crustacea.

Canthaxanthin is widely employed and gives a pinky orange colour. Astanxanthin, which will soon be put on the market, gives a much similar colour but a little deeper in colour which can be obtained quickly. In addition, and this is its strong point, it is the same pigment which gives natural salmon pink.

Canthaxanthin and astaxanthin are not only simple pigments but also provitamins A.

Thus, there is no justification for unfavourable prejudice against these synthetic products which exist in nature and which merit better than a dissuasive commercial denomination from the authorities.

These substances are employed to colour not only the flesh but also the eggs, which benefit from a noted preference by the fishculturers. We have seen that this preference is justified, by their performances, although the reasons for these good performances have yet to be defined.

f) SOURCES OF VITAMINS

1. Natural and synthetic vitamins

The ingredients used in the formulas contain natural vitamins, however the amounts employed can vary and are not very reliable. It is advisable to cover the needs, by adding vitamins to the premix. However, we must take into account the liposoluble vitamins contained in oil.

Premix contains not only vitamins, but also a neutral support (gluten or wheat middlings) so that its rate of incorporation in the food will represent 1 %. It would be indeed impossible to incorporate in a homogenous way the vitamins whose incorporation rates are from 10 - 5 to 10 - 7. Industries furnish such premix to food manufactures.

Certain rules must be abided by so as to obtain a good concentration of vitamins. The first and most important is not to put a vitamin premix in direct contact with an oligo-elements premix, as the latter is a powerful destructful agent to vitamins. These two products will be added separately when the food is being manufactured.

Another important precaution to take is the separate addition of cholin (Chlorine) which is a hydroscopic liquid which can also affect vitamins.

2. Technological problems arising by the incorporation of vitamins

Apart from vitamin C which we will study alone, the other preparations of vitamins are stable.

Vitamins are more or less fragile organic molecules which are prone to two types of aggressions:

1) During manufacture, the pressing process which agglomerates meal produces and the obligation of having to employ a die after humidification and heating. The process of extrusion causes yet more harm to the vitamins.

2) When stocked, the vitamins contained in the food mixture are in contact with more or less agressive elements: oligo-elements, peroxides. This, in hot and humid conditions can accelerate the process of degradation.

With the exception of vitamin C, solutions have been found in all the other cases, which permits their concentration in the food, for at least 6 months. Certain vitamins are presented in an oily solution form (A and E): If employed in this form, they will be quickly destroyed. The coating technique permits good conservation.

Certain vitamins have less resistance during the manufacture of the food:

Vitamin B_2 - 25 % can be destroyed during manufacture.

Panthotenic acid - This is an unstable and viscous solution. It is used in the solid form of calcium panthotenate, which has an activity of 46 %.

Nicotinic acid - 20 % can be lost during extrusion.

Folic acid - 10 % can be lost during the industrial processing.

Vitamin C - The vitamin C, furnished by industries, is, up to present, fragile. Losses of 88 % after 8 weeks of storage in food stocking rooms having a temperature of 21° C have been recorded: but only losses of 10 % are recorded if the stocking room is kept at 5° C. The losses during manufacture can vary from 20 % to 60 %, depending on the process of manufacture employed.

Deficiency in Vitamin C, in salmoniculture, is a common thing and is probably at the origin of the body deformations remarked. Controls carried out by us often show rates of less than 100 mg/kg.

For these reasons, it is advisable:

- To add a much larger amount of vitamin C than demanded: 500 mg/kg.
- To conserve the food for not more than one month in a place which has a temperature of not more than 20° C. The use of a cold storage room is advisable in the Mediterranean climate.
- To add vitamin C after the pressing or extrusion by means of an suspension coating. However, there is still hope of seeing it commercialized in a more stable form. Ascorbic acid sulphate would be the stocking form in the organism of vitamin C and ascorbic acid the active form. The organism mobilizes the active form from a pool of sulphate.

The sulphate form is much more stable and could be produced industrially. However, this view point is not the unanimous opinion of the specialists and tests are at present being carried out.

III - MINERALS

Two types of minerals which are strictly indispensable for living beings can be remarked.

1. MACRO-ELEMENTS which are the architectural elements of the organism. They are found in concentrations of 10-2 to 10-4: P, Ca, Mg, Na, K, S.

2. MICRO-ELEMENTS or OLIGO-ELEMENTS which intervene as enzyme molecule compounds and as biocatalyzers, their role can be compared to that of vitamins, with which they sometimes interfere. Their concentrations vary for 10-4 to 10-8: Fe, Cu, Mn, Zn, Co, I, Se.

The border between these two categories is not well defined, as certain elements are both structural and catalytic.

An essential difference must be remarked from a nutritional view point between animals with pulmonary respiration and those breathing through the gills. While with mammals and birds, the mineral supply is uniquely obtained through food, with fish the major part of minerals can be absorbed under soluble form through the gills and skin. For this reason, we must relativize the nutrition and requirements for minerals and take into account their tenor in the water.

Numerous experiments show that when fish are deprived of calcium in their food, they will normally have a provision in their organism: This element being directly absorbed from the water through the gills and skin.

The use of Ca^{45} with Carassius aurata has permitted to remark that 90 % of Ca absorbed by the organism can come from the water.

a) Calcium and phosphore

The most apparent role played by these two elements being the constitution of bone tissue, it is normal that they be associated.

With mammals, 99 % of Ca and 86 % of P are contained in the bones and teeth. With fish, an important part of these two elements is contained in the scales and skin:

around 40 % in trout. The relation Ca/P in the whole organism of fish varies from 1.5 to 2.1.

Ca, apart from its structural function it also ensures: the coagulation of blood, muscular contraction, nerve transmissions, and osmoregulation.

Phosphore is necessary for a great number of the essential metabolic functions. It consists of adenosin triphosphate (A.T.P), phospholipids, DNA and RNA. P plays a part in the energetic transformations, the control of permeability of the membranes.

The symptoms of deficiency in Ca are difficult to detect in fish due to the feeble diet requirement rate demanded. In using a yeast basis in the diet, especially lacking Ca, the input was 0.1 %. A loss of appetite, feeble growth and a poor consumption index was then remarked in trout and carp. In practice and with the employment of a classical diet consisting of fish meal basis, it is very improbable to find a deficiency in Ca.

As for phosphore, the water on general does not contain a high rate of this element, except in the case of polluted waters where P constitutes an eutrophic element. In marine waters, the rates are very low: 0.1 mg/1.

As there exists a tendency for economic reasons, to use more vegetable products instead of animal products, there often appears a deficiency in P, as this is then less assimilable.

The symptoms of deficiency in P have been studied for the Channel catfish and Cyprinus carpio. The signs generally remarked are poor growth, low consumption index and bone deficiencies. We remark in carp a frontal swelling and an increase in muscular fat.

The input of P for fish is that recommended in assimilable form. The normal food at disposal supplies remarkable quantities but often not very digestible.

In animal meal, P is found in the form of tricalcic phosphate of which the carp assimilates 13 % and the trout 64 %.

In vegetable products, P is essentially found in the form of phytate, which is not very well assimilated (except for peneid shrimp). In the soya bean, the digestibility of P varies between 29 % and 54 %.

The requirements in assimilable Ca varies according to the richness of the water, which, in any case, are very low: 0.10 to 0.25 of the ration.

The assimilable P must be supplied in amounts of 0.45 % of the ration for Channel catfish and 0.90 % for Tilapia nautica.

As there is very little information available and that phosphate is very important, it seems advisable to supply 0.80 % of P to the diet of fish, under the form of monosodic or monocalcic phosphate.

b. Magnesium

The role played by Mg is not well known but very probably of great importance. Deficiencies in Mg have been provoked in Channel catfish, carp, red sea-bream, rainbow trout. The symptoms are not very characteristic: poor growth, anorexia, apathy, muscular relaxation, mortality. Convulsions have been remarked in carp.

Apart from the absolute requirements in Mg, this must be supplied in proportion with Ca and P. According to the water and species, the food supplies must contain from 0.02 to 0.07 % of the Mg ration.

NEPHROCALCINOSIS is a disease which is characterized by white renal deposits of Co_3 Ca, which can reach the size of a walnut in trout and greatly decrease production. The apparition of this disease depends principally on the rate of carbonic gas found in the water. However, this disease occurs more often and has a greater effect in waters lacking Mg.

In the studies which were carried out on the transfer of fish to sea-water, Mg plays an important physiological role, when trout were submitted to stress, with a rise in the temperature and the salinity. This stress leads to a characterized syndrome which is followed by death. At the same time a high increase in magnesiemia (up to 300 %) was remarked, as if the membranes couldn't stop the entrance of ions. The hypothesis of blocking the transmission of the nervous influx by Mg was proposed.

c. Sodium, Potassium, Chlore

These elements are important on account of their participation in the essential physiological processus: osmoregulation, basic-acid balance, chlorhydric stomacal secretion, maintenance of the membrane, potential and transmission of the nervous influx.

Na, K, Cl are found in fresh water and in sea water in more or less great quantities, for this reason, it has not been possible to characterize a deficiency in any one of these elements.

Fish can tolerate excesses of Na Cl in the diet of up to 12 % without unfavourable effects occurring: Probably, because it is easy to excrete the excess through the gills and skin.

d. Sulphur

This element is found in certain essential amino-acids: METIONIN, CYSTIN,... The requirements in S will be covered when those of sulphured amino-acid are.

e. Iron

The deficiency in iron causes a microcytic hypochromic anemia in trout and carp, ... The requirements will be covered for trout with 150 mg/kg in iron.

Growth is not affected by a deficiency in Iron.

Ferruginous water can be toxic, ferric hydroxide forms a deposit on the gills. In such a situation, it is advisable to filter the water over pebbles before using it in fishculture, the ferric hydroxide will settle and the water will be fit for use in fishculture.

Dietetic form: Citrate, chlorine.

f. Iodine

Deficiency in iodine causes goitre, a swelling of the thyroid gland, corresponding to a hyperplasy of this tissue.

The requirements in I for fish are around 1 to 5 mg per kilo of ration.

Deficiency in I can occur in mountainous regions and with certain food: meaty food. In these conditions and in the absence of specific syndroms of deficiency, the

distribution of a supplement of iodized protein led to an increase in growth of 10 % with rainbow trout.

With the use of marine products in the rations, the danger of a deficiency in 1 is feeble. However, as fish meal is scarce, it seems advisable to add potassium iodide.

g. Copper

Copper is employed to form red corpuscles. The requirements in Copper are around 1 mg/kg of ration for carp, trout and Channel catfish.

Excess copper (16 - 32 mg/kg) leads to poor growth and slight anemia in Channel catfish. The common employement of sanitary baths with So_4 Cu in Salmon culture, gives reason to believe that there are probably more dangers of toxicity than of deficiency.

Vitamin C has a detoxifier effect concerning Copper and other different heavy metals.

h. Manganese

With carp and trout, tests have showen that the deficiency in Mn occurs with 4 mg/kg and causes feeble growth, withered fins and dwarfism. A supplement of 12 mg will suffice so as to ensure normal growth and development. The use of white fish meal will provoke a deficiency in Mn and cause cataracts to appear,

i. Zinc

Zinc is an important biochemical element which enters into the composition of different enzyms (cocarboxylase) and proteins (eye).

Its deficiency in trout causes reduced growth, cataracts, dwarfism, wasting away of the fins, and mortality.

As stated before, concerning ocular troubles, deficiency in Zn has occurred in fishculture, when white fish meal was employed. The symptoms can be prevented by adding 200 mg/kg ration of Zinc sulphate.

The use of white fish meal also causes, according to studies carried out in Japan, a deficiency in Manganese and Vitamin B_2, and in this case, it is advisable to supplement in consequence.

With carp and Channel catfish, the deficiency in Zn causes feeble growth and mortality, while no cataracts or dwarfism are remarked. The addition of 15 to 30 mg of Zn/kg ration prevents these symptoms.

j. Selenium

Selenium is the key element of glutathione-peroxidase which destroys toxic peroxides coming especially from rancid oil. The symptoms deficiency are similar to those when Vitamin E is lacking, the principal one being muscular dystrophy.

The requirements in Se are feeble, around 0.15 to 0.40 mg/kg for salmoneids. At rates of 13 mg, it becomes toxic.

k. Cobalt

This is found in the molecule of Vitamin B_{12} and does not seem to have a special fraction, when the requirements in vitamin B_{12} are ensured.

IV. CONCLUSION

Without vitamins, certain precautions concerning their conservation are necessary: In particular, never place in direct contact vitamins, oligo-elements, cholin chloride, but incorporate them into the other ingredients contained in the food.

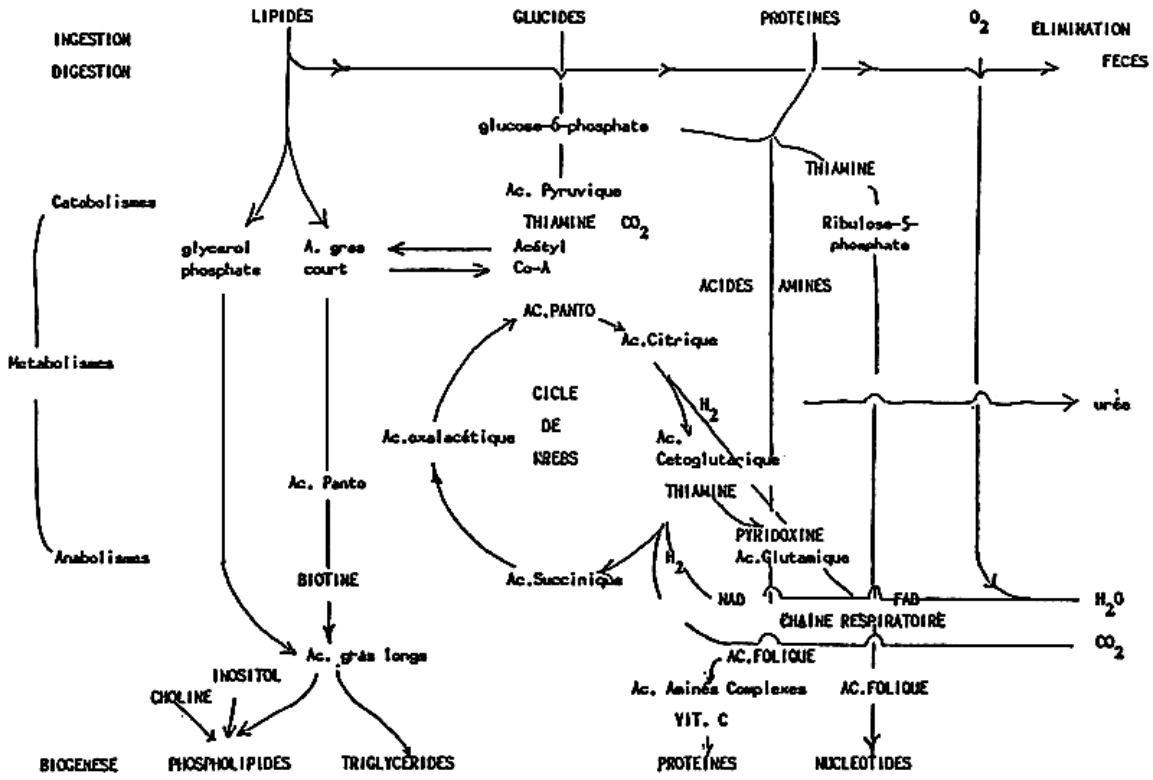
The food must be conserved in good conditions: away from humidity and in a temperature of not more than 20° C. In these conditions, it is advisable to renew fresh food every month. In Mediterranean climates, it is preferable to stock it in a cold room.

Vitamin C will continue to create a problem until a stable form can be commercialized. In these conditions, we must remember that a delay in growth and body deformations may be caused by a insufficient amount of Vitamin C resulting from the bad conditions of conservation.

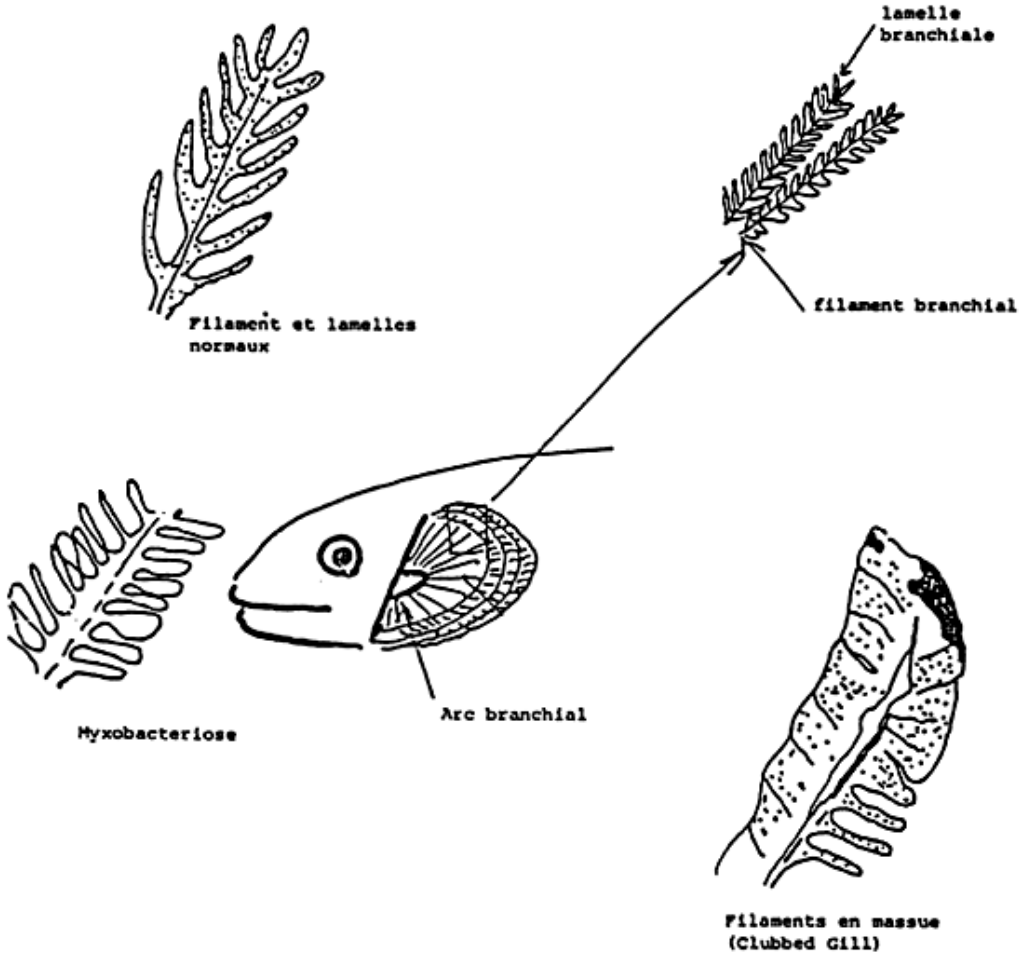
Experience has proven that important modifications in the formulation can give rise to certain deficiencies. It is normal to conjecture that the tendency to decrease animal products in favour of vegetable products will lead to the reconsideration of vitamin and especially mineral requirements.

Finally, vitaminotherapy, especially for specific and non specific immunostimulation, permits access to a research method which is only beginning to be explored and which is full of promise.

SCHEMA SIMPLIFIE DU ROLE DES VITAMINES DANS LES GRANDS METABOLISMES

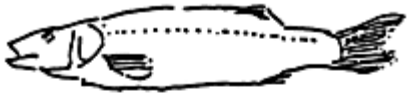


CARENCE EN ACIDE PANTOTHENIQUE



CARENCE EN VITAMINE C

Filaments branchiaux
tordus en spiral (Halver)

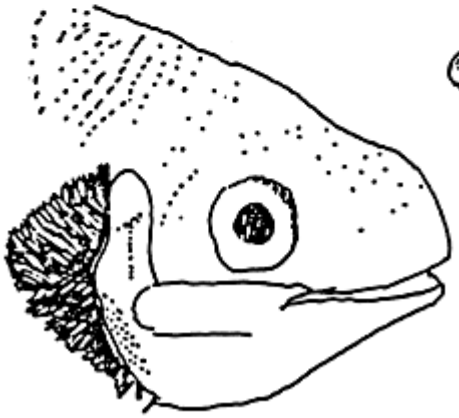


Bar normal

Bar carencé
DICENTRARCHUS LABRAX
(GODELUCK)

Lordose

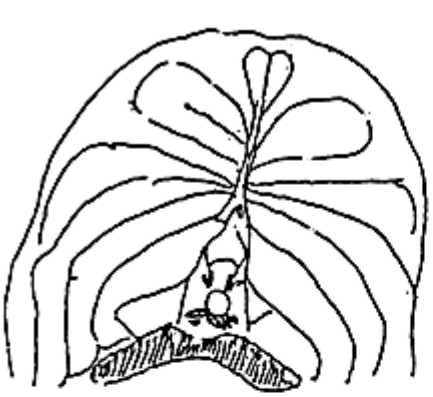
Scoliose



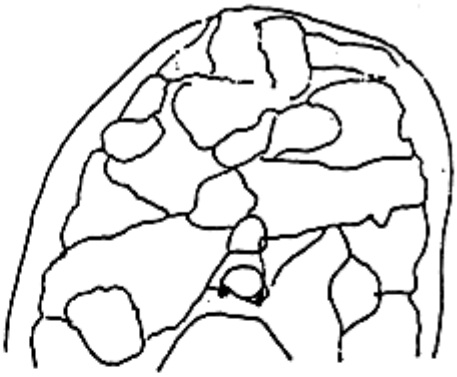
Raccourcissement de la tête
et de l'opercule (Halver)

DYSTROPHIE MYSCULAIRE

(Saumon atlantique)



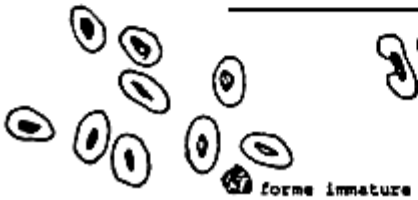
Normal



Carence en Vit.E et Se
(Hugh A. Poston)

Fibres musculaires
normales

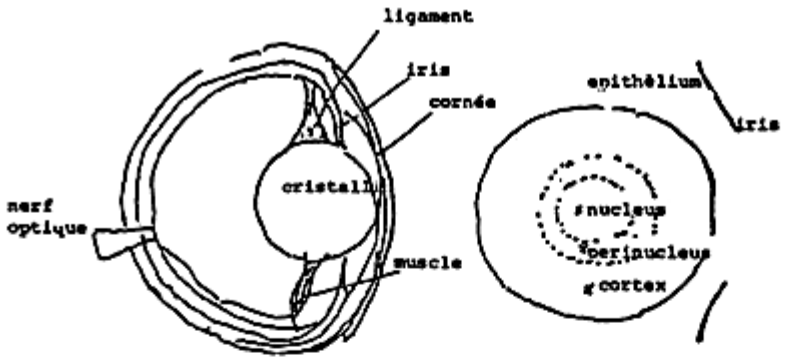
Fibres dégénérés



Globules rouges normaux (Coho)

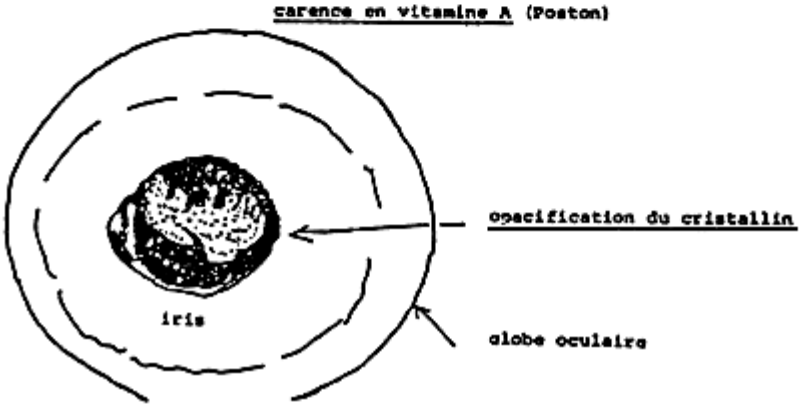
Carence en Acide Folique
(nucleus segmenté, pas de forme immature)

TROUBLES OCULAIRES

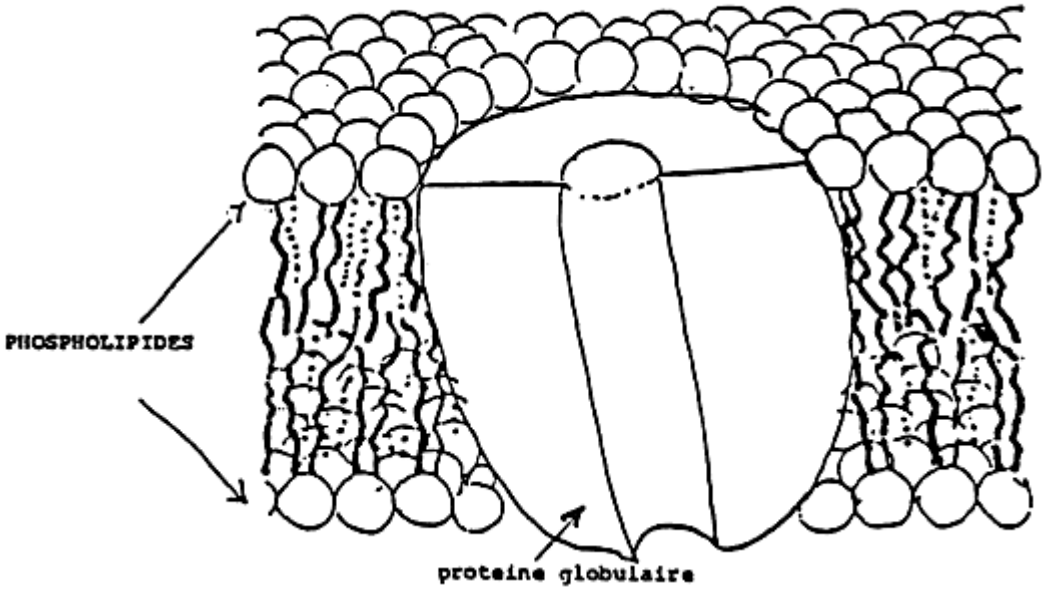


œil normal de téléostéen

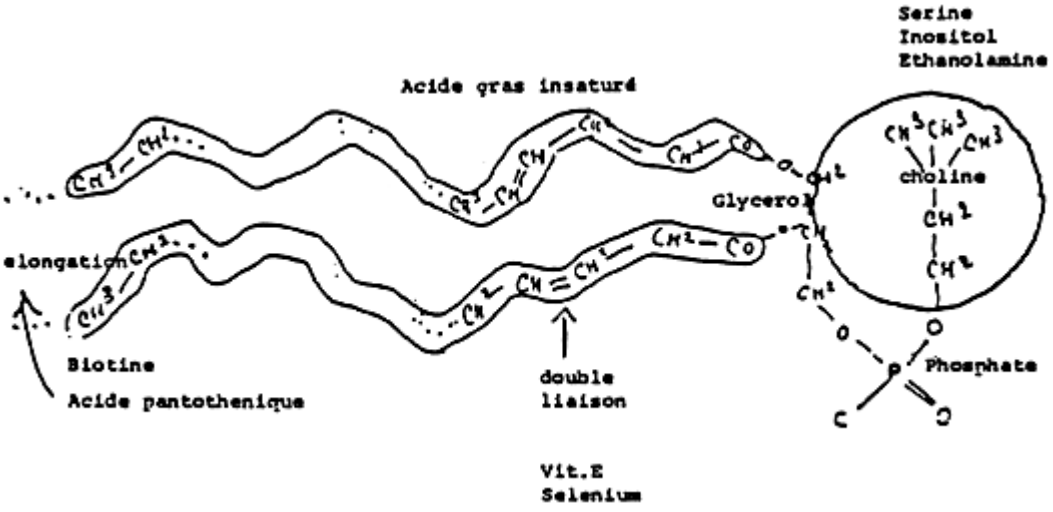
carence en vitamine A (Poston)



Structure de la membrane plasmique



MOLECULE DE PHOSPHOLIPIDE



COMPARATIVE NUTRITION FOR FISH AND CRUSTACEANS

DIGESTION AND DIGESTIVE SECRETIONS IN CRUSTACEANS

H.J. CECCALDI

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INTRODUCTION

The idea of comparing the function of nutrition in fish and in crustaceans seems at first impossible, as these two animals groups are so dissimilar. What can a sardine and a edible crab have in common? Skeleton, way of life, type of food, way of feeding, way of growth and this is only taking into account the principal aspects of their morphology or of their biology. However, these two animal groups which are so dissimilar possess two important common characteristics: they are both poikilothermic animals and most of them are aquatic species, adapted to living in fresh or sea-water. This report will deal especially with marine species.

LARVAL NUTRITION

After having given special attention to several homologous segments of the biological cycle, similarities can be clearly remarked between these two aquatic animal groups. First, it must be remembered that marine larval species coexist together in the plankton. They are submitted to the same conditions of environmental factors: salinity temperature, light, oxygen, nitrate and phosphate tenors. They are also submitted to the same variations from the external environment. They find the same phytoplankton species, they eat the same food particles, the same consumable protists.

In each case, the chemical composition and the biochemical characteristics of these food particles depend greatly on their origin, and these on the species to which they belong. The small size species will not have the same composition as large size species. Thus, when the larvae eat, they will obligatory select the small size particles, which mostly consist in phytoplankton cells.

The larger food particles are mostly of animal origin: large protozoa, small invertebrates, eggs or larval stages of other species. Thus the selection, according to size imposed by the dimension of the mouth on one hand, and by the grabbing capacity of these prey on the other hand, leads obligatory to the choice of food being made, the composition of which has been already defined. It must then be underlined that only species whose larvae have their nutritional requirements covered by the food available can survive in the pelagic environment.

QUALITATIVE NUTRITIONAL REQUIREMENTS

Apart from this similarity in composition when considered at a same period of time and during a same season, we must also take into consideration that the composition of these phytoplanktonic organisms varies with time especially concerning the sterol fatty acid tenors.(the length of the chains are more or less long, the gross insaturation of which is more or less high) or the tenor in carotenoid pigments We have only taken into account a few examples , taken from compounds of marine vegetable matter which have been studied correctly, although more often in a preliminary way. The small size animals which consume the phytoplankton see their own composition greatly modified. They themselves become prey for the larger larvae or post-larvae of fish and crustaceans. Thus is it a great similarity which exists between all plankton

eaters -most fish and crustaceans- as their food has the same basic composition and this composition evolutes with time in a similar way as for all consumers.

The ecological studies concerning the transfers in the trophic chains, rarely take into account this fact, although fundamental: the chemical composition of the elements which make up each of the segments of a trophic chain varies according to the different months of the year.

It is very probable that the larvae of different benthic species or the adult invertebrates of small size such as rotifers or copepoda have very delicate mechanisms for the sorting out, grabbing and selection of food particles, whether live or dead, animal or vegetable, having or not a bacterial covering. These mechanisms must be studied and explained in detail, thus enabling to quantify the influence of the composition of food ingested by the larvae, in other words, the particles available, for survival and vitality, the composition and growth of the animals. Survival and the good physiological state of crustaceans and fish at adult stage -until they reach the age for reproduction- will depend on the tenor in essential fatty acids or the indispensable amino-acids. It is most remarkable to observe that the requirements in long whain polyunsaturated fatty acids are very much alike for both fish larvae and crustacean larvae -at least for those which have been properly studied-.

The requirements in long chain polyunsaturated fatty acids, especially the linolenic series such as C 18: 3 w 3, C 20: 5 w 3, C 22: 6 w 3 correspond to well defined physiological functions, which have been recently identified and require more research.

These particular fatty acids, whose freezing point is situated at a much lower temperature than their saturated homologues, permit the membranes of diverse tissues to remain flexible and functional, even at very low temperatures. Marine animals must indeed permanently adapt themselves to the water whose salinity and temperature vary constantly. It has now been almost established that without a sufficient tenor in long chain polyunsaturated fatty acids, the survival of larvae along with the vitality of adults are greatly reduced. The mechanism and the method of action of these fatty acids have not yet been clearly established, although due to their presence in the membranes, the characteristics of the physical adaptation and control, at least partially, of the exchanges of the different ions between the external medium and the blood on one hand, and between the blood and the inter-cellular medium on the other hand.

This means how important the distribution of lipids of defined quality in the food is, for the survival of fish and crustaceans larvae, which are essential elements of marine trophic chains.

On the contrary, the requirements in sterols are quite different for crustaceans and for fish. The former are incapable of synthetizing the carbonaceous structure of sterols, although they have an essential requirement, both to synthetize their moulting hormones, ecdysones, and to preserve the integrity of their membranes. Without ecdysones, no exuvia nor growth take place. On the contrary, fish like all vertebrates, synthetize sterols and there is no need to add them to the compound food with which they are reared, as is necessary for crustaceans.

3. DIGESTIVE ENZYMS

An other fundamental aspect of marine animal larval nutrition is entailed by the evolution of their digestive enzyms during their larval growth.

The digestive enzyms -can be found in individuals even at egg stage- which start digesting the vitellus while the digestive tract is not yet distinguishable. It is remarkable to observe that it is the enzyms digesting the glucids and polysaccharides which appear first in the digestive tracts of marine animals.

Laminarises and amylases correspond in the mechanisms of larval feeding, to the composition of phytoplankton and particles which are available. These enzyms digesting glucides will progressively decrease and proteases of different nature, the list of which is but in beginning phase, appear progressively during growth.

A global index can be employed: For the report between the amylasic and proteasic activities which develop during growth and which present certain similarities in crustaceans and fish.

4. NUTRITION AND DIGESTIVE ENZYMS

Proteases exist in fish as soon as they hatch, even when their mouth is still closed. Their activity, feeble in the beginning, increases during their larval development. With herbivorous fish, for example carp, which live in fresh water, the amylasic activity maintains a remarkable level all through larval development. With carnivorous fish, the proteases/amylases ratio increases with age during larval and juvenile development. This ratio can constitute an index of the food diet. It reflects the digestive physiological potentialities of the species under study. Thus, we can differenciate the greatest and smallest degree of herbivoricity or carnivoricity of the different species belonging to a same family, Mugil or Penaeus for example. But this index is submitted itself to variations as the digestive enzymatic activities evolute themselves according to the quality of the food available and its composition.

Thus, when an adult crustacean eats food lacking proteins, its proteasic enzymatic activities are feeble. There is a better activity remarked when animals of the same species are fed protein-rich food. But when the protein tenor is too strong and when it reaches more than 45 to 50 %, the proteasic activities decrease, more so when there is a strong protein content in the food.

To our knowledge, there exists no detailed studies which has as objective the comparison of the molecule structures of the proteases in crustaceans and fish. Authors agree on the existence of trypsins in both zoological groups. Chymotrypsin has a very feeble activity in crustaceans and in decapoda, there exists a very feeble molecule weight protease, 11 000 daltons.

NUTRITION AND ENERGETIC BALANCE

There are many utilities for food, especially concerning the energetic expenditure: fish and crustaceans are confronted with the same biological problems: to ensure their growth, to fight the invasion of salts and thus maintain a compatible osmotic pressure with their physiology, to ensure their excretion to permit the development of their gonads and their reproduction, to permit compatible movements with their ethiology.

With fish as with crustaceans, it seems that proteins play a very important role not only for the supply of essential, the amino-acids employed for the biosynthesis of proteins, but also from a energetic viewpoint. In both cases, and due to the fact that they live in an aquatic environment, the catabolism of proteins is more economic on energy than for terrestrial animals, as 60 to 80 % of the nitrogen excreted is found in the form of ammoniac which is directly rejected through the gills into the environment.

There is a better growth return for fish than for crustaceans which suffer material losses through exuvia at each growth stage. These losses do not exist for fish, which make much better use of the food ingested, permitting much quicker growth and better conversion rate.

Locomotion is also better for fish, hydrodynamic animals generally do not employ a lot of energy to swim and have more flexible and efficient movements than crustaceans do. The latter have an articulated carapace, of rigid segments, which, only permits limited mechanical movements and sometimes permits the lighter ones to swim in a slightly similar way as fish. Except in the case of small size species like copepoda, or krill, the benthic character of crustaceans is ineffaceable. The complete opposite applies for fish which are less sedentary, and the species staying at the bottom, like pleuronects, living exclusively on benthic or soil-dwelling prey, are less numerous.

THE INTAKE OF FOOD

Filterer fish, such as sardines or herrings, in comparison to the numerous species that exist, are more scarce in proportion to filterer crustaceans, copepoda, euphausiacea and other pelagic crustaceans.

Due to the presence of multiple filters in the stomach of decapoda crustaceans, they can be considered as intense filterers, as they crunch their food into very fine particles. Only the fine particles have no access to the digestive tubules of the hepatopancreas, and are selected out by the filters, brushes, teeth and needles located in the posterior part of the pyloric stomach of this type of species.

Although lobsters have impressive sized claws and often agressive reactions they can not be compared to sharks whose jaws are of legendary ferocity.

Crustaceans can only swallow small size prey after they have masticated and dilacerated them by means of their anterior appendixes. On the contrary, certain fish can be very ravenous and extremly voracious and their eating habits depend greatly on the teeth which surround the anterior part of their digestive system.

The teeth of fish can vary considerably. From pointed teeth located in the buccal cavity of primitive fish such as lamprey, to the complete disparition of teeth in cyprinids, carps and gudgeons, there exists great differences in the masticating systems of fish. The group with the most efficient and rightly named "frightening" teeth is without doubt those of the shark, who changes its teeth periodically. Ray have their teeth set in rows with which they crunch the carapaces of sea-urchins and the shells of molluscs. Sea-bream have very hard teeth located on the palate with which the crunch the shells of lamellibranch-molluscs.

Blennys have very varied types of teeth and are heterodonta, as are sargos, borgues and oblades as like most omnivorous fish.

Most fish have similar teeth and are homodonta. These teeth can be found implanted not only in the maxillary arch but also in the roof of the mouth and even in the tongue as is seen in the pike.

Tetradons must adapt to their particuliar situation, diodons and parrot fish have four and sometimes only two teeth with which they browse on the coral reefs and catch prey which have found refuge in the deep cavities of the madrepores. Mullet have a wide open mouth which allows them to swallow debris and organic matter lying on the surface of the sediment. They can even suck in the particles which float on the surface of the water.

Among the other remarkable adaptations which fish present so to ensure the capture of food, we can quote the sea-bream and cat-fish barbels, the anglerfish traps, the apparition of denticles and asperities on the pharyngeal bones which play the role of tritural teeth for cyprinidae, the beaks of coral fish such as chaetodons, the blockated snouts of the hyppocampus, of Centriscus or oyster catchers, and of syngnathidae or of the fine and disproportioned teeth of numerous species of abyssal fish.

This list could be much longer. It would give an idea of the great diversity of fish teeth, which is quite the opposite to the simplicity and homogeneity of the means which crustaceans employ to collect their food.

DIGESTIVE TRACT

The digestive tract of crustaceans is more often very simple and rectilinear. Mastication takes place mostly in the stomach and absorption takes place in the tubules of the hepatopancreas. The digestive tract has not the same capacity of absorption as in fish and this is even more evident in mammalia as this function takes place in the tubules of the hepatopancreas.

In fish, the degree of differenciation of the digestive tract is very variable, starting with fish having a straight digestive tract and no stomach and arriving at fishing having sections which are very different morphologically, especially those with a stomach having chloride cells and areas for the secretion of pepsin. In this listing, we can thus find digestive systems which are quite similar to those of crustaceans and others which are closely related to the so-called superior vertebrates. During larval period, fish have simple digestive tracts. The digestion of proteins seems to be ensured by trypsin and chymotrypsin as is the case of crustaceans.

In most aquatic animals, salivary glands are missing. In most cases, the pyloric appendixes join up with the digestive tract of the pylorus. They vary in number from one species to another. Most sharks, rays and sturgeons have their absorbing surface extended by the presence in the mid-section of the intestine of a spiral valve which is not found in crustaceans.

Most fish families have a dorsal evagination of the digestive tract which is filled with gas so as to ensure a correct buoyancy and which permits the animal species to float between two waters. The crustacean does not possess an equivalent mechanism. Some, such as copepoda small in size, can contain, especially when they accumulate reserves, lipid drops, which enables them to float. But these lipids have no direct connection with the digestive tract.

NUTRITION AND TROPHIC MIGRATIONS

Both fish and crustaceans accumulate reserves at the end of a good season, when food is abundant. The reserves are accumulated in the form of lipids, principally triacylglycerols and glycogen. In fish, fatty tissue appears between the organs in the conjunctive tissue which supports the viscera. This is particularly remarked in sardines which can be very fat after the active consumption season of food. Glycogen can be stored in the liver and muscles. When an effort is made, it is the muscular glycogen which will be the first consumed. Migrating fish, especially salmon, store their reserves, after they leave the river where they were born, when they start their growth phase and acquire active feeding habits in the sea. When they begin their migration linked with spawning, they completely stop eating, so while in a completely fasting state, they swim up the estuary and water courses where they were born in the search of an original spot to spawn. After the emission of the gametes, becoming thin and fatigued, they die. We

see how important the active feeding period is, it completely conditions the whole reproduction phase. Trophic migrations have been observed for numerous species. They take place without changing the environment, at sea like for herrings, sprat, anchovy, mackerels and again sardines, cod, albacores and tuna fish. On the contrary, eel actively feed in fresh water and go to the sea to spawn, for example european eel go to the Sargasso Sea to spawn.

The similarities with crustaceans are very limited. Generally having very limited swimming capacities, migrations are not quite so important. The migrations of tropical lobsters have not yet been clearly explained. Indeed, the active feeding phases are very limited at stage C of the intermoulting cycle, which corresponds to a phase of active consumption of food for superior crustaceans and especially decapoda. If the quantity of food required has as consequence the accumulation of enough reserves, growth will be good during the following exuviation. If on the contrary there is no accumulation of reserves growth will be average or non-existant.

With small planktonic crustaceans, active consumption phase depends especially on vertical migrations and the food available during the circadian movements in the different water masses which they cross through.

NUTRITION AND REPRODUCTION

One of the ultimate objectives of the nutritional activity linked with the reproduction of each animal consists in accumulating reserves in its ovocytes. Each female must consent to making this effort without realizing that the survival of the species depends on this. It is necessary that each one enable the genetic programme to create an embryo, the latter must have at its disposition all the reserves necessary, both in quantity and quality, which will enable the larva to hatch in good conditions, and find its food. In this way, the biological cycle of each species is settled.

Both for crustaceans and fish an important part of this food ingested is directed towards the ovaries or testicles. The mechanisms of transport, the detailed structure of the carrier macromolecules, the vitellogenesis, the place and the mechanism of their synthesis require yet still numerous research especially for the part of the reproduction cycle which is situated on one hand between the digestive tract and the metabolic reserves of the animal and on the other hand, between the tissues which synthetize the vitellogenesis and the ovary. It is important however to underline that depending on the quality of the food absorbed and especially in accordance with the lipidic Part of lipoproteins, the reserves accumulated in the ovocytes will permit the acquisition of eggs of more or less good quality.

NUTRITION AND RESERVES

Reserves are consumed according to requirements. It is known that fish employ rarely their hepatic glycogen, only in the case of necessity, even if they are fasting. They prefer to catabolize the proteins and preserve their hepatic glycogen.

Crustaceans do not abide by the same rules for the consumption of reserves according to the different groups or the different environmental conditions which were studied: lipids are sometimes consumed first, glucids in other cases. Triacylglycerol, diacylglycerol, muscular glycogen which are abundant in crustaceans are the first to be consumed. The principal reserves are located in the hepatopancreas and are liberated during the moulting season.

NUTRITION AND AQUACULTURE

The aquaculture operations simulate, either by accelerating or delaying, the biological phases which occur in nature. Thus it is not surprising that, at least at larval stage, the animals reared are placed into environmental conditions similar to those found in natural conditions, or the most suitable conditions for the species in question which may not be identical. The food furnished must be more or less the same as that found in natural conditions or at least, suited to the species in rearing.

Beside these natural biological requirements, economic rules must be followed; it is, in particular, necessary to make proper use of the facilities existing, which represent more often very important investments.

In a hatchery, it is absolutely necessary to have under control algae cultures, which will be used at the first larval stages, both for fish and crustaceans. Besides this, algae in a rearing environment will permit the natural elimination of excretion matter coming from the animals in rearing. Artemia and rotifer cultures such as briachionus, will then be employed for the rearing of both fish and crustaceans. Finally, particles of animal origin, such as the minced washed flesh of molluscs or compound food particles will be used to feed both fish and crustaceans, the former however will adopt to this food more quickly.

In any case, the reconstitution of the different stages of the natural biological cycle in controlled conditions always nearly requires very similar hatchery and production structures, even for the way in which they are operated, sequential lighting, regular spaced out feeding, environmental control.

CONCLUSION

To conclude, the cyclic variations of the environmental conditions, the existence of the same natural food and prey in the biocenoses bring about a similarity, concerning the feeding methods employed, the feeding behaviour and the coverage of the physiological requirements, between fish and crustaceans, especially at larval and post-larval stages. This great original similarity develops during their ulterior differenciation, as they grow, both in natural environments as in aquaculture.

As for compound food, there exist many common points: crustaceans and fish require the same ten essential amino-acids and a rather high protein content rate in their food. Very often, the same basic meal employed in the manufacture of compound food will be used. So as to cover the fatty acid requirements, the same appropriated oils are employed. Research on the eventual origins of proteins or of lipids used in the manufacture of commercial type food, whether for fish or crustaceans, have turned out to be much more general and of major interest for the rearing of these aquatic animals.

Thus we see, that even though of very different phylogenic origine, fish and crustaceans have sufficient points in common so that the aquaculture structures where they are reared can be similar or even employed for both fish and crustaceans. Their integration in very similar trophic systems, in the natural environment, is a basis for the ressemblances of the rearing structures in aquaculture.

GENERALITIES

Digestion can be defined in many ways depending on the specialist studying it. Indeed, nutrition typically includes, the intake of food, from the exterior environment, its assimilation and its role in the maintenance of the growth of the organisms which ingest it. This is the most typical way in which to consider nutritional mechanisms.

But digestion can be studied in a more precise manner, in taking into consideration the processes which take place at cellular level or the metabolic cycles, the enzymatic activities and carrier mechanisms in the interior medium.

Finally, we can enlarge yet more the examination of phenomena linked with nutrition by examining the endocrinological regulations linked with digestion, along with the excretion and respiration processes which are in control of what happens to the food after it has been assimilated.

This report will not take into account the excretion and respiration functions.

A REMINDER CONCERNING THE PHENOMENA LINKED WITH GROWTH IN CRUSTACEANS

Growth occurs with successive moults in crustaceans. The moment when the animal looses his old tegument or exuvia, is known as exuviation- DRACH, 1938, to his merit developed a system permitting a clear comprehension of the evolution during the different stages which takes place from one exuviation to the next. Immediately on loosing his exoskeleton, the animal increases in volume by absorbing great quantities of water. Being very limp, it cannot eat for several hours or even days, and will rely solely on its reserves, which it has accumulated during the previous stages, to survive and overcome this natural physiological crises. The moults become more and more spaced out as the animal grows older. It must be remembered that with crustaceans, growth is discontinuous and is characterized by a particular cycle: The constitution of reserves, the consumption of reserves, from one exuviation to the next, in other words, during each intermoulting cycle.

The reserves of crustaceans are found essentially for adults, in the cells of the digestive glands. In decapoda, this gland, located at mid-gut level, is known as the hepatopancreas. This denomination is not exactly valid, and certain authors have rightly tried to define it otherwise in proposing, for example, as term the mid-gut gland. The reserve substances are principally made up of glycogen, lipids, vitamins, pigments and mineral salts, principally calcium. Glycogen reserves can also found in the muscle.

The reserves found in the eggs during their development are constituted by the vitellus which develops during the ovogenesis. As for many species, the embryo, void of a digestive tract, but consuming however reserves, sustains on the vitellus. When there is a feeble quantity of vitellia reserves, the larvae hatch quite quickly after the spawning has taken place, when the last vitellin of the egg has been consumed. Young larvae feed on phytoplankton more or less at first.

It is of prime importance to know the quantitative and especially qualitative variations of the food which is consumed by the crustacean planktonic larvae during growth. In natural environments, the first larvae choose cells coming from diverse species of phytoplankton, according to the size of their mouths or the shape or hardness of these cells. From then onwards they choose either organic particle or more often small living animals, larvae or small invertebrates which live in the plankton. They thus acquire progressively a carnivorous behaviour and physiology as they get older.

The feeding behaviour of adults is practically acquired as soon as the benthic way of life has been established by the species in question.

DIGESTIVE SYSTEM IN CRUSTACEANS

Food diets

The food diets of crustaceans vary greatly from one group to another. Indeed certain species consume algae, while others are strictly carnivorous, detritivorous, necrophagous or filterers of particles.

Appendixes employed to obtain food

For Decapoda, the most anterior appendixes are generally employed to obtain food, in a very developed and specific way as they are located near the mouth. The appendixes located on the cephalic metameres; the mandibles, maxillas, maxillulas surround the mouth and dilacerate the food before the latter is introduced into the oesophagus. The two pair of antennas: antennules and antennas, play a role in the chemical reception, in other words in the reconnaissance of food thanks to dissolved molecules that are released into the environment.

For Decapoda, the three anterior pairs of thoracic appendixes are adapted to the function of nutrition: they transform into claw jaws or maxillipeda. These appendixes also, manipulate and dilacerate food, while the posterior appendixes, five pairs, are employed for the locomotion.

The food thus dilacerated reaches the stomach where it is reduced to a very finely minced gruel.

Digestive tract

The digestive tract in Copepoda is rectilineal. The anterior part is generally enlarged while at the same time, it does not really form a stomach. Copepoda do not possess a specialized digestive gland. The absorbing cells and the reserve cells constitute the walls of this digestive tract.

Isopoda have generally three pairs of digestive caecums located on either side of the digestive tract and which are constituted by a basement membrane which supports an epithelium. Numerous cells with brushy borders constitute the epithelium. The absorption and the storing of reserves take place in the caecums.

The digestive tract of Decapoda is divided in three parts, the head-gut or stomodaeum, mid-gut or mesenteron, the hind-gut or proctodaeum. The stomodaeum and proctodaeum are coated in chitin and this coating is rejected at each exuviation.

The stomach

The stomach comprises two pouches, the anterior cardiac pouch (or stomachic bag) where the food ingested accumulates and the posterior pyloric pouch which possesses calcareous parts, bristles, needles, filters along with recesses and proeminences where the food will be pulverized successively as it passes through each one.

The posterior part of the cardiac stomach and the pyloric stomach is reinforced and supported by a group of articulated calcareous parts, plates and ossicles. These parts and areas are thickened by the chitinous coating of this organ.

The mucus of the stomach is similar to that of the oesophagus. The walls of this organ are not flat and include a great number of recesses of diverse form and size. The

height of the cells constituting the stomach walls vary from one place to another in this organ. There are also a certain number of bristles and needles located inside the stomach, of different size depending on where they are located.

The masticatory parts of the stomach.

The anterior and posterior portions are lined inside with a proteic chitin membrane which joins up with the exoskeleton. It is also subject to periodical moults like the exoskeleton. The anterior part of the digestive tract thus possesses a reinforced coating, on the inner surface, prominences, which can submit the food to extreme trituration. These prominences can become calcareous and create numerous skeletic parts of different forms. The whole lot form a real inner skeleton with a very special articulation. Each part is activated by the muscles located on the outside of the stomach wall, the movements of which are controlled by a group of characteristic nervous elements. The prominences and recesses differ in form and disposition from one group to another. These systems have been more or less largely described over the past century. The studies carried out show that the stomach framework can be summarily compared to a "three forked claw that the food must pass through to reach the pylorus" (MILNE - EDWARDS, 1834). HUXLEY (1880) compared the stomach system to a gastric mill and the pyloric portion to a filter.

The stomach thus comprises, on its inner surface hard elements or ossicles, with the same function as teeth, forming a triturant or masticatory device and a group of recesses and valvules. There also exists near the posterior part of the stomach and the pylorus, bristles, needles and tubules which play the part of a complex filter.

Structure and terminology of the ossicles

All the ossicles with the exception of those situated in the symmetry axis of the stomach, are symmetrical, bilateral and in twos. A general terminology for ossicles was proposed by MOCQUARD (1883). It is still valid to-day and has been reemployed by MAYNARD and DANDO (1974) with some modifications which were proposed by COCHRAU (1935).

Thirty three ossicles at least have been described during the studies carried out on compared anatomy. They can be divided into seven categories, depending on what role it is beleived that they must play. Indeed their exact role requires to be defined in detail.

Seven fundamental categories have been described and show great variations joinings and expansions. In certain species, the ossicles form calcified plates: their form and size can vary greatly, which makes their homology difficult from one species to the other.

The masticatory parts have thus roles, differing in number and in quality, depending on where they are located respectfully. The first fifteen parts located in the cardiac bag constitute a first anterior sub-system often known as the gastric mill. The anterior parts, stronger and more calcified are special ossicles which take the name of teeth. The second sub-system, made up by at least eighteen parts, which are smaller and less calcified, participate in the functioning of the filter sub-system in the pylorus region.

Several specialists have admitted that the efficiency of a stomach depended on its complexity. Finally, it must be remarked that the complexity of the mandibles varies in an inverse manner to that of the stomach.

Trituration in the stomach

The food passes down the digestive tract through complex passages. Movement depends greatly on the size of the food.

The cardiac bag of the stomach is separated from the pyloric bag by a cardio-pyloric valvula, thus giving two adjacent bags. Big particles remain in the cardiac bag; they are digested, by the movement of the stomach muscles, in the dorsal part of the bag where the gastric mill parts intervene.

The particles can pass beyond the cardio-pyloric valvula while remaining in the plane of symmetry of the stomach and enter into the dorsal part of the pyloric bag. The finer particles pass into the mid-gut gland. The bigger ones are retained back by a filter located at the entrance of the hepatopancreas and are directed later on towards the intestine.

The rhythmic movements of the different regions are ensured by a striated musculation, the contractions are entirely controlled by neurons.

The stomato-gastric nervous system of Decapoda crustaceans is doted with an important ganglion which innervates the anterior part of the digestive tract. This ganglion can control two neuronic systems:

- The system which ensures the rhythmic motricity of the gastric teeth (12 neurons in the Palinurus vulgaris) spiny lobster.

- The system which ensures the rhythmic motricity of the pyloric region and which comprises 12 neurons (14 neurons in the Palinurus vulgaris) spiny lobster.

These systems organize alone all the rhythmic activity of the head-gut.

The rhythmic activities of the digestive tract

The cardiac bag of the stomach is the centre of rhythmic contractions, with a frequence of one contraction every 8 to 10 seconds. These movements are ensured by the activity of the 12 neurons: 10 motor neurons and 2 interneurons. The side teeth, fixed laterally, in the most posterior part of the cardiac bag, have transversal rhythmic movements which are controlled by four motor neurons.

The medium tooth fixed on the roof of this bag at the level of the lateral teeth, has coordinated rhythmic movements. It has longitudinal movements controlled by six motor neurons.

The pyloric bag is characterized by construction movements, the first in the anterior part. These successive and coordinated movements ensure the filtration mechanisms and permit the progression of the food towards the mid and end-gut. The contractions take place every 1 to 2 seconds.

When food is given to a Jasus lalandei lobster, it swallows the food although the rhythmic movements of the cardiac bag do not commence immediatly.

The pyloric bag, which was the centre of contractions of regular intensity and with frequencies of around 1 every 3 seconds, becomes the centre of rhythmic contractions which are more regular and shorter, one every second, which commence within one minute after the ingestion of food. The rhythmic contractions of the cardiac bag don't begin until 3 hours later, although the food lies there.

The apparition of the rhythmic contractions of the cardiac bag could be linked with the secretion of the digestive enzyms from the tubules of the hepatopancreas to the stomach.

It is in the stomach that the food is transformed into a liquid gruel, and here again that the greater part of the chemical digestion of the food takes place.

In the other groups of free crustaceans, the mechanical degradation depends on the food diet and on the morphology of the appendixes. For example, filterer Copepoda and Cirripeda have certain appendixes doted with byssus with which they collect the food particles in suspension in the water. Parasites, on the other hand, possess special organs for each group.

FOOD REQUIREMENTS

Global requirements

Crustaceans must cover important requirements: locomotion, exuviation, the constitution of an new exoskeleton at each moulting period, growth, excretion, maintenance of the osmotic pressure and especially for females, the production of gametes. The latter is important as the ovaries represent a very remarkable volume of the body weight.

These requirements are rather badly known, even from a quantitative view-point. The Copepoda Calanus finmarchicus filters 70 ml of water per day so as to ensure its food, in water which has an average planktonic resource.

Studies have proven that planktonic crustaceans barely succeed in feeding themselves correctly with the quantities of plankton available during the year. They sometimes acquire a cannibal behaviour. The same applies for crustacean larvae during growth, in both natural and hatchery environments.

The requirements vary greatly depending on growth. The oxygen consumption per weight unit, which is a convenient way to define the metabolism, is all the greater when the animals are small in size.

It seems that larvae of Decapoda crustaceans feed at all hours of the day and night. The post-larvae of Peneidae take four meals per day while adults are satisfied with two or even one meal per day. The progressive acquisition of cyclic feeding activities develops at the same time with the acquisition of the possibilities to ensure food reserves and the establishment of a digestive enzymatic secretion rhythm.

The quantities of food that must be furnished to the same species of animals of different size, varies according to the size. The following formula is employed :

$$Q = n P^i$$

where Q is the quantity of food absorbed per day,
P is the weight of the animal,
n is the quantity of food per time unit in given conditions,
and i is the rate increase of the food absorbed, when the weight of the animal increases.

Food quantities

Few species have been studied from this point of view. As has been already stated here above, the oxygen consumption is one of the best ways to define the metabolism of animals, but the food absorbed by the food canal (and not swallowed)

must be taken into account, as the food rejected by the organisms studied and not digested must be taken into account.

Quantitative requirements in basic nutriments

They vary greatly depending on the food diets of the species taken into consideration. Thus, the more carnivorous species can not be reared if the food furnished has not a high protein tenor. We can give as very approximative values for the global composition of food, the following figures: proteins 45 %, glucides 30 %, lipids 10 %, mineral mixture 5 %, vitamins 2 %, binders 3 %, indigestible ballest 5 %.

It must be remarked here that the protein requirements vary according to the size, thus the age of the animals. On general, the optimum protein tenor decreases during growth. REGNAULT showed with Crangon that the best growth rates were obtained with food containing 60 % of proteins for juveniles, while 30 % was sufficient for older animals.

As for the amount of food, the requirements will also decrease during growth. During the rearing of Peneidae shrimps, juveniles wegning between 0,2 and 0,5 g consume daily 50 % of their live weight in food. The values decrease and reach 25 % between 1 g and 2 g, 15 % at 10 g, and 5 % at 20 g.

Qualitative requirements

The essential amino-acids have been identified: arginin, threonin, methionin, valin, isoleucin, leucin, lysin, histidin, phenolalanin, tryptophan. The protein consumed in natural environments or employed in rearing must contain these diverse amino-acids in sufficient amounts. In certain cases, basic or sulphur amino-acids are added to the mixture so as to try and compensate deficiencies. The results obtained are not always better and sometimes they are even very bad.

Lipids play an important part in the metabolism of crustaceans: a certain number of fatty acids prove to be necessary. Long chain unsaturated fatty acids, especially the eicosapentanoic (C_{20} : 5) and docosahexanoic (C_{22} : 6) fatty acids are especially important. The linolenic series of fatty acids are more effective than the linoleic series of fatty acids. Those whose first double link is found in position n 3(ou w 3) are more effective than those with their first double link in position n 6 or n 9.

The fatty acid composition of reared crustaceans varies depending on the lipid composition of the food ingested.

The vitamin requirements are not known precisely; However, certain works carried out have proven that crustaceans must receive in their food vitamin C, pantothenic acid, sterols. The latter plays a particular important part, as the moult hormones, the ecdysons, are synthetized by crustaceans from sterols.

Natural food diets

In a natural environment, the crustacean populations depend on biotops in which they find both in quality and quantity, food which is apt to cover their requirements.

As these requirements vary during the growth of each animal, the biotop where they live must not only be able to supply their instantaneous requirements but also those for later on.

Research work has been carried out on crustaceans in rearing concerning their stomach content, in spite of the fact that the effect of the masticatory parts of the stomach make the organisms during ingestion and ingested, unidentifiable.

BEN MUSTAPHA (1962) obtained the following composition for species consumed by shrimp:

- Crustaceans 14.2 %
- Polychaeta 16.6 %
- Molluscs
  - Pelecypoda 39.5 %
  - Gasteropoda 9.3 %
  - Scaphopoda 17.5%
  - Cephalopoda 0.4 %
- Echinodermata 2.2 %

The stomach also contained a non negligeable fraction of sandy-muddy sediment.

On the other hand, the composition of the benthic populations and the number of crustaceans in nature depends especially on the granulometry and hydrodynamics of the beds of the zones in question.

FOOD EMPLOYED IN AQUACULTURE

Natural food

The development of natural populations is always limited by the production of food available in the biotop where they are found. In intensive rearing, the food furnished is obtained from another biotop, where it was gathered and transported to the rearing tank where it will be consumed.

The animals the most frequently employed as food are lamellibranch molluscs for example Venerupis (= Tapes) philippinarium, or Mytilus, in Japan. Cephalopoda mollusks, such as frozen squid, are employed in the United States, euphausiacea crustaceans fished in cold seas (krill) are employed in Japan as food in tank rearings. Waste fish from trawler fishing is also often added.

Research carried out by HUDINAGA and KITTAKA (1967) on Penaeus japonicus post larvae has permitted to obtain relations between the live weight of Venerupis/the weight of P 21 post-larvae of Penaeus equal to 2.4 for 21 day-old post-larvae after metamorphosis.

Natural compound food

For economic reasons as well as to cover adequately the requirement of crustaceans in rearing, food manufactured while employing natural ingredients which have been reduced to power form and processed by the use of physico-chemical methods seem to be becoming more and more renown. The animal or vegetable meal which contains protein and glucid tenors capable of covering the requirements of the animals, is the basic point of this compound food (NEW, 1976). Lipids, vitamin and mineral salts are then added. The mechanical cohesion and maintenance in the water is ensured by diverse binders, whose properties have sometimes been studied in detail (FOSTER, 1972).

Proteins represent the prime fraction of the composition of compound food. Traditional proteins may be employed in feeding. The meal of animal origin which is employed, is fish meal of crustaceans obtained from the cephalo-thorax of shrimp, cephalopoda meal obtained from squid, and soluble concentrated meal from fish. The proteins of vegetable origin usually employed is soya or copra meal. Proteins obtained by the employment of

more recent methods, for example from Spirulin algae, yeast growing on alkans, or brewers' yeast are also employed.

The glucids employed most frequently are corn meal, wheat, lucerne, rice. Different starches are employed, but their digestibility by the amylases of the digestive tract of crustaceans varies greatly from one starch to another.

The lipids employed are vegetable oils or oils obtained from marine organisms, such as cod liver oil especially, or other fish liver oils or squid liver oil. It is necessary, that the lipid furnished, contain sterols and carotenoid pigments. The food is completed on one hand by vitamins presented in the form of mixtures which have been previously prepared, and on the other hand in the form of mineral mixtures which contain principally elements such as calcium, phosphorus and certain metallic oligo-elernents such as copper, zinc, iron, manganese for example, which are also added to the compound ingredients during manufacture.

When the compound food possesses the basic ingredients obtained by means of processing or new technological methods, it is sometimes incorrectly called synthetic food.

Presentation

Pellets must have a consistance so that the crustaceans may easily manipulate them by means of their appendixes, while not crumbling them into bits; also they must be able to remain in the water a rather long time; several hours. It is thus necessary to employ binders which permit the fine particle ingredients to stick together. These binders can consist in polysaccharides obtained from algae, such as agar, alginates, carragheen or terrestrial vegetable by-products such as pectine gum and guaranates. Other binders of animal origin such as gelatine or chitine by-products, and even synthetic binders manufactured by chemical industries such as carboxymethy-cellulose or polyvinyl are also employed.

In order that the food be not only accepted but also searched for by the animals, the pellets are coated with appetizers. These compounds are generally marine organism extracts such as polycheta, cephalopoda, or lamellibranches; organic; molecule mixtures, the appetizing properties of which are known, are also employed for example certain amino-acids or certain nitrogenous molecules such as trimethylamin oxide.

Vacuum processing is employed for the manufacture of compound food. This method permits the acquisition of pellets which can be conserved and handled more easily than fresh food.

However, soft food presented in the form of pastes or jellies, ensures a better growth than that obtained with dry pellets.

The chemical degradation of food

The chemical degradation of food is caused by the action of the digestive enzyms from the hepato-pancreas in particular. This complex organ ensures several functions. As a rule, we generally admit that along with its functions in the secretion of digestive enzyms and in the temporary and cyclic retention of reserves, the hepato-pancreas is the principal organ which ensures the absorbtion of digestive products (GIBSON and BARKER, 1979).

The hepato-pancreas consists of a set of blind end tubules; The open ends permit the products by secretion to be discharged into the stomach.

The walls of the tubules combine cells of several types: absorbtion and accumulation cells, secretion cells, embryonic cells and fibrillar cells.

The secretion cells or B cells (taken from the german word Blasenzellan) have a basal nucleus and large cytoplasmic vacuoles containing an acid matter which flocculates easily. They have a striated border. These cells have a restricted quantity of reserves in the form of lipids, glycogen, and calcium of phosphate. The mechanism of secretion differ depending on the groups: apocrin in Panulirus, holocrin in Astacus or mecrocrine in other species. The mechanism of enzym evacuation is not well known in detail, due to the difficulty to observe the phenomena which take place inside a massive organ. It is at present believed (LOIZZI, 1971) that R cells (from the german word restzellen) or absorbing cells, accumulate the nutriment which are found in the light areas of the hepato-pancreas tubules, and that they synthetize glycogen and lipids. F cells described as being "fibrillar" synthetize the digestive enzyms and keep them in reserve in a supernuclear vacuole. The latter will fatten by pinocytosis, in taking the nutriments from the light areas at the tubules until they form a typical B cell.

When the B cell are full, they are the most voluminous cells of the hepatopancreas. They contain an unique central vacuole, representing at least 4/5 of the cell volume. The cytoplasm is dense and thin, containing myelinic forms, groups of parallel filaments and other varied inclusions. There is a complex apical which separates the vacuole of the tubule light, having a brushy border, small vacuoles of pinocytosis, dense cytoplasm, small mitochondries, microtubules. When at full maturation stage the vacuole will compress the basic cell nucleas. No glycogen granulas nor lipidic drops are found present in cell B.

It is believed that the secretion is of merocrine or apocrin type in normal physiological conditions but that in intense stimulation it can be of holocrin type.

The expulsion of digestive secretions from cell B could be caused by the contracting and constricting muscle network which surrounds the external wall of the tubules (LAEVITT and BAYER, 1982).

The secretion of cells has been remarked as simultaneous for several cells located at the same level in each tubule of the hepato-pancreas (BOCHEN, comm. pers.)

The digestive enzyms

The digestive enzyms secreted, differ greatly from one type to another. Although some studies have been carried out on enzyms, during the past century, the identification and detailed description of each one has only been commenced. We know however that several proteases exist in certain peneid shrimp, such as the Astacus. Carboxypeptidase A and B activities (GATES and TRAVIS, 1969, 1973; GALGANI, 1985) similar to trypsin activity have been remarked and show a molar mass of 34 200. Aminopeptidases and dipeptidases were discovered after a chromatographical or electrophoretical separation (DE VILLEZ, 1965; DEVILLEZ and BUSCHEN, 1967; LEE, 1980; MURAMATSU and MORITA, 1981). There exists especially a very active protease of a feeble 11 000 Daltons molar mass (PFLEIDERER et al., 1981); this enzym seems only to exist in Decapoda. It has the same role as that of pepsin, the latter being absent in the hepatopancreas.

In certain crustaceans such as Penaeus japonicus, there exists a feeble collagenolytic activity.

The trypsine studied in the same species is made up of 6 isoenzyms. Its molar mass is 25 000 Daltons. It alone represents more than half of the proteasic activities of the hepatopancreas in Peneid crustaceans. An immune serum obtained with a trypsin of Penaeus japonicus reacts positively against the trypsins of eight other species of peneids. The sequence of the trypsin from the Astacus fluvialitia Crayfish has been recently defined (TITANI et al., 1980) and presents around a 50 % homology with bovine trypsin.

The echymotrypsin activity, revealed in several species of crustaceans is generally feeble (BRUN and WOJTOWICZ, 1976; TRELLU and CECALDI, 1977; GALGANI, 1985). In Palaemon serratus, chymotrypsin has an average activity and appears as soon as embryogenesis begins.

Some enzyms capable of hydrolizing native collagen has been revealed and characterized in several species (GRANT et al., 1981; GALGANI, 1985).

Enzyms digesting glucids and polysaccharides also exist; amylases, maltases, saccharases, and sometimes cellulases. In Palaemon serratus, beta-glucosaminidase, beta-glucosidase, alpha-mannosidase, B-fructofuranosidase and alpha-fucosidase have been defined.

The α-amylase of Palaemon serratus has a molar mass of around 50 000 (VAN WORMHOUDT, 1980). It is made up of 2 and sometimes 3 isoenzyms depending on their geographical origin.

Three glucuronidases of 235 000, 275 000 and 370 000 molar masses have been defined in Palaemon serratus (TRELLU and CECCALDI, 1976).

The digestion of lipids is ensured by lipases and esterases. The lipases have an effect on the lipids present in the form of emulsions and the esterases continue the enzymatic digestions on the hydrosoluble products presented. This has been established for more than a century by HOPPE-SEYLER (1877) who demonstrated the digestion of olive oil by the digestive juice of the Astacus lobster. Several esterasic activities have been discovered in the digestive juice of different species. Although the energetic metabolism of crustaceans is largely under the influence of lipids, tnere also exists a righ number of esterases. Each one plays a role, which is sometimes very specific and much greater, on the molecules to be digested having ester functions. TRELLU and, CECCALDI (197§) have proven the existance of 20 sets in using as substratum α-naphtyl-acetate in Palaemon serratus.

Chitinases also exist, permitting the digestion of the chitin which forms the exoskeletons: numerous crustaceans are indeed predators of other crustaceans and on the other hand, some of them consume their own exuvia after moulting. Taking this biochemical definition of chitin as an example, JEUNIAUX defined real chitinases as enzyms which liberate, by their action, n-acetyl-glucosamine.

Other enzymatic activities, such as desoxyribonuclease of 33 000 molar mass, ribonuclease of 25 000 molar mass and alkaline phosphatases have been defined.

Crustaceans also have emulsifying compounds which play the same role as bile in mammals, in other words it disperses fats before their digestion. These compounds have been defined and studied by VONK. They are formed of taurin by-products, taurocholic and taurodesoxycholic acids.

The optimum pH activity of the different enzyms is very variable, ranging from 5.5 to 9. In most cases, optimum pH is much higher than in vertebrates and especially in mammals.

The global proteolytic activity of the hepato-pancreas of Penaeus kerathurus has an optimal pH of 8.5 for gelatine and 9.5 for casein.

The optimal temperature of this activity is 50° C (GALGANI, 1985).

The trypsic activity of 5 peneid species has an optimum pH around 8.3, a carboxypeptidase A activity of 7.5, a carboxypeptidase B activity of 9.2.

α- amylase shows a optimal pH of around 6.3 to 6.8.

Variation of digestive enzymatic activities

Variations during the intermoult cycle

During the intermoult cycle defined by DRACH (1939), the cycle of reserve storage - reserve consumption which characterizes the physiological variations caused by the exuviation and the formation of a new exoskeleton, is closely connected with the digestive activities. The digestive enzyms indeed show variations in their activities during the intermoult season (BAUCHAU and MENGEOT, 1965; VAN WORMHOUDT et al., 1972 b; TRELLU and CECCALDI, 1977).

Feeble, at stages A and B, after exuviation, they increase during the storing periods of reserves, during the different stages of stage C. During the active growth of crustaceans, in other words during the Summer months and beginning of Autumn, two digestive enzymatic activities are at maximum during the intermoult season, the first during stage C, the second during stage D_1 - D_2. On the contrary, during the cold months, only one maximum during the intermoult cycle at stage D_0 is remarked.

Variations during the circadian cycle

During the circadian cycle, the digestive enzymatic activities are submitted to important variations. Two maximums have been defined in some species such as Penaeus japonicus and Palaemon serratus: The first maximum takes place in the morning, the second one in the evening, twelve hours after the first. It has been established through recent research that the first maximum was started by the beginning of the light phase and was produced around five hours after the passage obscurity-light (VAN WORMHOUDT, 1977).

The daily duration of the photophase plays an important role in the circadian cycle of the digestive enzymatic activities. Preliminary studies were carried out, but this data must be specified by systemized studies (VAN WORMHOUDT and CECCALDI 1974; TRELLU and CECCALDI, 1980 b). The length of the light wave employed also plays an important role on the digestive enzymatic activities, the blue green light being beneficial (VAN WORMHOUDT and MALCOSTE, 1976).

The circadian rhythm of digestive enzymatic activities is not acquired at first larval stages during which irregular cyclic variations are remarked. The enzymatic activities pass through a tetracircadian phase at Zoea stage 4, and then a bicircadian cycle by a progressive decrease of both the digestive enzymatic activity peaks. Some observers have indicated that enzymatic activity increases from 1 to 4 hours after feeding.

Variation during larval development

It is of prime importance to feed the larvae during their growth, food compositions which have been adapted to the physiological digestive capacities of the different larval stages. In particular, the composition of the food must correspond to the enzymatic activities of the digestive tract of the larvae and of their evolution during growth. Specific activities are generally expressed in milligrammes of soluble proteins of the heapto-pancreas.

The Palaemon serratus has a high growth rate in the first stages, which decreases during the last larval stages. On parallel, the activity of the digestive enzyms is reduced, along with that of the amylase which greatly decreases. The relation amylase/prolease thus increases from Zoea 1 to Zoea 4 stage and then decreases until metamorphosis takes place. Later on, it again increases (VAN WORMHOUDT, 1981). At first larval stage the larvae don't feed. First Zoea stages are fed mostly phytoplankton rich in polysaccharides. The increase of proteases takes place at Zoea 4 stage when the larvae begin to feed on live animal prey.

The Macrobrachium rosenbergii, at first larval stages; Zoea 1 and 2 do not feed and live solely on their reserves. When they reach the following stage the amylasic and proteasic activities increase greatly at first and from then afterwards in a more regular and moderate way. At Zoea stage 6, the specific activity of the amylase increases and is multiplied by 5 while that of proteases is only multiplied by 2.

The feeble digestive enzymatic activity of Penaeus japonicus during nauplius stages is greatly increased at metanauplius stages. The increase in the specific amylasic activities is multiplied by 15 while it will only increase 5 times between Zoea and Mysis stages. The digestive enzymatic activities are at maximum at Mysis stage 1; They decrease until the metamorphosis into post-larvae takes place, and then increase once again progressively (LAUBIER - BONNICHON et al., 1977).

On general, these variations take place at the same time as the change in food diets is carried out. Simultaneously, special neuro-secretions appear in the neuro-secreting tissues of the ocular peduncle in certain species (BELLON - HUMBERT et al., 1978). In addition, these digestive enzymatic activities are correlative with nucleic acid tenors (REGNAULT, 1977).

These biochemical variations are connected with the ecology and feeding behaviour of the species studied in their natural environment. When low protein content food is given to the animals in rearing, their proteasic activity is feeble. Progressively as the protein tenor is increased, their proteasic activities will also increase until they reach their maximum value. Then when protein tenors go above 45 %, the proteasic enzymatic activities will keep decreasing as the protein tenor increases. A similar mechanism was defined for amylases, but the optimal percentage permitting a maximum amylasic activity is around 6 to 8 % of glucids in the food.

The variations in salinity have hardly no effect on the digestive enzymatic activities.

The acclimatation of crustaceans to temperatures which are different from those of the biotop from where they come, lead also to adaptations at molecule level (VAN WORMHOUDT, 1980; TRELLU and CECCALDI, 1980 a). These regulation mechanisms of the digestive enzymatic activities can be carried out in many ways, the principal ones being the modification of the cinetic parameters of the enzyms and the quantitative variations of isoenzyms.

Endocrine regulation of the synthesis of digestive enzyms

Recent research has permitted the establishment of new facts in this sphere, but a lot of work remains to be developed.

Gastrin, localized through immunocytochemistry in the walls of the stomach, in the neurosecreting cells and in the sinus gland, increases the synthesis of digestive enzyms and especially -amylase. It provokes an increase in the proteic synthesis of the hepato-pancreas.

Ecdysteroids secreted by the Y organ stimulate the synthesis of digestive enzyms.

Cholecystokinine or CCK, peptidic hormone, is also present in the neurosecreting cells and the sinus gland of the ocular peduncle. It increases the synthesis of the digestive enzyms. The same applies for secretin, localized through immunocytochemical techniques in the neurosecreting cells of the ocular peduncle and which increases the synthesis of the digestive enzyms.

On the contrary, the Molt inhibiting Hormone (M.I.H) present in the neurosecretions, provokes an inhibition of the proteic synthesis by blocking the ecdysteroids in the Y organ; It will also inhibits therefore the digestive enzyms synthesis.

The use of immunocytochemical techniques in employing vertebrate antiserums has permitted to localize several hormonal activities in the neurosecreting cells of crustacean Decapoda. This is how leucine - enkephalin activities substance P, glucagon have been defined.

Endocrine regulation of the physiological functions linked with nutrition

Little precise data exists in this particular sphere. The Crustacean Hyperglycemic Hormone or C.H.H. (known, some years past, as Hyperglycemic Hormone or H.C.H) is synthetized by the neurosecreting cells and is located in the gland of the sinus of the ocular peduncle. Its role is to increase the glucid tenor of the hemolymph.

Inversely, insulin of mammals has no glycostatic effect but a stimulating effect on the synthesis of glycogen.

CONCLUSIONS

Although research should is carried out so as to understand in detail the physical, chemical and biochemical processes which take place when crustaceans start feeding. The metabolic cycles, the regulations caused by the modifications of the environmental factors, the hormones implicated in these mechanisms, the roles they play, the implications on the ecology of the animal in question, the biochemical studies concerning the structure and action method of the enzyms, the comparisons of the phylogeny and the biochemical evolution should certainly give new very interesting and promising results (CECCALDI, 1982). Their application would lead to the perfection of efficient cheap compound food for both extensive and intensive rearing along with other interesting developments.

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FEEDING AND DIGESTION WITH BIVALVES

Jean-Marc DESLOUS-PAOLI

SUMMARY

Introduction

On the contrary to the U.S. where they represent but 4.6 % of the total marine production (CHEW and DONALDSON, 1985) (Table 1), mollusc, principally bivalves, can no longer be considered as a marginal production. In France, 100 000 ton of oysters, 98 % of which is represented by Crassostrea gigas (HERAL et al., 1985a) and 85 000 ton of mussels (Mytilus edulis, Mytilus edulis galloprovincialis) (DARDIGNAC, 1985) are produced yearly. Elsewhere, new rearings are being developed, such as pectinidae and Ruditapes philippinarum, on the Atlantic and Mediterranean coasts.

The species presently cultured, some of which depending only on natural collections (Crassostrea gigas, Mytilus edulis, Mytilus galloprovincialis while others depend on hatchery production, either partially Crassostrea gigas in the U.S., Pecton maximus, Ostrea edulis, or totally (Ruditapes philippinarum, Crassostrea virginica, Argopecten irradians (Table 1-2)

In addition, the development in laboratories of mollusc strains with higher growth potential, or showing more resistance to disease, will be derived exclusively from hatchery productions.

Furthermore, the decrease in growth performance and in quality of Crassostrea gigas cultured in the big French shellfish ponds (HERAL et al., 1985b) and the successive mortalities through epizooties which struck Crassostrea angulata and Ostrea edulis, requires a better comprehension of the relations existing between the rearing environments and the molluscs cultured there or those which live naturally in competition. Due to the fact that these organisms are sedentary, the comprehension of these relations require the knowledge of not only the seasonal and daily variations of the quality and quantity of food available and which is brought by the currents, but also the knowledge of the nutriment requirements linked with the physiological evolution of the individuals.

Thus the studies carried out on food, whether those pertaining to larvae produced in hatcheries, or living in natural environments, or hatchery adult broodstock or those cultured in natural environments, become necessary so as to guarantee larval and adult productions of quality and at lower prices and so as to implement the rational management of the molluscs stocks exploited.

1. RESULTS AND THE ORGANIZATION SCHEME

From the general equation by WINBERG (1956) which has been reexamined and modified by different authors (RICKER, 1968; CRISP, 1971; GRODZINSKI et al., 1975; LUCAS, 1982; HOLMES and Mc INTYRE, 1984) the transfers can be synthetized by the following equation (Fig. 1).

$$C = R + F + U + P$$

where C : Consumption (filtration or captured)

R,= Re + Rs + Ra : respiration

Re : overheating

Rs : standard metabolism

Ra : activity

F = F’ + F" : biodeposits

F' : pseudofaeces

F" : faeces

U = Ua + Uc : excretion

Ua : non assimilated

Uc : products from catabolism

P = Pg + Pr + Ps + Pe : production

Pg : growth

Pr : reproduction

Ps : secretions (mucus, shell, byssus)

Pe : eliminated tissue (predation, desquamation)

2. ANATOMY

2.1. Organs employed for the capture and sorting out of particles

2.1.1. With larvae (see BAYNE, 1971, 1976)

Larvae do not start eating until the veligerous stage has been reached (Table 3). Until the metamorphosis takes place, they ensure the capture of particles by means of the muco-ciliary mechanism of the velum (Fig. 2). When the metamorphosis takes place, the velum regresses and is anatomically replaced by the labial palps which are employed to sort out particles before ingestion (Fig. 3). The function of the food particle retention is then ensured by the gill filaments which are developing and whose ciliary quivering ensure the intrapalleal circulation of the sea-water.

2.1.2. With juveniles and adults (See OWEN, 1974 ; PANDIAN, 1975 ; PURCHON, 1974 ; MORTON, 1983)

Three main families can be distinguished by their predator organs (OWEN, 1977). These are the prosobranchia, the septibranchia and the lamellibranchia.

The prosobranchia feed by means of their ciliary tracts which they stick out of the sediment. The labial palps permit a quantitative sorting out. Ctenidia are present but their importance for the supply of food is unknown.

Septibranchia have modified Ctenidia forming muscular septa which can open and close. These animals feed on debris and big particles.

Lamellibranchia can use the food deposited on the bottom or that floating in the water, depending on whether they have or have not siphons. The principal organ for filtration is the gill. Mussels have (DARDIGNAC, 1985), two gills. (Figure 4). Linked to the visceral mass by the branchial axis, each one has two rows of flat filaments. These filaments face the ventral side of the mollusc (direct or descending branches, bend round abruptly, and then turn round and up facing the dorsal side (reflexed or ascending branches. On the contrary to the oyster, the extremities of the reflexed branches are not connected to the visceral mass; also, all the filaments are similar to one another and placed in uniform rows (smooth gills). Each direct branch is linked to the corresponding reflexed branch by three very flexible bridges which are tissue expansions. Otherwise, clumps of cilia link each filament to the neighbouring one and delimit the spaces between each, these spaces are known as ostia (Fig. 5). The lateral sides of the filaments (Fig. 6) are garnished with frontal, Iatero-frontaI and lateral cilia, which by their

movement, create and maintain the circulation of the water in the palleal cavity. The current penetrates in between the lobes of the mantle, crosses through the gills while passing by the ostia and flows out through the exhaling siphon.

From 1851 onwards (DRAL, 1967) the gill was considered as a sieve, whose meshes were formed by the latero-frontal cilia. However, no one could figure out how smaller sized particles than the meshes were retained. In 1905, it was remarked that the latero-frontal cilia were covered by a layer of sticky mucus, which explained how the particles were kept back, as they stuck to the cilia. The study carried out by DRAL (1967) on the movement of the latero-frontal cilia and the retention of particles by the Mytilus edulis L shows the power of retention of molluscs depends both on the movement as well as on the frequence of the quivering of the cilia.

When the particles are captured by the gills, they are directed towards the marginal grooves (junction of the direct and reflexed branches of the filaments) or dorsal grooves (extremities of the reflexed branches) and conveyed towards the labial.palps which direct them towards the mouth where they are ingested.

2.2 The digestive tract

2.2.1. Anatomy

After 48 hours with D.shaped larvae, the different regions of the digestive tract differ from each other and prefigure the digestive tract of the adults (Fig. 2 - 3). It is at pediveliger stage that the digestive gland starts showing two lobes which are the anlages of the two caecals of the adult gland. The morphology of the digestive gland will only be complete when young adult stage is reached

The Crassostrea gigas (LESBENERAIT, 1985), at adult stage, opens its mouth between the labial palps. In its mouth follows the oesophagus, which bends slightly and leads to the stomach. The latter communicates by means of canals, with the digestive gland, a pigmented khaki colour mass (Fig. 8).

The stomach is extended posteriorly by means of a cylindric caecum in an oblique position : The stylet bag in which lies the cristalline stylet, protrudes largely into stomach cavity.

Half separated from the stylet bag by two typhlosoles, major and minor, which come out of the stomach, the intestinal pipe is very slack and in a spiral shape which runs lengthwise along the stylet bag. At the posterior end of the tract, the intestinal pipe opens onto the intestine, into a corresponding groove, while the stylet bag, is prolonged a little longer to the rear and opens into the other groove of the intestine; both organs, pipe and stylet bag, are then separated in the terminal region.

From the beginning, the intestine presents a recurrent route (ascendihg branch) in the shape of a dorso-ventral curl around the stomach and the digestive gland, and then directs towards (descending branch) the posterior end of the animal. From the intestine then follows the rectum which runs along the dorsal external side of the adducent muscle and is finished off by the anus, which opens into the palleal cavity.

2.2.2. Histological structure

With the exception of the gastric shield, the digestive tract is lined with ciliated epithelium of numerous glandular cells, having a glycoproteinic secretion. The muscular wall is not very well developed. Thus the histological structure of the digestive tract appears very uniform, the only variations, depending on the regions, being the height of the ciliated cells and the density of the glandular cells. The gastric shield is an

exception. It comprises a layer of high cells, covered with a thick, chitinous and sclerotic cuticle (ARNOULD, 1976).

The digestive gland is made up of a great number of tubules at the blind extremity, communicating with the stomach, by a series of ramified channels (Fig. 8). The glandular tubules open onto a short secondary channel which is the same for many tubules, the channel then continues on to the principal channel. The principal channels join up together in channels of larger and larger diameter before entering into the stomach.

The glandular tubules contain two categories of cells : the digestive and secretory cells.

The digestive cells are more numerous. These are high cells, whose apical part shows some microvillosities (Fig. 9). The nucleus is basal, the mitochondria are quite numerous. The presence of heterophagical vacuoles at different stages of evolution, characterize these cells. In the routine metabolism, they have the functions of intracellular digestion, which are followed by the functions of extracellular digestion, by the emission of fragmentation spheres when the feeding conditions vary.

The secretory cells are characterized by a cytoplasm rich in vesicles of rough endoplasmic reticulum. The nucleus has a developed nucleolus (Fig. 10). It has an intense synthesis and proteinic secretion.

The different canals and tubules of the digestive gland are surrounded by a reserve tissue formed of cells of conjunctive nature, with lots of lipidic and glycogen granulas.

The digestive gland of Mytilus galloprovincialis larvae changes very early (LUBET,1978). 48 hours after fecundation (larvae D), it contains the different cellular types, characterizing the adult gland : secretory and digestive cells. The process of digestion and the absorbtion starts when stage D is reached. The activity of the gland is remarked by the formation of lipid globules which are evacuated by exocytosis and can be taken back by the amoebocytes.

3. THE SOURCES OF FOOD

3.1. In aquaculture

3.1.1. Algae fodder

One fifth of the phytoplanktonic species belonging to 37 types, have been listed, to feed mollusc larvae and juveniles in hatcheries and nurseries (CHRETIENNOT-DINET et al., 1986). However, from these species, only ten of them are used regularly in aquaculture (Table 4). These algae have been selected in accordance to three criteria. They must be of adequat size, good nutritional value, and relatively easy to culture.

Size is a limiting parameter concerning the diameter of the mouth and the oesophages of larvae juveniles and adults.

The nutritional value depends on the species which are to be fed, and their development stages. Indeed, the larvae can be more or less demanding (in descending order : Crassostrea Ostrea Mytilus and Mercenaria) In addition, the requirements of the animals develop with time, and a species of poor nutritional quality for the young stages can be of better quality for the older stages. The nutritional quality also depends on the intrinsic quality of the phytoplanktonic species used. However, the biochemical

composition of an algae varies, depending on the function of the culture (LAING and MILLICAN, 1986), while NEWKIRK and KAYARAT (1985) remark that an increase in survival and growth of Ostrea edulis larvae. It has also been demonstrated that the kind of lipids in the food ration will intervene in the notion of the nutritional quality. Certain unsaturated fatty acids, in particular, those of 6 w 3 group favour the growth of oyster larvae and of juveniles. At present, the nutritive quality of an alga can not however be explained by only its biochemical composition, but all authors acknowledge the superiority of a plurialgal type food concerning the growth of larvae, and of the water supply containing natural elements (HELM et al., 1973).

The facility in the production of cultures is very important in hatcheries and nurseries of industrial type where the quantities of algae produced can reach 10,000 liters per day (example SATMAR in France).

3.1.2. Other sources of food

In order to feed larvae in the same way as marine bivalve juveniles, different nutritional sources have been tested (Yeasts, minced macrophytes, vegetable and animal extract, etc...) but these have all turned out to be of little effect. On the other hand, the importance of bacteria has been remarked by MENGUS (1978) and PRIEUR (1981). Also the use of lyophilized algae and enriched microcapsules seem more promising. Nevertheless, at present, only live unicellular algae produced in culture contain all the suitable elements for the feeding requirements of bivalves at young stages in either hatcheries or nurseries.

If the presence of inorganic particles in the matter held in suspension does not supply any digestible matter, it can however contribute, either negatively or positively to the development of molluscs, depending on the concentration and type of mineral used. The addition of argillaceous suspension can be even recommended so as to facilitate the ingestion of artificial preparations which are given in the first fattening of oysters (LANGTON and BOLTON, 1984) and their digestion when there exists feeble concentrations of algae (EWARD and CARRIKER, 1983). This also helps to reduce the costs of artificial rearings (URBAN and LANGTON, 1984) and to increase the growth rates of oysters (ALI et PRUDER, 1983) (Fig. 11).

The dissolved substances which are contained in the sea-water or in the phytoplanktonic culture mediums also play a leading part. WILSON (1979) demonstrates that the filtrates of Isochrysis galbana cultures, at stationary phase, speed up the capture of cells by the bivalves, while the filtrates at declining stages are inhibited from doing so.

3.2. In the natural environment (HERAL, 1985)

3.2.1. Particulate material

In the studies in situ, if one of the first explicative factors for growth is the temperature, this is but the third explicative factor for the production of flesh of Crassostrea gigas. Thus HERAL et al (1984) demonstrate a close connection for the production of flesh with phytoplankton and phytobenthos whether in a degenerated form (pheopigments) or live (chlorophyl). On parallel, LELONG and RIVA (1976) demonstrate in situ the action of phytoplankton on the growth of Ruditapes decussatus. These relations with phytoplankton are confirmed for the fattening of oysters (DESLOUS-PAOLI et al., 1982) (Fig. 13) for the growth of mussels (KAUTSKY, 1982) and for the energetic tenor of Ruditapes decussatus (BODOY and PLANTE-CUNNY, 1983).

Otherwise, the pernicious influence of a too elevated sestonic load has been demonstrated with the production of flesh by VAHL (1980) for Chlamys islandica, by WILDISH et al., (1981) for different lamellibranches and by DESLOUS-PAOLI et al (1981), HERAL et al., (1983) and DESLOUS-PAOLI and HERAL (1984) for Crassostrea gigas. On the contrary, the importance of bacteria (PRIEUR, 1981) often associated with particles, has been pointed out by MARTIN (1976) for Ruditapes decussatus and by AMOUROUX (1982) for Venus verucosa.

3.2.2. Dissolved organic substances

In the coastal waters, the levels of dissolved amino-acids vary between 0.2 and 2.9µ moles per liter (NORTH, 1975). While, JORGENSEN (1982), at Isefjord, found variations between 0.4 and 2.5 µ moles per liter. In the Marennes-Oleron basin, HERAL et al. (not published, fig. 14), find fluctuations between 0.2 and 10 µ moles per liter but not presenting significant seasonal peaks, the daily variability during a tidal cycle being superior than the annual variation. The same applies for the dissolved fulvic and humic carbon (FEUILLET et al. (1979). These great variablilities could be caused by the synthesis of absorptions and excretions by molluscs but also by all other organisms.

4. FEEDING FOR BIVALVES

4.1. With larvae

For the larvae, the ingestion takes place in a continuous way. However, the filtration and ingestion rates (Table 5 - 6) can vary depending on numerous parameters

The efficiency to retain particles depends on the size of these particles For mussel larvae, the optimum capture of particles is situated around ∅ 3.5 um, (Fig. 15)(RIISGARD et at., 1980; SPRUNG, 1984a). The filtration rate depends on the cellular density in the environment and permits to regulate the ingestion (Fig. 16) while decreasing the energy losses caused by the effort made to capture the particles. The ingestion of cells by the larvae depends on the size of the larvae. However, the increase in number of cells ingested, corresponds to the decrease of the percentage which is represented by the ration, with regard to the body weight (Fig. 17) Ingestion also depends on the temperature, thus, on the metabolic level of the individuals. Indeed, UKLES and SWEENEY (1969) register an ingestion of 134 cells of Monochrysis lutheri by Crassostrea virginica larvae of 75 um par day at 10° C while it is 457 cells per larva per day at 27-30° C. Likewise, LUCAS and RANGEL-DAVALOS (1981) remark that the Crassostrea gigas larva ingests 400 cells per day at 21° C and nearly twice as much at 24° C when it is fed a mixture of Isochrysis galbana and Monochrysis lutheri.

For Mytilus edulis fed on Isochrysis galbana, the assimilation efficiency (100 * (respiration + growth ingestion) varies little around 40 % for concentrations of between 5 and 40 cells per liter. On the other hand, it increases up to 75 % for lower cell densities (SPRUNG, 1984b) (Fig. 18). On parallel, the net efficiency of growth, in other words the percentage of energy assimilated for growth, increases from the cell density permitting the acquisition of a maintenance ration, up to a plateau of about 6,5 % from 10 cells per ul (Fig 19). Indeed, the relation between the larval density in rearing and the availability of food is an important factor for the growth of the larvae (Fig. 20) and must be adjusted according to the size of the larvae.

4.2. For juveniles and larvae (See BAYNE and NEWELL, 1983; DESLOUS-PAOLI, 1985)

In feeding conditions, which are much similar to those found in natural environments, the filtration level depends on the physiological state of the animals. Indeed, the high metabolic demand induced when its time for the spawning period and for the reconstitution of gamets of Mytilus edulis to take place brings about a remarkable increase in filtration rates. These drop to a plateau as soon as spawning ends in the month of June (BOROMTHANARAT, 1986) (Fig. 21). During this period, the amount of material consumed depends on the sestonic load present in the environment.

However, the filtration level depends on the size of the particles having served to define this filtration level. Indeed, the retention of particles is more than 50 % for particles of 1 µm for Mytilus edulis (Fig. 22) and of 4µm for Crassostrea gigas.

A schematic aspect of the relations existing between the sestonic load and the feeding is given by WIDDOWS et al (1979) (Fig. 23). When a certain level of the sestonic load is reached, the quantity of matter retained by the gills exceeds the ingestion possibilities and causes the production of pseudo-faeces. It is still difficult to conclude on whether a qualitative sorting out is made or whether the size of the particles is taken into consideration by the individuals. However, certain authors (KIORBOE et al., 1981; NEWELL and JORDAN, 1983) have remarked a relative enrichment of the ration ingested in comparaison to filtered particles and LOPEZ and CHENZ (1983) pointed out that Nucula annulata ingests selectively the organic and especially the bacterial fraction of the ration consumed. However, they point out that this selectivity can be modulated by sedimentological factors. If the sestonic loads are above values of 200 mg per liter, one will remark a decrease, or even a complete arrest for the capture of particles. This has been remarked for Crassostrea gigas in natural environments, when high sestonic loads were caused by Winter storms. (Fig. 24). On the contrary, when there are low sestonic loads, the efficiency with which the particles are retained increases (Table 7) This mechanism can be used to maintain an optimum ingestion and continuous digestion PALMER, 1980b).

The relations between filtration, ingestion and assimilation are schematized for Mytilus edulis by NAVARRO and WINTER (1982) (Fig. 25). In this case, point corresponds to the cellular load for which ingestion and assimilation are at optimum. Above this, the assimilation rate is maintained constant by the progressive decrease of the filtration rate. However, with other bivalves, such as Aulacomyasater, the filtration rate remains constant, which leads to an increase in the cell density. The resulting increase of the digestive transit leads to a decrease of the digestion efficiency which little by little decreases the assimilation (Fig. 26).

The intertidal lateral bivalves have imposed feeding activity rythms by the periodical emergence caused by the tides. However, for species which are constantly submerged the tidal rythms or nychthemerals have not been clearly defined. Certain authors have registered some (SALANKI, 1966; MORTON, 1977; PALMER, 1980; COPPELO, 1982) (Fig. 27), while others have not remarked any (LOOSANOFF and NOMEJKO 1946; WINTER, 1978; HIGGINS, 1980). It seems however that the feeding and digestion rhythms are controlled by the variability of the feed at disposal (LANGTON and GABBOT, 1974; OWEN, 1974; WILSON and LA TOUCHE, 1978; ROBINSON and LANGTON, 1980).

5. DIGESTION WITH BIVALVES

5.1. The digestive transit and rhythms of digestion

5.1.1. The digestive transit

The mechanical aspect of digestion is similar in larvae and adults. It is a trituration, caused by the combined action of the cristalline style of the gastric shield, of the food which is separated and mixed with the enzymes (LUBET, 1978) Only the fluids and the macromolecules, which result from the extracellular digestion exist in the gastric cavity, are capable of entering into the diverticula (OWEN, 1974). They are then absorbed by the pinocytose and digested by the intercellular passage. The intracellular wastes are rejected by the desintegration of the digestive cell.

The part played by the cristal line style has not yet been clearly described, it seems to act as a never ending screw which carries the fine particles to the epithelium level of the cristalline style sack, for absorption purposes.

At intestine level, digestion and absorption exist in addition to the role of mucilaginous secretion which is used to form and transport faeces.

With larvae, the evolution of the digestive gland takes place according to two schemas, depending on whether the feeding takes place continuously or not (LE PENNEC and RANGEL-DAVALOS, 1985). In the first case, all the Pecten maximus larvae ingest and digest at the same time and continuously, after 7 hours of feeding at 16° C, and after 5 hours 20 mn, at 18° C (Fig. 28 b). In the second case, digestion commences 6 hours after ingestion, and finishes after 10 hours at 17° C for Pavlova lutheri (Fig 28 a). The time for digestion varies however according to the algal species employed. Mytilus edulis larvae require 15 hours at 10°C to digest 80 % of the Isochrysis galbana cells and 13 hours with Monochrysis lutheri cells. Digestion is also 2.6 times quicker at 20° C than at 10° C (LUCAS and RANGEL-DAVALOS, 1981).

For Crassostrea gigas adults, the dynamic of the digestive transit takes place in three phases, when a sequential feeding method is employed (LEBESNERAIT, 1985; BOUCAUD-CAMOU et al., 1985).

The infilling of the digestive tube (Fig. 29 a) :

This infilling takes place rather quickly, and one can remark intact algae in the stomach, in the principal channels of the digestive gland and in the intestine. It takes around 5-3 hours between 10° C - 20° C for the algae to completely pass through the digestive tube. Then, they are excreted along with a lot of mucus through the anus (Fig. 29 a 1). The temperature has a great influence on this stage (Table 8).

The start of the digestion (Fig. 29 b) :

As soon as the algae enter into the channels of the digestive gland, they are affected (within an hour) ; while live algae can be observed rather a long time in the stomach (6 hours) and especially in the intestine (8 to 16 hours after feeding). Three to six hours after the commencement of the experiments, the first residues of the digestion appear in the faeces, mixed with numerous live algae (Fig. 29 b 2). Progressively, the percentage of residues will rise, while the faeces solidify and mould themselves into the shape of the rectum. The residues accumulate into a pleated ribbon and are clearly separated from the straight ribbon formed by the intact algae (Fig. 29 b 3). Both ribbons are probably moulded together in the rectal bag so at the start of the digestion at least there are supposedly separate transit tubes for the algae and residues.

The end of the digestion (Fig. 29 c) :

The residues will dominate the intact algae, invading all the lumen of the rectum, and after around 10 hours, homogeneous faeces will be obtained (Fig. 29cl) but we must wait for at least 40 hours to find no more live algae in the faeces. They will no longer be excreted continuously, but intermittently, mixed with a lot of mucus. The digestive tract will be completely empty after 50 hours at 20° C, but the digestion can last for 75 hours at 10° C.

As stated by WIDDOWS (1978) the efficiency of digestion and so absorption, depends on the quantity of food available. Indeed, the more food available, the less the need to complete the digestion so to ensure the energy gain necessary for Mytilus edulis. However, as the quantity of food influences the efficiency of digestion so does its composition. BERRY and SCHLEYER (1983) on Perna perna, BRIEELJ and MALOUF (1984) on Mercenaria mercenaria and BOROMTHANARAT (1986) on Mytilus edulis reveal the increase in efficiency of digestion with an increase of the organic fraction in the food (Fig. 30), thus pointing out the gene that is represented by the mineral seston for the digestion when this makes up 80 % to 90 % of the food ration, as is often encountered in the rearing sectors on the French Atlantic coast. (Fig. 31). Thus, as numerous authors have remarked with different bivalves, the efficiency of absorption varies with the seasons. These variations are probably due to the environmental conditions on one hand and to the requirement of the molluscs on the other. Indeed, it appears that during Wintertime sugars are not used, while during Spring and Summertime around 50 % of the sugars consumed are digested by Mytilus edulis and Crassostrea gigas (DESLOUS-PAOLI and al., 1986) (Table 8), while the lipids are greatly employed during both Summer and Winter. The digestion of the different energetic substrates of the food is thus undouptably induced by the implementation of enzymatic organs adapted to the food requirements. These nutritional requirements are probably linked with the physiological seasonal state of the bivalves.

5.1.2. The rhythm of digestion (See MORTON, 1983)

The study of the dissolution rhythm of the cristallinestyle of Rasaea rubra (MORTON, 1956) and of the stucture of the digestive tubules (Mc QUISTON, 1969) along with the results obtained by other authors (MORTON, 1973; LANGTON and GABBOTT, 1974; LANGTON, 1975; MATTEWS, 1976; MORTON, 1977) give to believe that the digestion phases, extracellular in the stomach and intracellular in the digestive tubules are organized in the repetitive phases linked with the tidal cycles. For example, MORTON (1970, 1971) described for Cardmium edule and Ostrea edulis a cycle linked with the tides: The feeding will take place during the high tide cycle, and the matter ingested will not pass through the digestive diverticular before the next high tide, when it will be submitted to intercellular digestion. It is thus during the low tide period that the extracellular digestion is produced in the gastric cavity. The intracellular digestion during out flowing waters and low tide periods will be followed by the fragmentation of the digestive cells and the preparation of the tubules, for the new flow in, of matter during the following high tide cycle.

But numerous authors pointed out that the rhythm of digestion was lost when the animals were placed into continuous immersion or feeding conditions (LANGTON and GABBOT, 1974).

Thus it appears that the synchronism of the digestive processes is regulated by the availability of food (OWEN, 1974; MORTON, 1977; ROBINSON and LANGTON 1980; MORTON, 1983; HILY, 1985) (Fig. 32), the maximum size of the cristalline style is

reached when the stomach is full, and the minimum size, when it is empty (LANGTON and GABBOTT, 1974). This does not differ from the results showing that the rhythm of digestion were under the control of the environmental varients, such as tides or day and night alternance. Indeed, the food levels fluctuate in unatural environment, especially in relation with the tides for intertidal species.

5.2. The enzyms

5.2.1. With larvae

MASSON (1975) states that from the ovocyte stage, numerous enzymatic activities commence, apart from some lypolitic enzyms which will not develop until after the metamorphosis has taken place, and with the exception of the amylasis which will only commence activity during the pelagic life.

5.2.2. With juveniles and adults (See OWEN, 1974; MORTON, 1983)

The studies carried out by HILY (1985) on Ruditapes philippinarum and by BOUCAUD-CAMOU et al., (1985) on Crassostrea gigas will serve as a basis to regroup enzymes according to the actions in the different stages of digestion.

The glucanases (amylase, cellulase, laminarinase) digest the walls of the algae and the reserve substances (starch, laminarine) (BOUCAUD-CAMOU et al., 1985). These glucanasic activities are found in all the epithelium of the digestive system and more so in the digestive tubules. It appears, when digestion commences, that there is a amylase secretion in the stomach, and the glucanases can be found on the surface of the cristallinestyle. The latter then seems to, incorporate the enzymes secreted by the stomach wall (ARNOULT and BOUCHEZ-DECLOUX, 1978).

Lipases and proteases in feeble quantities are found present in the lumen of the digestive tubules and of the stomach. Normally proteases are found in the intestine. The proteins seem to be digested by the enzymes at optimum acid pH (BOUCAUD-CAMOU et al., 1985).

An intracellular digestion continues in the bordering brushy cells and in the digestive cells by the action of the lysosomale enzymes like the D.glucosidase for glucides (HILY, 1985), the acid phosphatase, the acetyl- glucosaminidase and the peptidase (BOUCAUD-CAMOU et al., 1985). There also exists, at the brushy border level of the digestion canals and in the stomach epithelium, membranar enzymes (peptidases, alkaline phosphatases) which must have a relation with absorption.

Thus, a plan of the digestion of Crassostrea gigas (Fig. 33) is proposed by BOUCAUD-CAMOU et al., 1985): "Oysters seem to fill their digestive tube completely as soon as the food is taken. A flow of particulates enter simultaneously into the stomach and into the canals of the digestive gland. All the substances directly assimilable seem to be then absorbed by the membrane enzymes of the bordering brushy cells. The algal walls are attached as soon as they enter into the digestive canal, due to the action of the glucanases which are particularly active at this level, then progressively in the stomach due to the mechanical and then chemical action of the cristalline style, with the help of the enzymes secreted by the stomach wall and the digestive gland. The food digested in the stomach can then continue into the digestive gland or else be digested and absorbed by the stomach wall". The absorption and the intracellular digestion continues in the intestine.

5.3. Dissolved absorption

While the experimental work carried out by PEQUIGNAT (1973) points out the nutritional role of the amino-acids and the dissolved sugars, the energetic supply that they represent has, up to present, not been quantified in the energetic balance of molluscs.

Indeed, the branchial epiderm of the lamellibranches is where a strong absorption of the dissolved organic molecules, such as the amino-acids, sugars and fatty acids, takes place. Numerous experiments emphasize these mechanisms (JORGENSEN, 1982, 1983; WRIGHT and STEPHEN, 1982; GOMME, 1982; NELL et al., 1983). Thus this absorption is carried out principally at gill, mantle edge, stomach and small intestine level (GOREAU et al., 1973; BAMFORD and GINGLES, 1974; STEWARD and BAMFORD, 1976). Mytilus edulis can thus absorb half of the amino-acids contained in the sea-water which passes through the branchial cavity at concentrations of 1 umole per liter (JORGENSEN, 1983). JORGENSEN (1982) also shows that the absorption of amino-acids from natural water can suffice and supply more than twice the amount of energy necessary for the filtration activity of the gills. On parallel WRIGHT (1982) estimates that the absorption of amino-acids supplies 6 to 60 % according to the concentrations available in the water, of the oxydation requirements of the metabolism expressed by the respiration. This mechanism thus permits to satisfy the requirements of 11 essential amino-acids for Mytilus californianus with principally the L-methionine and L-Lysine Ncl (HARRISON, 1976) as well as taurine which represents 70 % of the pool of intracellular free amino-acids of the gills (ZURBURG and DE ZWAAN, 1981). On the contrary, according to NELL et al., (1983), while for amino-acids there is an active absorption; for glucose, the absorption appears like a passive diffusion which does not contribute greatly to the carbohydrate needs of the oysters. On the other hand, FANKBONER and DE BRUGH (1978) and FANKBONER et al., (1978) point out that oysters and mussels accumulate the organic carbon dissolved in the sea-water and PHLEGER and ROSSI (1982) point out that Hinnites multirugosus juveniles can concentrate 150 times in 24 hours.

On parallel, a certain number of dissolved organic substances can be absorbed in the same metabolic manner and don't play an energetic role but a growth substance role, as does cholin chloride and vitamins (NELL et al., 1983). COLLIER et al., (1953) also pointed out the great beneficial influence of carbohydrates, which are present in marine environments, on the pumping rate and the intervalvular activity of the oysters and THOMSON and BAYNE (1972) on the filtration rates of mussels. These constatations brought about the development of the first artificial diets, employing sugars, lipids and vitamins (CASTELL and TRIDER, 1974; TRIDER and CASTELL, 1980; NELL and WISELY, 1983).

CONCLUSION

The variety of food found in natural environments, and its variability in a controlled environment, make it difficult to comprehend the nutrition of bivalves

Indeed, if bivalves are classed as suspensivores and deposivores, this classification is no longer applicable when dealing with animals living in an intertidal environment. The re-suspension of the water-sediment interface leads to an as important participation of the phytoplankton in the food rations of suspensivore animals, such as oysters and mussels for example.

In addition, in a hatchery, a nursery or experimentally, the variability of a same phytoplanktonic species, depending on the factors of the culture environment, or the age of the populations make it difficult to comprehend of the cause-effect relations.

On the other hand, the existing controversy between OWEN (1956) and MORTON (1973, 1983) concerning the rhythms of nutrition, are due more probably to the study of the secondary factors (tides, nycthemerals) rather than the primary ones (food). Indeed, those animals whose feeding rhythm is cyclic in intertidal environments, adapt to continuous feeding when they are fed constantly. Indeed these two authors agree that the digestion depends on the availability of food.

However there exist some points where particular efforts are required, if one wishes to fully understand the feeding mechanisms of bivalves. The first is the quantitative and qualitative estimation of the ration really ingested. Up to present, most of the studies have been carried out in conditions which do not bring about the apparition of pseudo-faeces. This means that the totality of the matter filtered was ingested. This is not what normally happens in natural intertidal environments, where the particulate loads are often relatively high. Also the level of digestion is probably linked with the quantity and the quality of the food ingested. Indeed, without a doubt a connection is produced between the time of transit of the food in the digestive tract and the level of the different enzymatic activities, which gives an optimization of the energy acquired, depending on the energetic requirements of the animals.

Therefore, the nutrition of bivalves is the result of successive adaptation of the filtration functions, ingestion and digestion, to the amount and the quality of the food available permitting a animal of given physiology to satisfy its requirements in the best way possible.

Table 1. Estimated Five Year Average (1978-1982) of Commercial Landing s for Mollusc in the United States (from Chew and Donaldson, 1985)

		Metric Tons (Meat weight)
Oysters		
Pacific Crassotrea gigas[1]		2,950
American Crassostrea virginica[2]		27,400
Others		50
	Oyster Total	30,400
Clams		
Hard: Mercenaria mercenaria[1]		6,325
Surf: Soisula solidissima		18,850
Ocean guahog: Arctica islandica		14,740
Soft: Mya arenaria		3,970
Manila: Tapes japonica[2]		500
Others		1.315
	Clam Total	45,700
Scallops		
Bay: Argopecten irradians[2]		595
Calico: Argopecten gibbus		2,490
Sea: Placopecten magellanius		12,960 5
Others		5
	Scallop Total	16,050
Others		
Squids, Octopus, Abalone[2], Mussels		42,200
	GRAND TOTAL	134,350[3]

[1] Dependent on hatchery seed for portion of commercial production.

[2] Seed produced in the hatchery and sold regularly or irregularly to commercial shellfish growers.

[3] Estimated average 5 year total of commercial marine landings of all species of fish and Crustacea (round or live weight) and mollusc (meat weight) is 2,920,000 M.T.. Mollusc is only 4.6% of the total production.

Tableau 2 : From Lucas (1981).

— Bilan annuel des ventes de naissain par la Seasalter Shellfish, Whitstable (G.B.) exprimé en milliers d'individus

Années	*Ostrea edulis*	*Crassostrea*	*Rudhapes decussates*
1975	2 374	17 185	236
1976	5 134	11 402	0
1977	1 860	16 374	357
1978	9 324	13 121	1 072
1979	9 772	45 0S5	7 385
1980	21 451	57 556	753

— Bilan annuel des ventes de naissain par la SATMAR, Bartleur, exprimé ea milliers d'individus
(1) Bilan de juin à juin. (2) Bilan par année civile.

Années	*Ostrea edulis*	*Crassostrea*	*Rudhapes philippinarum*
(1)			
1974-75	0	323	1 979
1975-76	198	999	1 470
1976-77	1 579	I 470	1 459
1977-73	1 765	5 166	2 063
(2)			
1978	?	15 3S3	4 707
1979	1 448	29 122	8 534
1980	1 527	41 075	18 983

TEMPS APRES LA FECONDATION (en jours)	TAILLE (en µm)	STAGE	CARACTERISTIQUES GENERALES	ORGANES DE NUTRITION
1	80	Trochophore (Trochophore)	Forme de toupie. Couronne ciliée. Glande coquillière, mais pas de coquille.	Pas d'alimentation Un archenteron en forme d'U, mais pas de différenciation du tractus digestif.
1^{+}	80-90	Post-trochophore (Young veliger)	Sécrétion d'une coquille monovalve	Pas d'alimentation Apparition d'un velum. Tube digestif en voie ce différenciation.
2 à 14	90 à 150	Véligère Stade 0 (0 shaped or straight-hinge veliger]	Coquille formée de 2 valves à charnière droite: Prodissoconque I (sécrètée par la glande coquillière) puis Prodissoconque II sécrètée par la manteau	Velum dévelopée retractable entre les valves. Tube digestif différencié (cesopnage, estomac avec sac du stylet, intestin en U). Glande digestive impaire
15 à 25	150 à 230	Véligère umconée (Veliconcha)	Présence d'un umbo sur la prodissoconque II	Comme ci-dessus, mais glance digestive formant progressivemant 2 lobes.
26	220 à 240	Véligère geillée (Eyed veliger)	Tâche pigmentaire ou "oeil" dans les lobes du manteau. Le pied se différencie.	Comme cidessus
27 à 29	240 à 260	Pédivéligère (Psdiveliger)	"Oeil" présant. Pied développé et fonctionnel	Velum en regression progressive. Appareil digestif inchangé.
30	250	Plantigrade (Plantigrade)	Pied. Byssus. Vie benthique. Filaments branchiaux	Cavité palléale active. Palpes labiaux. Appareil digestif inchangé.

Tableau 3 : Stades larvaires de *Mytilus edulis* en élevage à 20°C. (Lucas, 1982b)
Remargues : les durées des différents stades et les tailles correspondantes ne sont dannées qu'à titre indicatif, en raison d'une forte variabilité d'origine génétique et écologique. La taille est la plus grande dimension de la coquille sur une ligne parallèle à la charnière, Les termes anglais sont empruntés à Bayne (1964 et 1976). Il faut capendant signaler que cet auteur donne un sans restraint au stade D en la faisant correspondre à la prodissonconque I (durée : 1-3 jours); en conséquence le stace veliconcha débute à la prodissoconque II (durée: 2 semaines).

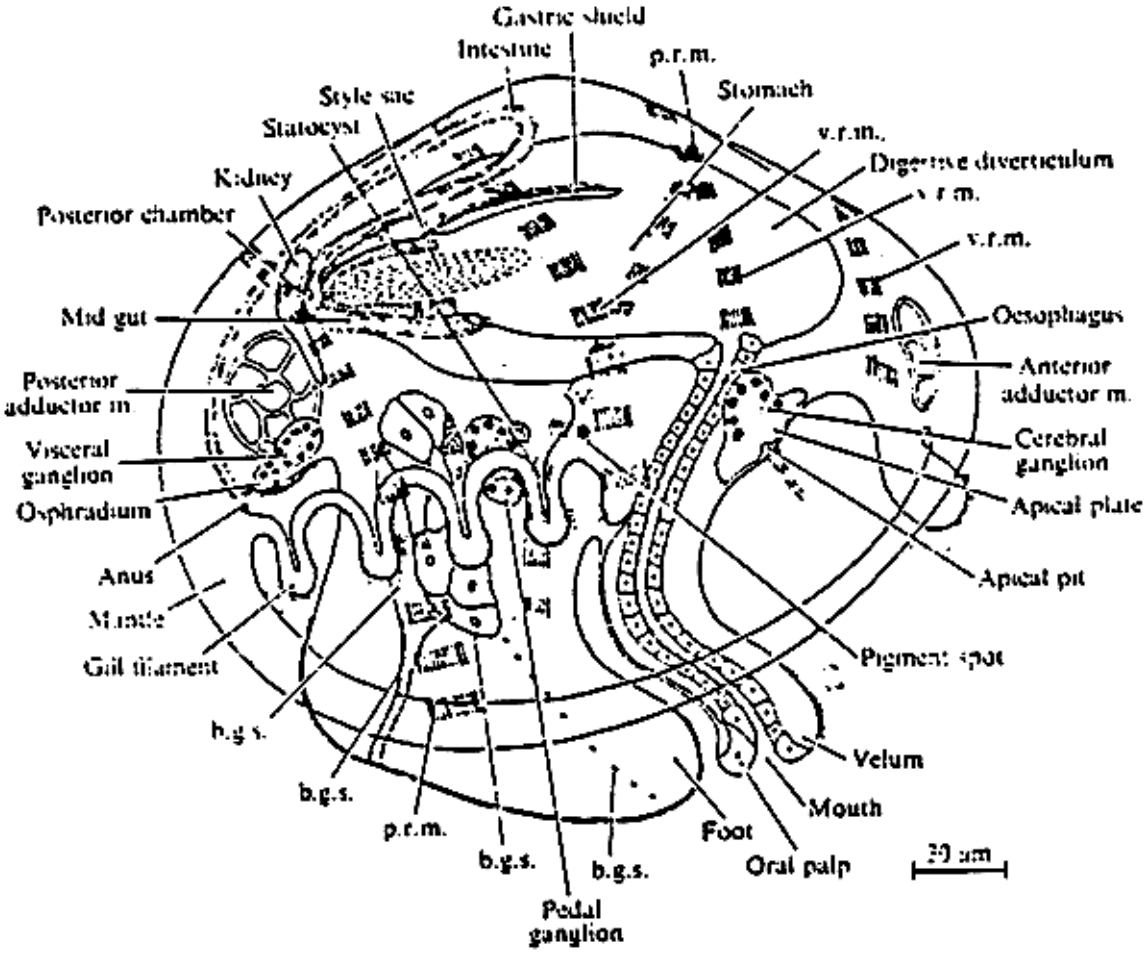

Figure 2 : A diagrammatic reconstruction of the pediveliger larva of Mytilus edulis. p.r.m. pedal retractor muscle; v.r.m. velar retractor muscle; b.g.s. byssal gland system. (from Bayne, 1971)

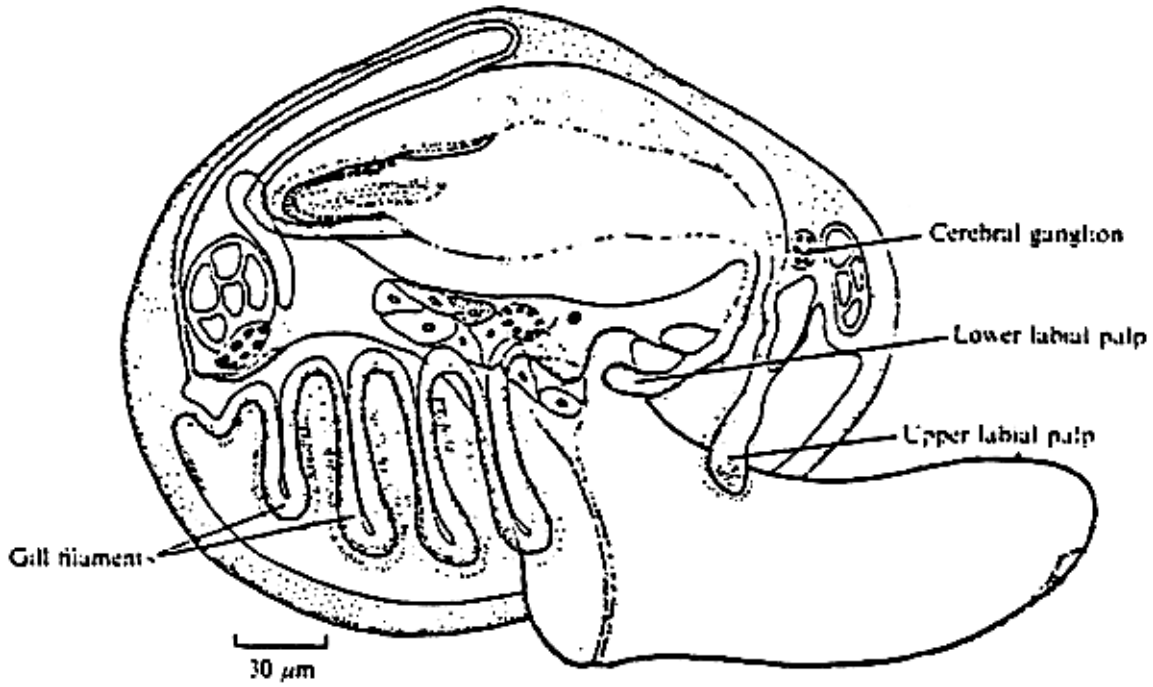

Figure 3 : A diagrammatic reconstruction of an early plantigrade of Mytilus edulis, immediatly after metamorphosis and before secretion of the dissoconch shell has begun. Organ systems not labelled are as in fig.2 (from Bayne, 1971)

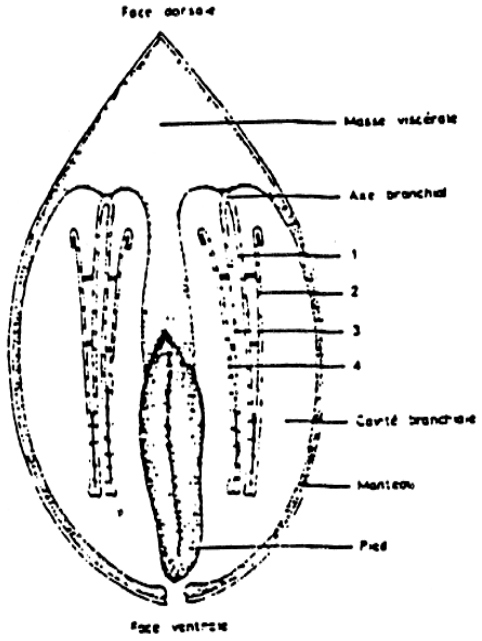

FIGURE 4 : Diagrammatic transverse section of a mussel :
(1-2) outer demibranchs
(3-4) inner demibranchs
(from Dardignac, 1986)

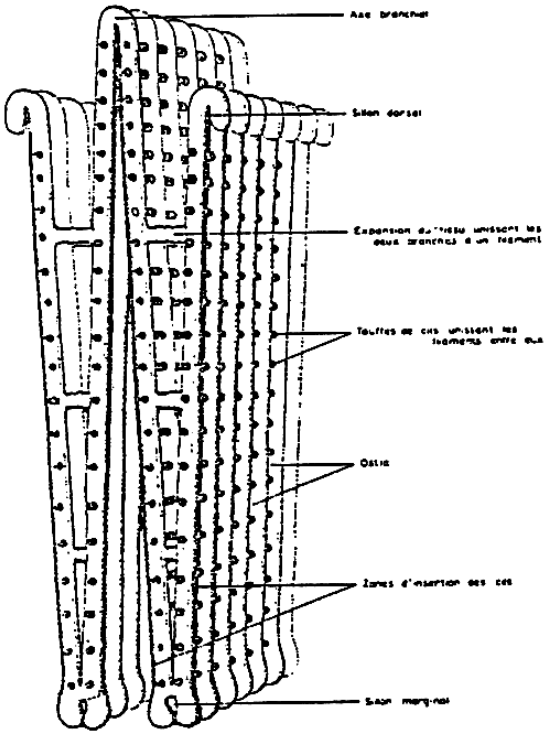

FIGURE 5 : The gill of Mytilus edulis.
(from Dardignac, 1986)

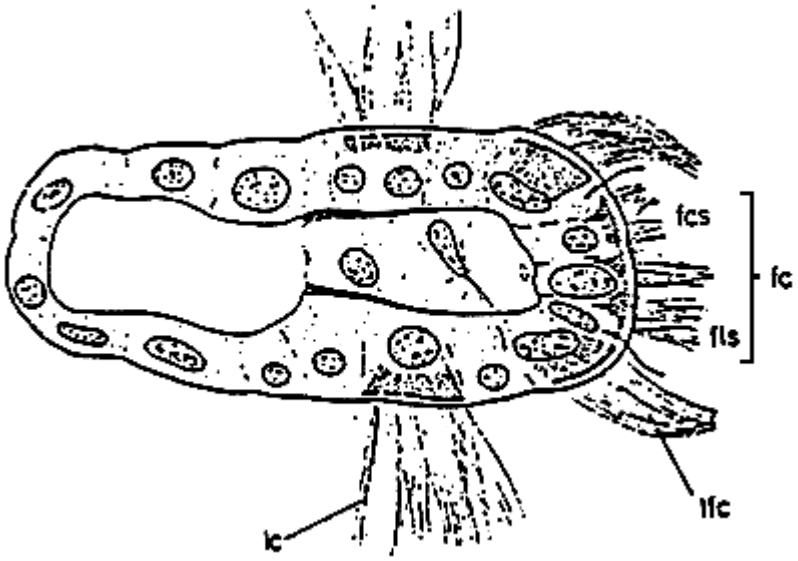

Figure 6 : Semidiagrammatic transverse section of a lamellibranch gill filament showing ciliary tracts : fc, frontal cilia fcs, frontal tract of short cilia; fls, frontal tract of long cilia; lc, lateral cilia; lfc, laterofrontal cilia. (from Owen, 1966)

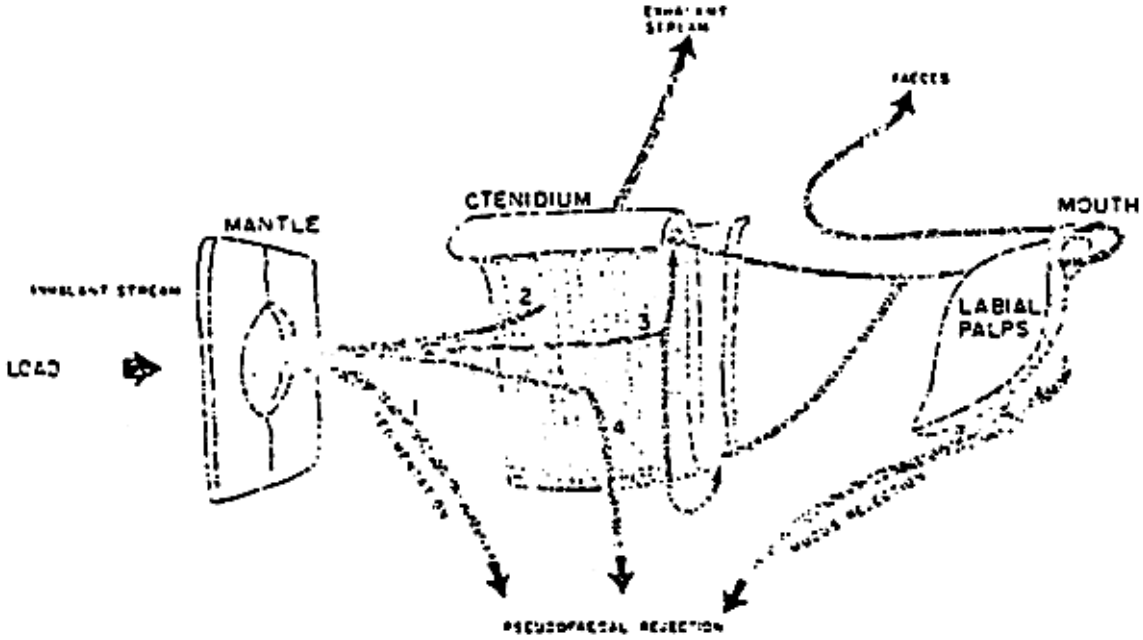

Figure 7 : Schematic representation of paths and fate of particulate material drawn into the inhalant pallial cavity. 1: sedimentation of partcles; 2: passage through the ostium; 3: impingement upon ctenidium and transportation on frontal mucus bands to food grooves; 4: rejection of large mucus masses. (from Bernard, 1974)

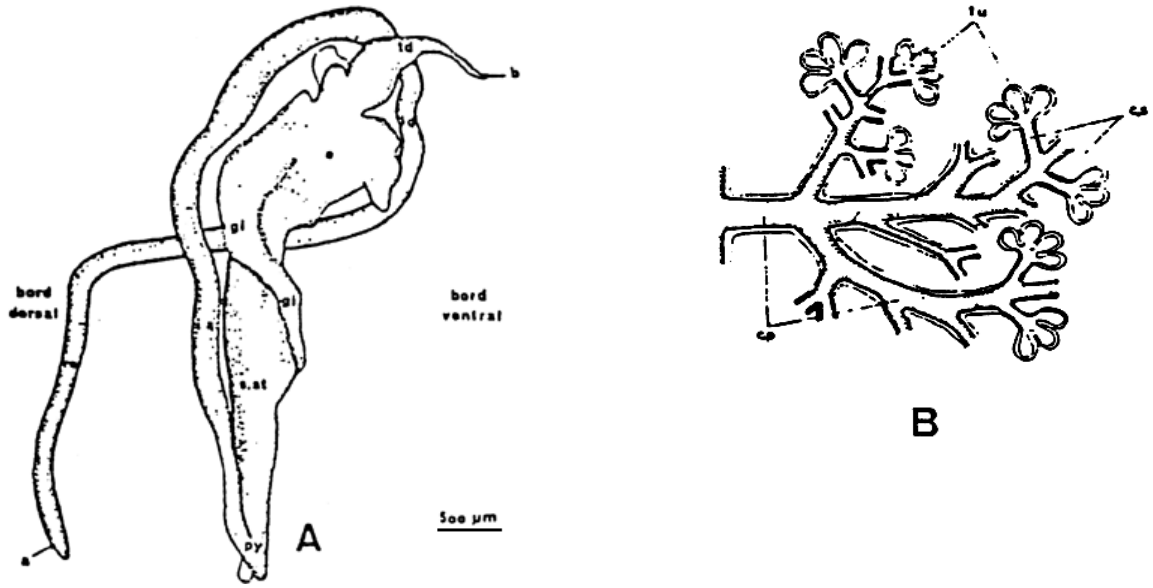

Figure 8 : Schematic representation of the digest tract (A) of Crassostrea gigas, and of the digestif glande (B). (from respectivly Boucaud-Camou et al., 1985 and Owen, 1955)

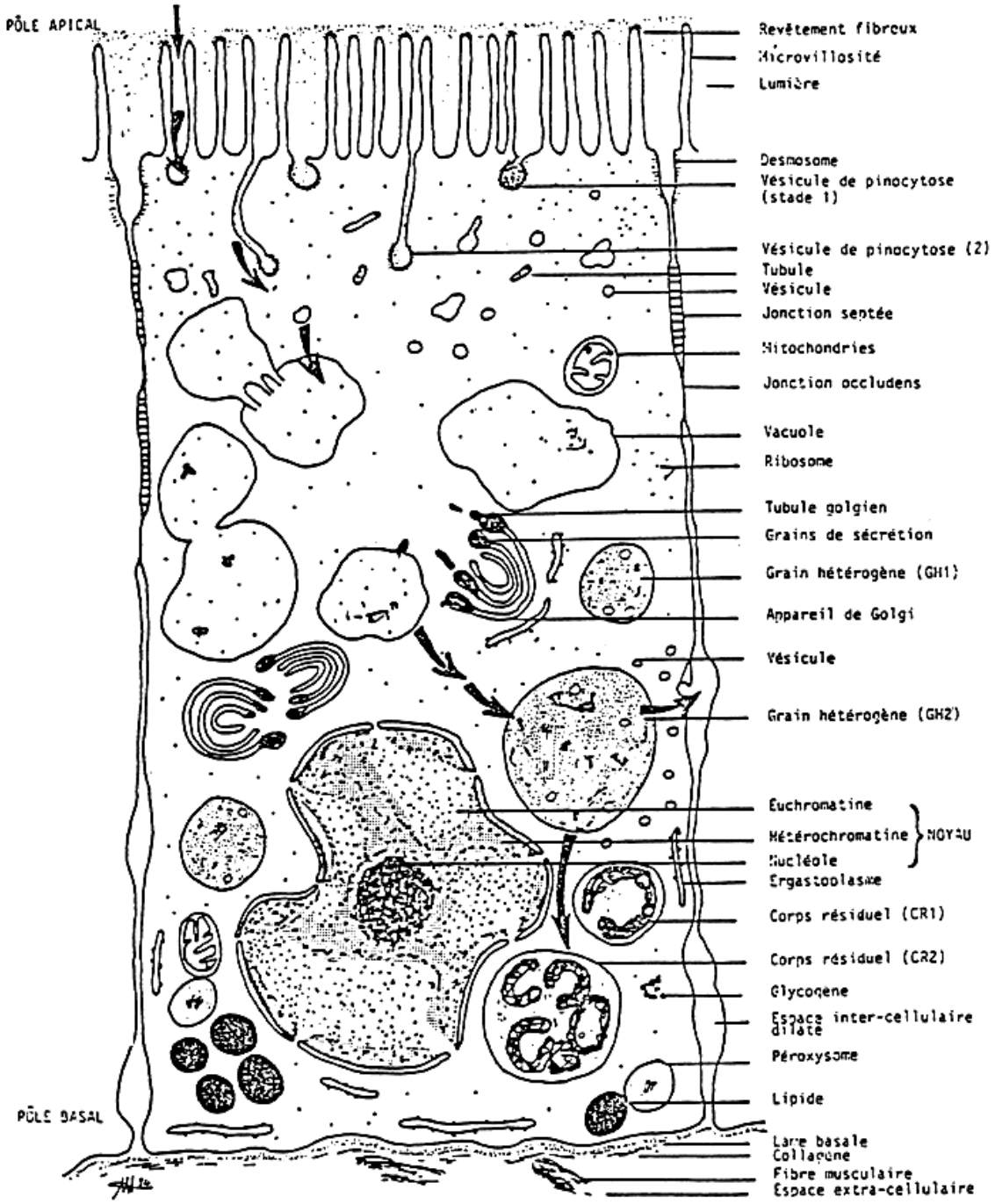

Figure 9 : from Henry, 1984a

Schéma d'interprétation ultrastructurale da !a cellule digestive des tubules digestifs de la palourde *Ruditapes decussatus* en métabolisme da routine.

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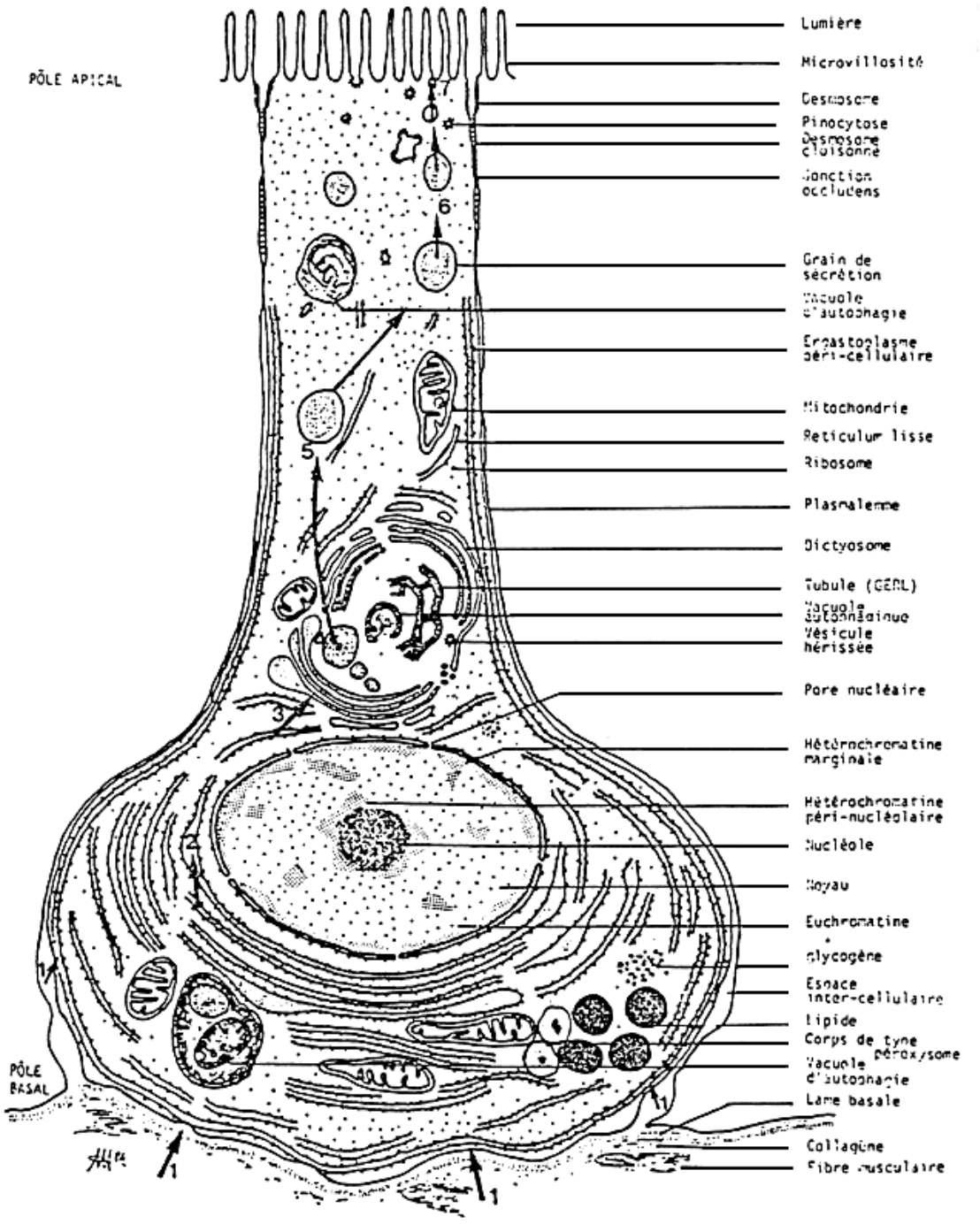


Figure 10 : from Henry, 1984b
Schéme d'interprétation ultrastructurale de la callule sécrérice des tubules digestifs de la palourde Ruditapas decussatus an métabolisme da routine

Tableau 4 : Percent of use of the main algae in the commercial hatcheries. (from Chretiennot-Dinet et al., 1986)

Nutritive value : *** : very good
** : good
* : mean

ALGUE-FOURRAGE	Fréquence d'utilisation (en pourcentage) d'après LUCAS, 1980	Fréquence d'utilisation (en pourcentage) d'après WALNE in COST,1978
Chaetoceros calcitrans ***	37,5	40
Dunaliella primolecta *	25	0
Iaochrysis galbana ***	75	80
Isochrysis aff. galbana "Tahiti" **	0	20
Nannochloropsis oculata *	25	0
Pavlova lutheri ***	62,5	70
Phaeodactylum tricornutum *	12,5	50
Pseudoisochrysis paradoxa **	62,5	50
Pyramimonas virginica *	37,5	0
Skeletonema coatatum **	12,5	20
Tetraselmis suecica ***	25	60
Thalassiosira pseudonana ***	62,5	40

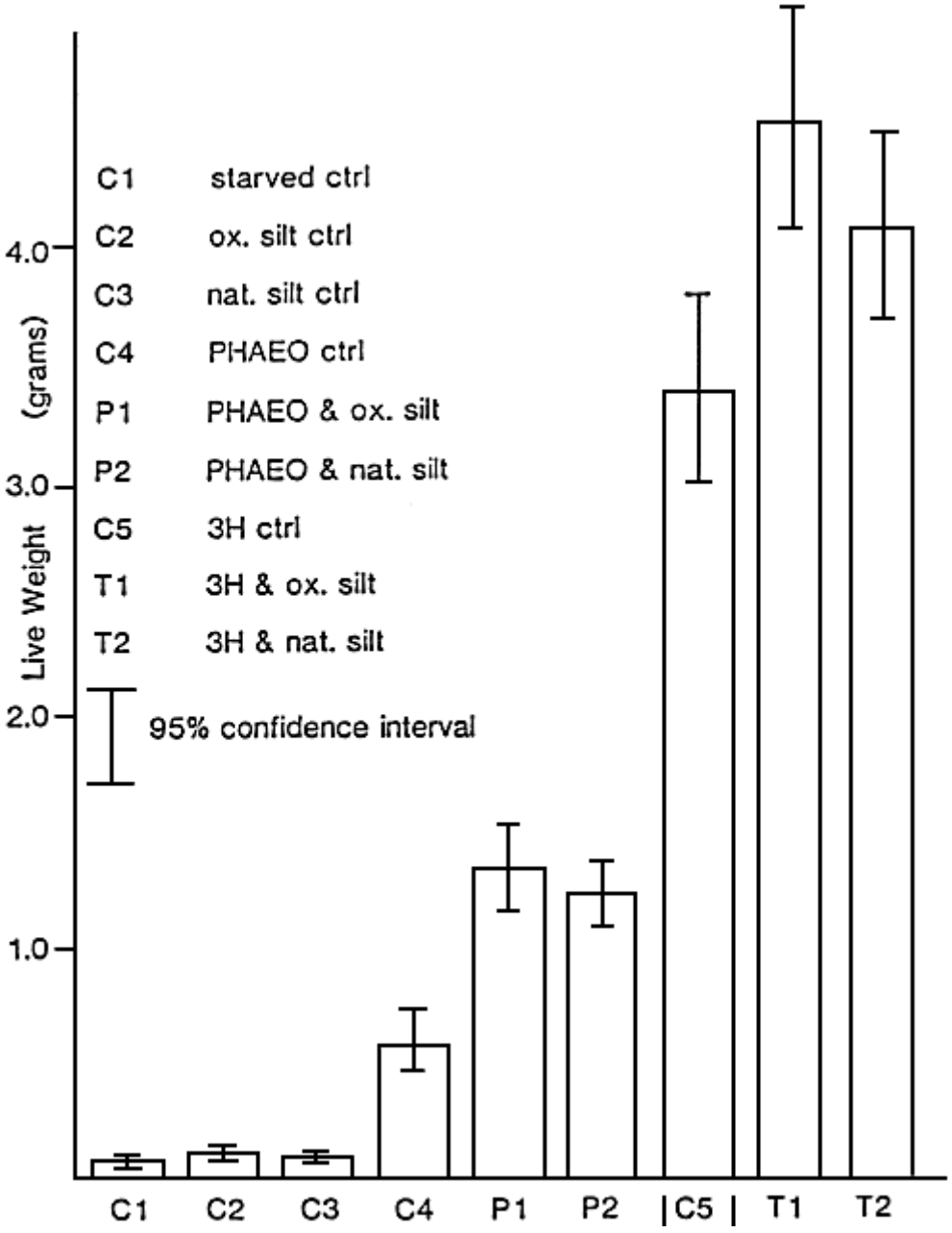


Figure 11 : Average increase in individual live weight of oysters fed with Phaeodactylum tricornutum (PHAEO), Thalassiosira pseudonana (3H) and natural and oxydized silt for 7 Weeks. (from Ewart and Pruder, com. pers.)

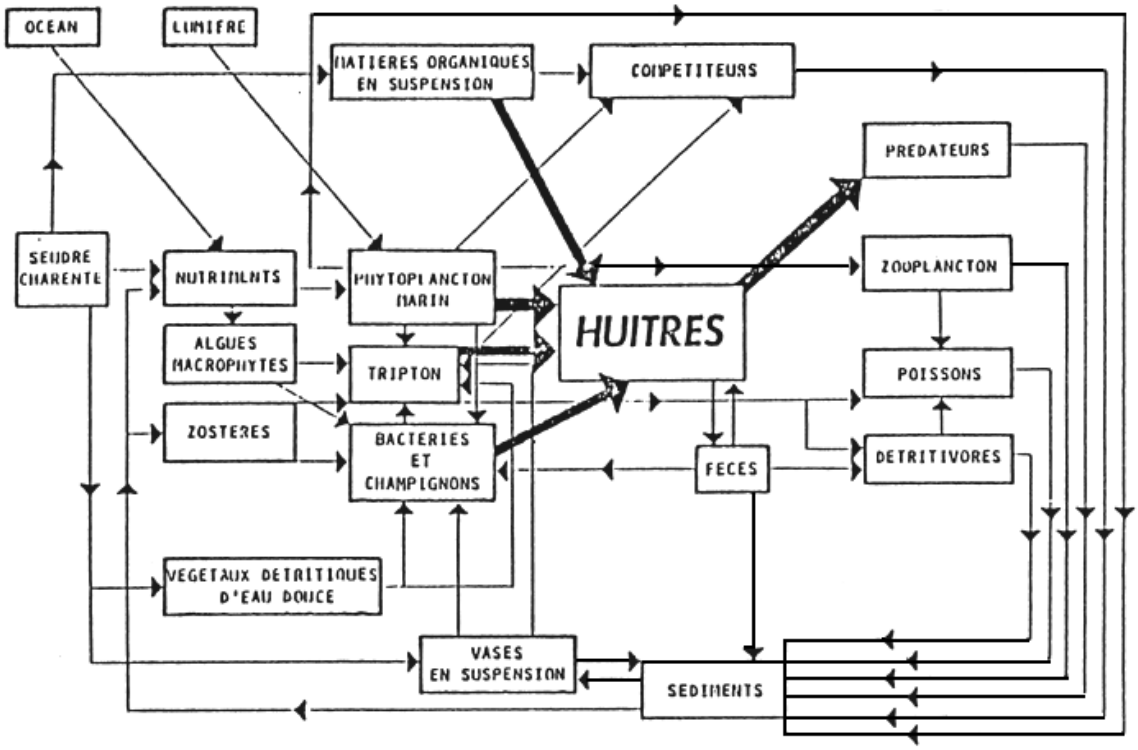


Figure 12 : schematic diagramme of an oysters' ecosystem in the bay of Marennes-Oléron. (from Heral, 1977)

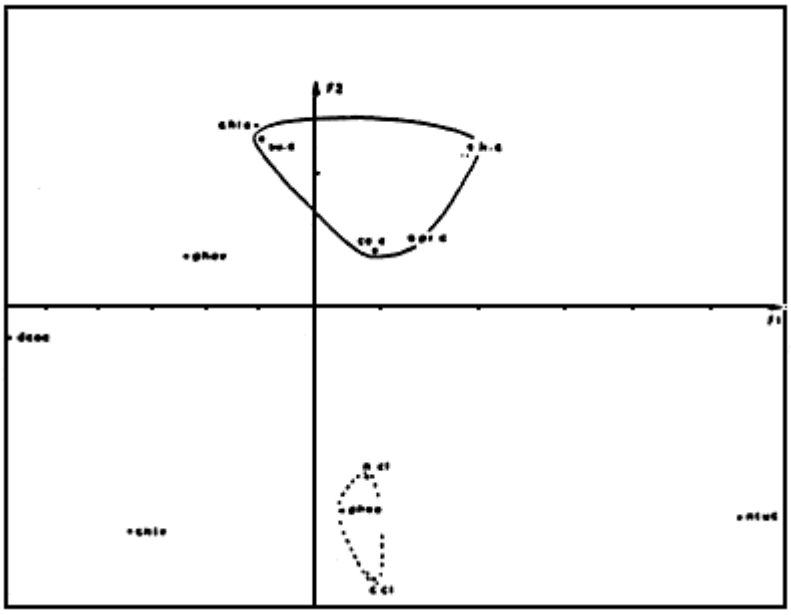


Figure 13 : Factoriel analysis of the relation between oysters and foods (from Deslous-Paoli et al., 1982)
For the oysters: protein (pr.c), lipid (li.c), ash (ce. c), carbohydrate (su.c)
For the foods: turbidity (ntuc), particulate C (C.cl), particulate N (N.cl), chlorophyl in water (chle) and on the bottom (chlv), pheophitin in water (phee) and on the bottom (phev), DCO (dcoc)

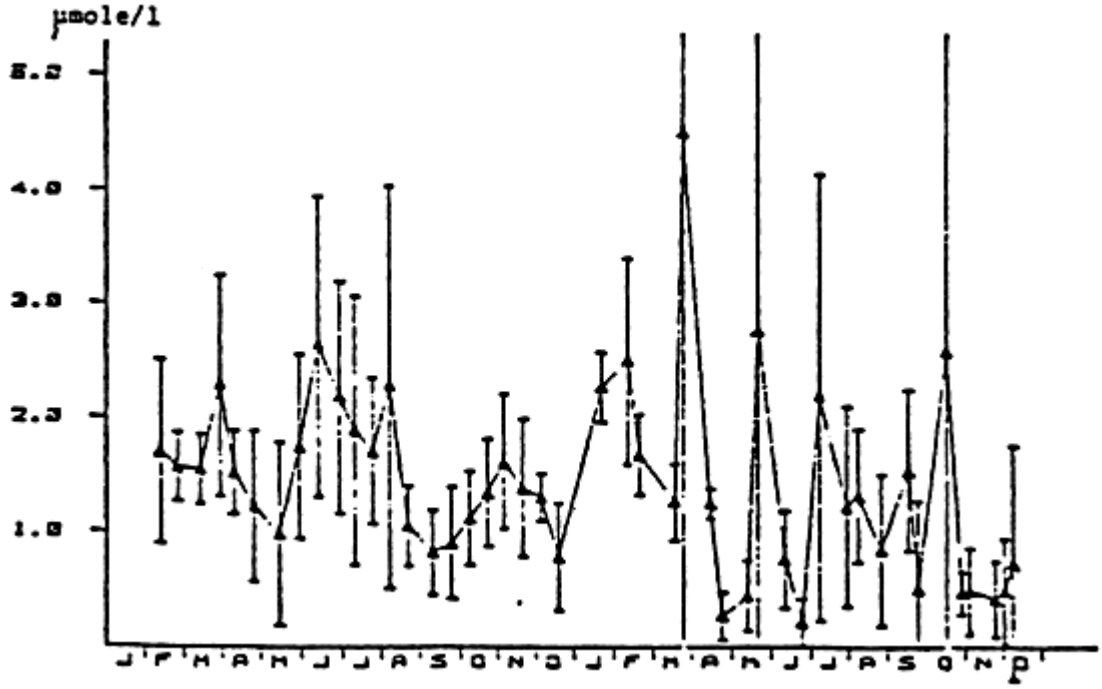


Figure 14 : Variation of the dissolve amino acids in the water of the bay of Marennes-Oléron (from Héral, 1986).

Table 5 : Bivalve larvae : filtration rates reported in literature. (from Sprung, 1984a)

Species	Shell length (µm)	Temperature (°C)	Food concentration (cellsμl^{-1})	Food alga	Filtration rate ($\mu l\ h^{-1}$)	Source
Ostrea edulis	200	20-22	15-26	*Flagellates*	27.1	Jorgensen (1943) with data from Bruce et al. (l940)
Ostrea edulis	218-280	19-25	31-54	*Isochrysis*	18-20	Walne (1956)
Ostrea edulis	219	23-24	8-123	*Isochrysis*	15-42	Walne (1965)
Ostrea edulis	178-184	24	8-230	*Isochrysis*	0.8-10	Walne (1965)
Ostrea edulis	231	21-22	0-123	*Isochrysis*	13.8-27.5	Walne (1965)
Ostrea edulis	180-260	21	20-50	*Isochrysis*	12.5-25.0	Walne (1966)
Ostrea edulis	228	?	50-100	*Isochrysis*	0.3-9	Wilson (1980)
Ostrea edulis	250	?	40-220	*Dunaliella*	1.8-5.4	Wilson (1980)
Crassostrea gigas	87-151	25	100	*Isochrysis*	2.8-7.0	Gerdes (1983)
Crassostrea gigas	89-294	25	50-50	*Isochrysis-Chaetoceros*	2.3-93.5	Gerdes (1983)
Mytilus edulis	170-260	18	25-380	*Isochrysis*	4-25	Bayne (1965)
Mytilus edulis	260	16	64	*Isochrysis*	12.5	Bayne (1965)
Mytilus edulis	260	11	60	*Isochrysis*	2	Bayne (1965)
Mytilus edulis	150	12	1.5-5.5	*Isochrysis*	11.4	Riisgård et al. (1980)
Mytilus edulis	120-250	15	3-6	*Isochrysis-Monochrysis*	16.2-141	Riisgård et al. (1981)
Mytilus edulis	120-250	17-19	3-12	*Isochrysis-Monochrysis*	10.6-85.3	Jespersen and Olsen (1982)
Mytilus edulis	120-250	6	1-5	*Isochrysis*	4-21	This paper
Mytilus edulis	120-250	12	1-5	*Isochrysis*	10-61	This paper
Mytilus edulis	120-250	18	1-5	*Isochrysis*	17-52	This paper

Table 6 : Bivalve larvae : ingestion rates reported in literature. (from Sprung, 1984a)

Species	Shell length (µm)	Temperature (°C)	Food alga	Ingestion rate (cells h^{-1})	Source
Ostrea edulis	180-195	20-22	Flagellate I	1000	Bruce et al. (19400
Ostrea edulis	218-280	19-25	*Isochrysis*	1040	Walne (1956 1959)
Ostrea edulis	178-184	24	*Isochrysis*	133-600	Walne (1965)
Ostrea edulis	219	23-24	*Isochrysis*	591-1517	Walne (1965)
Ostrea edulis	231	21-22	*Isochrysis*	456-2333	Walne (1965)
Ostrea edulis	180-260	21	*Isochrysis*	830-2500	Walne (1966)
Ostrea edulis	228	?	*Isochrysis*	90-900	Wilson (1980)
Crassostrea gigas	>200	20	*Isochrysis*	2600	Malouf and Breese (1977)
Mytilus edulis	150	12	*Isochrysis*	81-89	Riisgrd et al. (1980)
Mytilus edulis	Whole size spectrum	15	*Isochrysis-Monochrysis*	150-800	Jespersen and Olsen (1982)
Mytilus edulis	120-250	6	*Isochrysis*	18-80	This paper
Mytilus edulis	120-250	12	*Isochrysis*	41-292	This paper
Mytilus edulis	120-250	18	*Isochrysis*	38-408	This paper

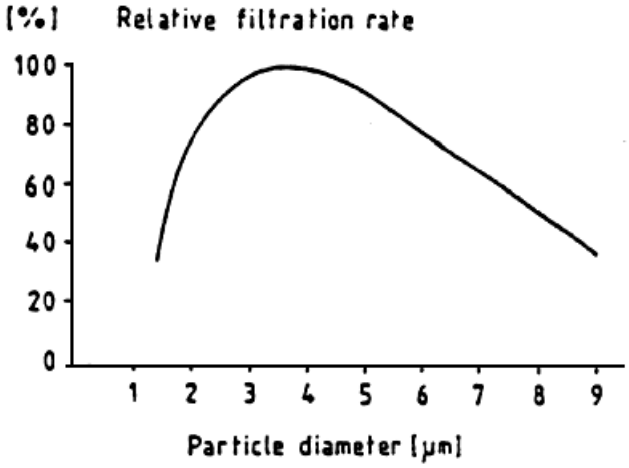


Figure 15 : Mytilus edulis larvae. Size spectrum of particle retention. Mean of spectra corrected to a maximum of 100 % : $y=-49.31+49.89x-4.17x^2$
x: channel of Coulter counter
y: retention efficiency (from Sprung, 1984a)

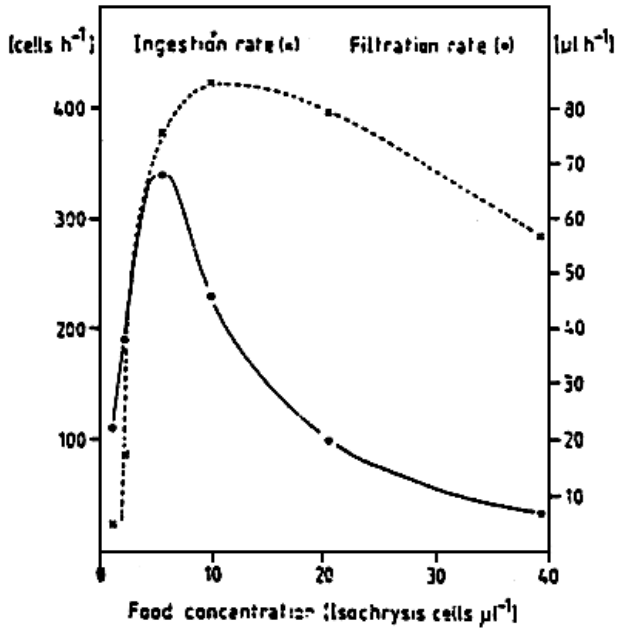


Figure 16 : Mytilus edulis larvae of 251 um shell length. Interrelation between ingestion and filtration rate at 12°C. (from Sprung, 1984a)

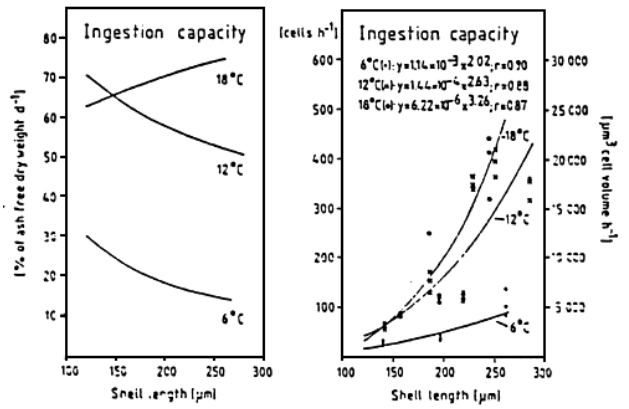


Figure 17 : Mytilus edulis larvae. Ingestion capacity of larvae of different sizes at the experimental temperatures.
y: ingestion capacity (cells.h^{-1})
x: shell length (um)
r: correlation coefficient (from Sprung, 1984a)

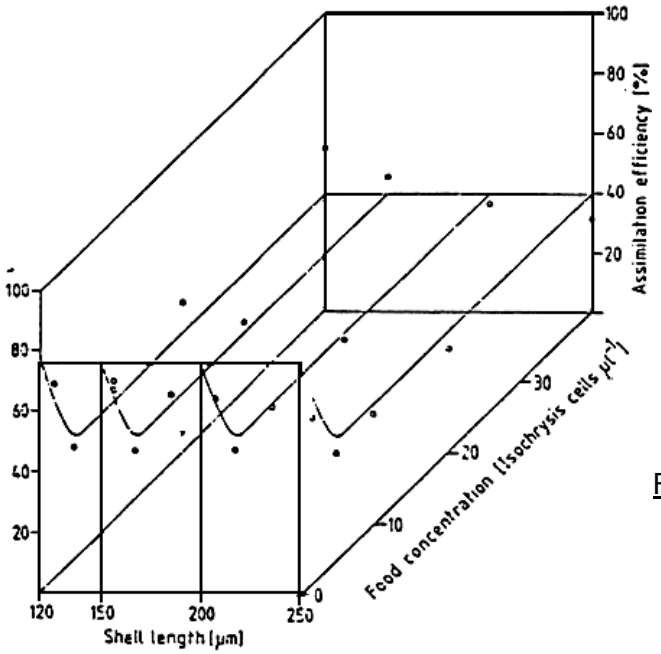


Figure 18 : Mytilus edulis larvae. Assimilation efficiency (%) at various food concentration and larval sizes. Curves fitted by eye. (from Sprung, 1984b)

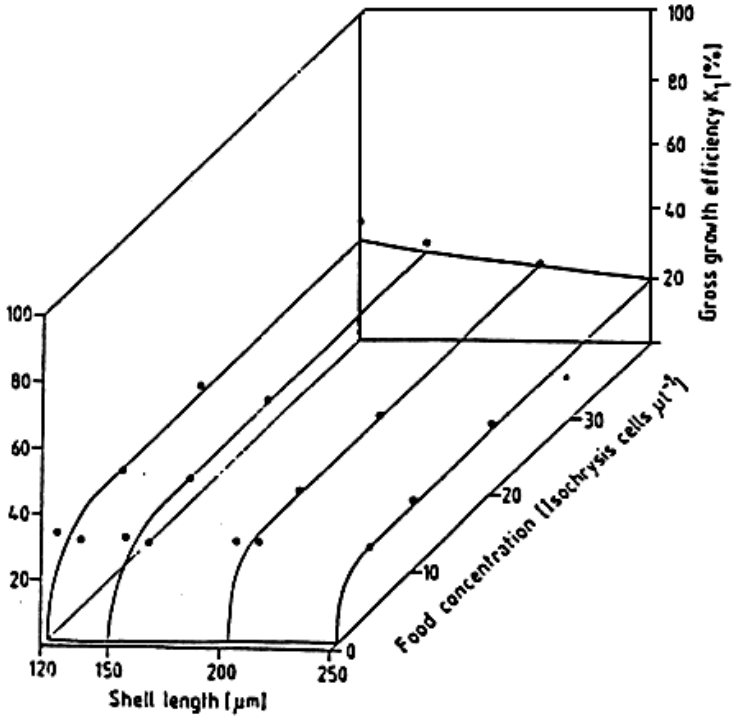


Figure 19 : Mytilus edulis larvae Gross growth efficiency K_1 (%) at various food concentration and larval sizes. Curves fitted by eye. (from Sprung, 1984b)

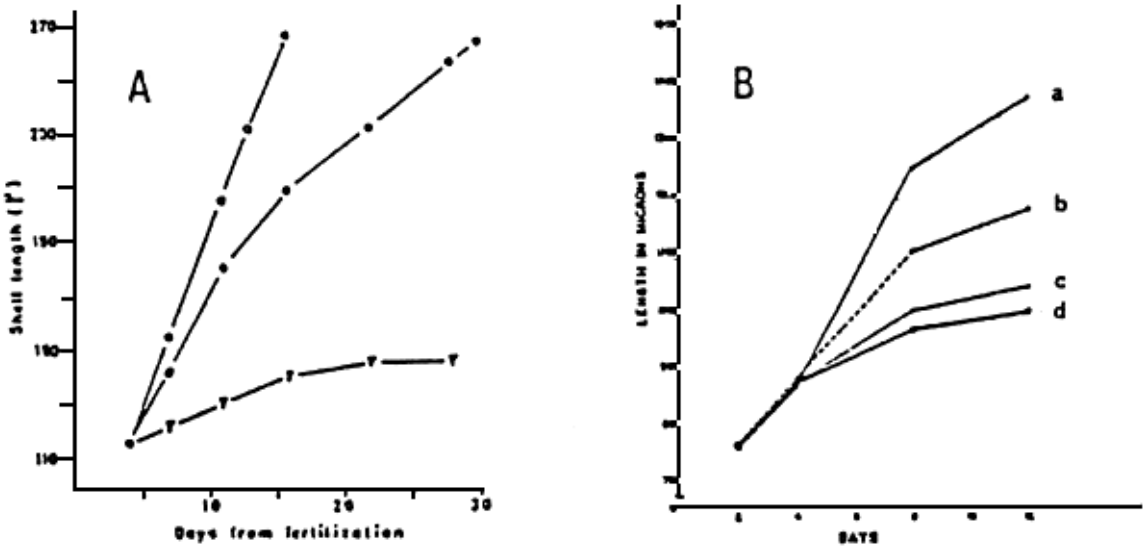


Figure 20 : Growth of Mytilus edulis larvae :

A-Growth of larvae feed with Isochrysis galbana

▼ : starved

● : 25 cells.ul^{-1}

○ : 100 cells.ul^{-1}

(from Bayne, 1965)

B-with an injection of 50 cells.ul^{-1}.day^{-1}

a : 440 larvae.1^{-1}

b : 2700 larvae.1^{-1}

c : 16500 larvae.1^{-1}

d : 32900 larvae.1^{-1}

(from Davis, 1953)

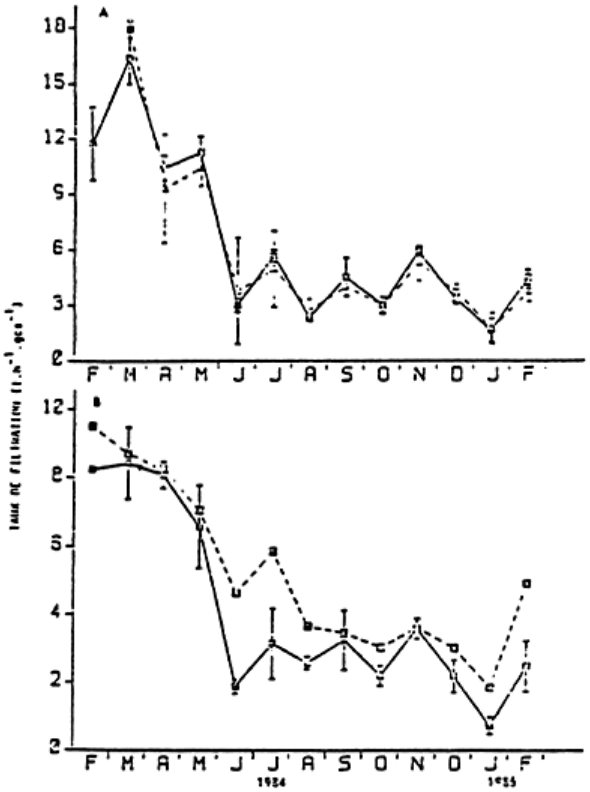


Figure 21 : Monthly variations of the filtration rates for Mytilus edulis feed with natural food. Measured from
A (—) Coulter counter
(---) chlorophyl and pheophitin
B (—) Total seston
(---) biodeposition
(from Boromthanarat, 1986)

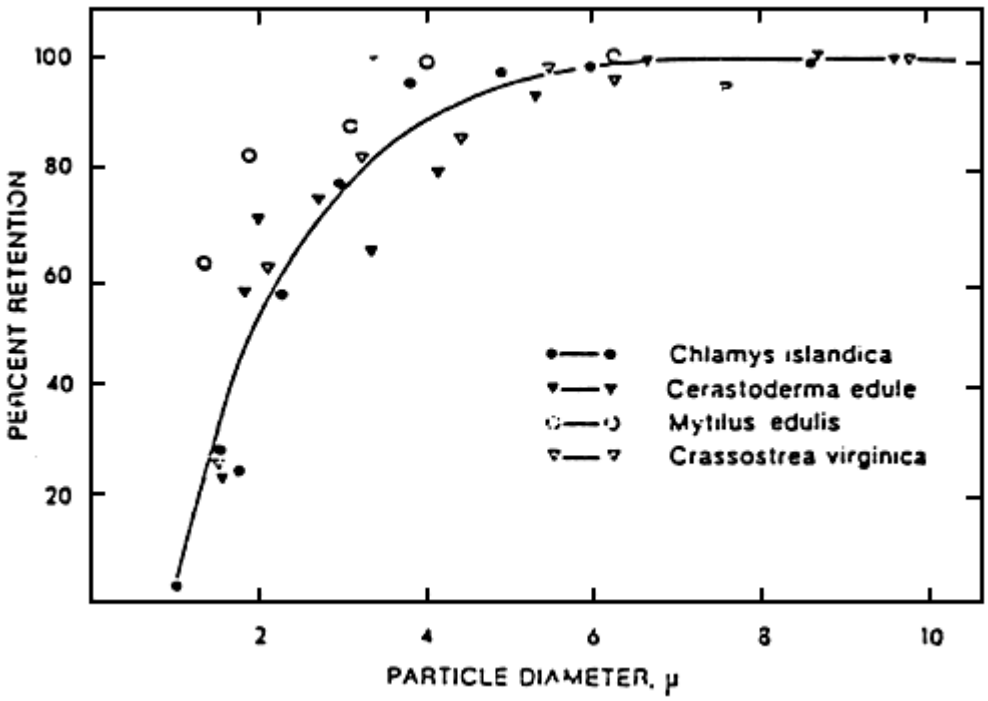


Figure 22 : Graph showing the particle retention efficiency of a variety of bivalves as a fonction of particle size. Maximum efficiency of each species = 100 % (from vahl, 1973)

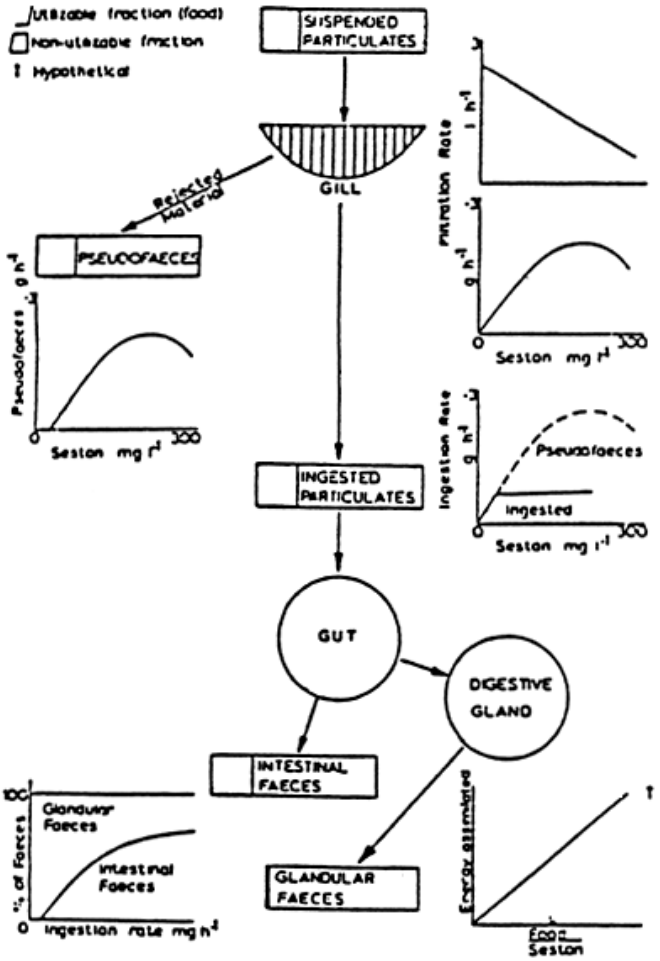


Figure 23 : *Mytilus edulis*. Schematic diagram summarising effect of particle concentration on feeding and digestive system (from Widdows et al., 1979).

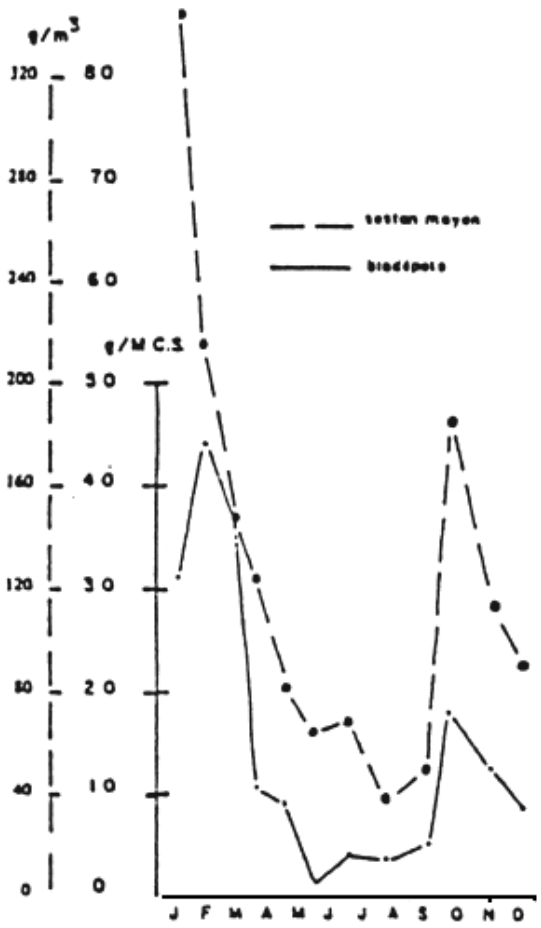


Figure 24 : Seasonal evolution of biodeposit product by *Crassostrea gigas* (g/g dry flesh weight) and of average seston (g/m^3) (from Sornin et al., 1983).

Table 7 : Efficiency of particle retention of *Crassostrea gigas* undor hight natural seston (20/2/84 ; 19/3/80 ; 14/5/84) and low natural seston (27/2/84; 27/3/84; 22/5/84) in relation with temperature.(°) : standard deviation. (Deslous-Paoli, 1985)

Date	20/2/84		27/2/84		19/3/84		27/3/84		14/5/84		22/5/84	
	rétention %	nb particules n 10^3 – n 1^{-1}	rétention %	nb particules n 10^3 – n 1^{-1}	rétention %	nb particules n 10^3 – n 1^{-1}	rétention %	nb particules n 10^3 – n 1^{-1}	rétention %	nb particules n 10^3 – n 1^{-1}	rétention %	nb particules n 10^3 – n 1^{-1}
0.6–0.8	0	-	0	556(20)	0	291(13)	7.4(1.8)	029(31)	0	332(30)	0	745(23)
0.8–1.0	0	-	1.2(6.7)	207(18)	0	360 (8)	6.7(2.5)	322(41)	0	387(16)	14.4(7.5)	343(23)
1.0–1.2	0	60(3)	7.4(1.7)	91 (8)	0.9(1.4)	350 (5)	6.5(8.9)	70(11)	0	358(11)	13.3(12.5)	123(26)
1.2–1.5	0	74.5(2.6)	6.4(0.9)	53 (4)	0.3(4)	221 (7)	8.3(10.3)	22 (3)	8.6(2.2)	238 (9)	24.2(18)	65(11)
1.5–1.9	1(2)	80(1)	16(5.9)	28(3.3)	13.6(9.8)	158 (7)	15.3(12.1)	12(1.5)	20.3(1.5)	157 (5)	29.2(24)	19(2)
1.9–2.4	16(3)	56(1.6)	22.5(3.4)	10(2.4)	19.5(14)	73 (5)	26.4(12.5)	6(0.7)	31.2(1.5)	70 (4)	37(23)	11(1)
2.4–3.1	29(3)	32(1.6)	37.4(10)	4.2(0.8)	26.8(5.2)	36(1.4)	41.6(12)	3(0.3)	39.1(5.0)	28(2.5)	49.6(19)	6(0.7)
3.1–3.9	39(3)	14(1)	62.9(9.5)	2.4(0.3)	39.2(5.5)	17 (1)	41.3(7.3)	3(0.3)	50.1(4.4)	15(1.2)	65.3(14)	3(0.3)
3.9–4.9	50(1)	5.3(0.4)	53.3(10)	1.2(0.1)	51.7(6.7)	7(0.5)	48.7(6.4)	1.1(0.2)	60.7(0.4)	7(0.4)	76.1(12)	1.6(0.1)
4.9–6.1	65(2)	2(0.2)	62.1(11.5)	0.6(0.1)	62.3(5.3)	2.7(0.2)	58.5(13.6)	0.3(0.1)	70.4(3.7)	3(0.1)	84.9(9.1)	1.1(0.1)
6.1–7.7	76(4)	0.8(0)	64.6(11.9)	0.3(0)	71.8(5.2)	1(0.1)	64.8(12.5)	0.2(0)	79.9(4.8)	1.4(0.1)	89.5(7.7)	0.8(0.1)
7.7–9.7	78(8)	0.2(0)	64.1(9.3)	0.2(0)	78.9(7.1)	0.6(0)	72.2(9.4)	0.2(0)	85.7(6.2)	1.8(0.2)	82.4(9.0)	0.2(0)
9.7–12.3	-	-	-	-	74.4(10.5)	0.2(0)	66.3(17.3)	0.1(0)	53.2(5.8)	0.7(0.1)	-	-
12.3–13.5	-	-	-	-	67.6(11.6)	0.1(0)	51.2(31.8)	0.02(0)	81.6(5.1)	0.3(0.1)	-	-
13.5–19.5	-	-	-	-	69.1(6.4)	0.1(0)	-	-	78.9(5.2)	0.1(0)	-	-
Seston mg1^{-1}	-	10	-	4	-	7.56	-	2.93	-	8.16	-	2.07
température °C	0 °C		5 °C		11 °C		10.5°C		16 °C		17 °C	

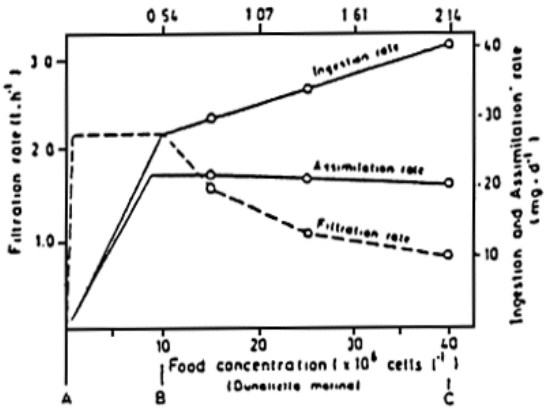


Figure 25 : Mytilus edulis. Concept of the interrelationships existing between filtration rate, assimilation efficiency and food concentration.
(from Navarro and Winter, 1982)

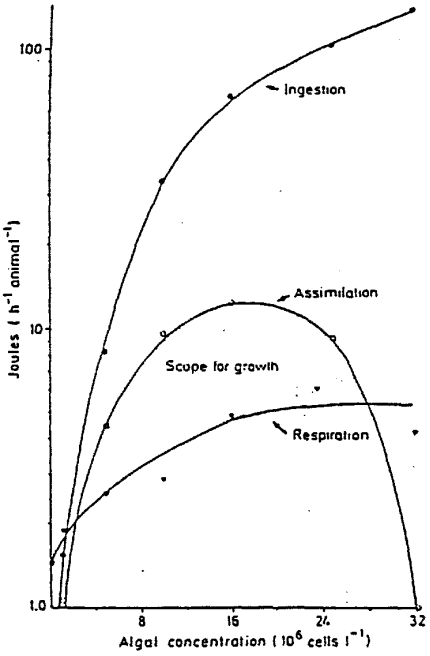


Figure 26 : Aulocomya ater. Ingested ration, assimilated retion oxygen consumption and scope for growth in 50 mm mussels, expressed as functions of food concentrations. (from Griffiths and King, 1979)

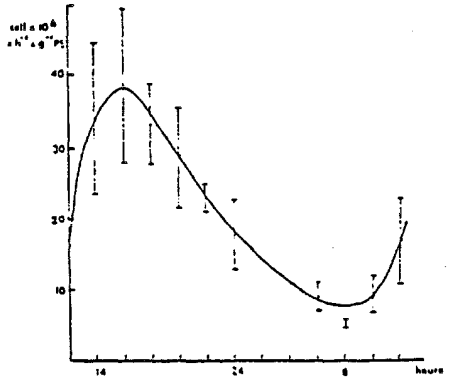
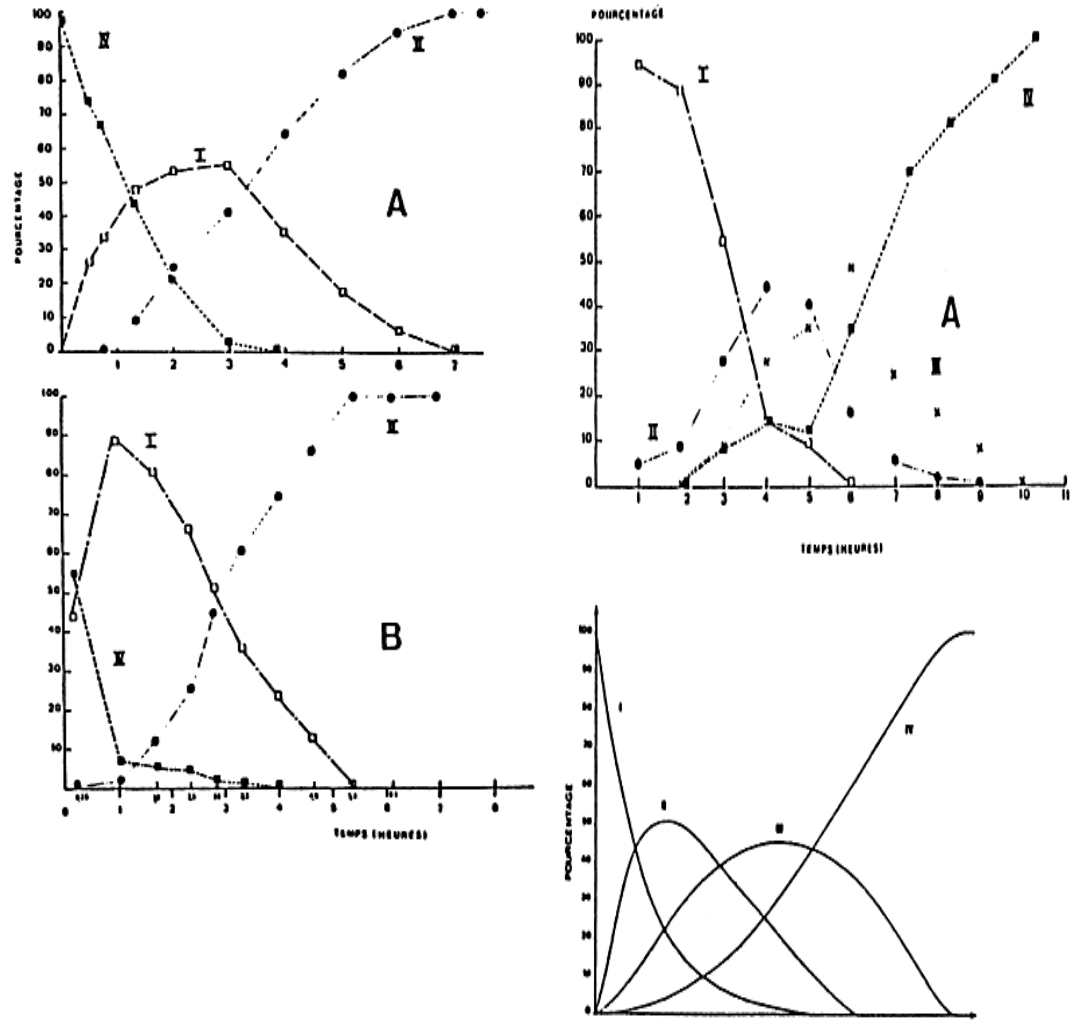


Figure 27 : Daily rhythm of the filtration rate of Crassostrea gigas. (from Coppelo, 1982)



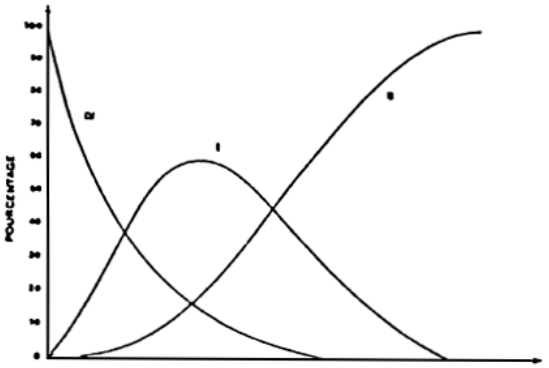


Figure 8a : Synthetic model of the ingestion and digestion during discontinuous feeding of larvae of of Fecten Maximus, (A) at 17°C. (from be Pennec and Rangel-Davalos, 1986)

Figure 8b : Synthetic model of ingestion and digestion during continuous feeding, (A) at 16°C, (B) at 18°C

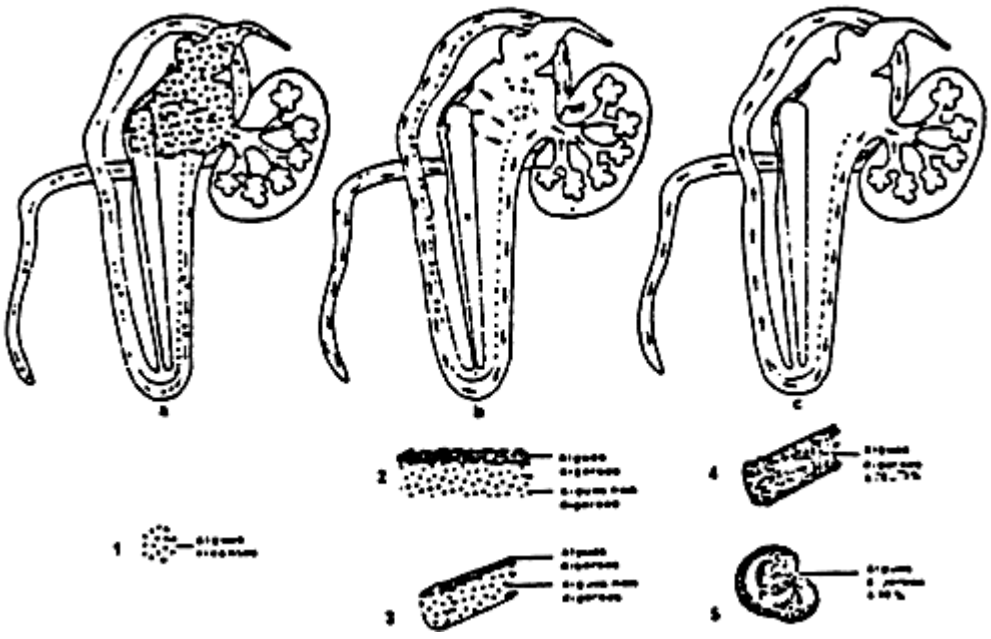


Figure 29 : Schematic representation of the digestive transit in Crassostrea gigas (for explanation see text). (from Boucaud-Camou et al., 1985)

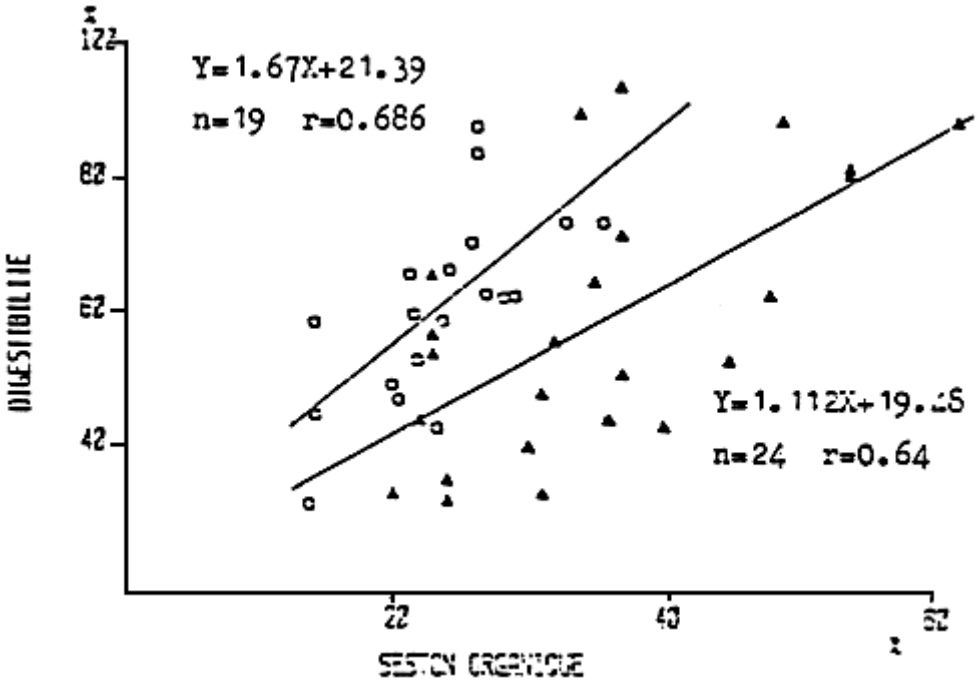


Figure 30 : Relation between the organic fraction of the food and digestibility for Perna perna (Berry and Schleyer, 1984)(o) and for Mytilus edulis (Boromthanarat, 1986)(▲).

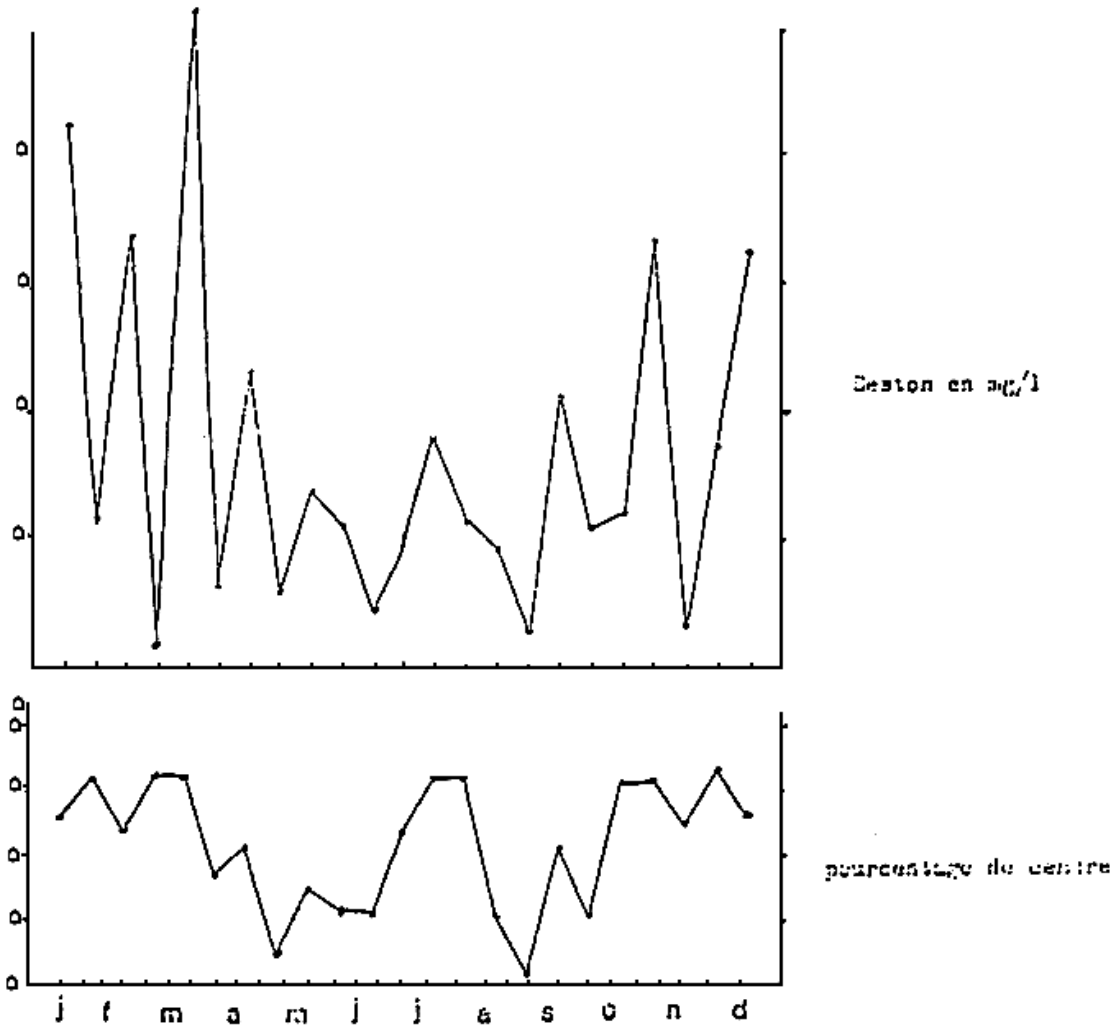


Figure 31 : Variation of the total seston and of its mineral fraction in the bay of Marennes-Olérorn. (from Heral et al., 1980)

Tableau 8 : Evolution saisonnière des pourcentages digérés (DC), des quantités d'éléments consommés et absorbés ainsi que du taux de filtration pour *Mytilus edulis* (A) *Crassostrea gigae* (B) et *Crepidula fornicata* (C).

		A				B				C			
	Période	Juil. 1982	Nov. 1982	Févr. 1983	Avril 1983	Juil. 1982	Nov. 1982	Févr. 1983	Avril 1983	Juil. 1982	Nov. 1982	Fevr. 1983	Avril 1983
DC %	Org	49,0	34,3	31,3	20,6	54,7	38,4	1,2	23,0	37,6	33,2	23,5	12,0
	Prot		40,5	41,9	75,3	11,8	43,0	17,2	62,7	15,8	33,8	0	74,4
	Lip	88,3	93,5	18,4	19,6	90,9	88,9	100	32,7	100	100	100	100
	Glu	46,3	0	0	50,5	41,2	0	0	26,2	21,1'	0	0	40,6
	Chloro + phéo	64,9	60,7	48,5	58,4	26,7	53,0	10,0	61,2	53,8	65,7	12,6	45,8
CONSOMMES	Org (mg)	129,9	234	1 572	813	162,3	451	543	1 107	36,8	75,4	106,2	86,6
	Prot (mg)	4,9	14,5	95,6	62,8	6,1	28,0	33,0	85,5	1,4	4,7	6,5	6,7
	Lip (mg)	2,5	6,4	12,9	14,0	3,1	12,4	4,5	19,1	0,7	2,1	0,9	1,5
	Gluc (mg)	10,5	10,6	59,7	64,6	13,2	20,5	20,6	87,9	3,0	3,4	4,0	6,9
	Chloro + Pheo (ug)	255	133	760	678	319	257	263	923	72	43	51	72
ABSORBES	Org (mg)	63,7	80,5	492	168	88,8	173	6,5	255	13,8	25,0	25,0	10,4
	Prot (mg)	-	5,9	40,1	47,3	0,72	12,0	5,7	53,6	0,2	1,8	0	5,0
	Lip (mg)	2,2	6,0	2,4	2,8	2,8	11,0	4,5	6,2	0,7	2,1	0,9	1.5
	Glue (mg)	4,9	0	0	32,6	5,4	0	0	23,0	0,6	0	0	2,8
	Chloro + (ug) Phéo	166	81	369	396	85	136	27	564	39	28	6,5	33

S	Energie (EPLG) (joules)	169	376	1 042	1 787	220	719	311	1 910	44	125	34	225
	Temps (h) immersion	14	16,5	18,5	18,5	14	16,5	18,5	18,5	14	16,5	18,5	18,5
	Taux de Filtration 1/h/gCs	2,03	2,63	1,75	5,52	53	5,07	0,6	7,52	0,57	0,85	0,12	0,59

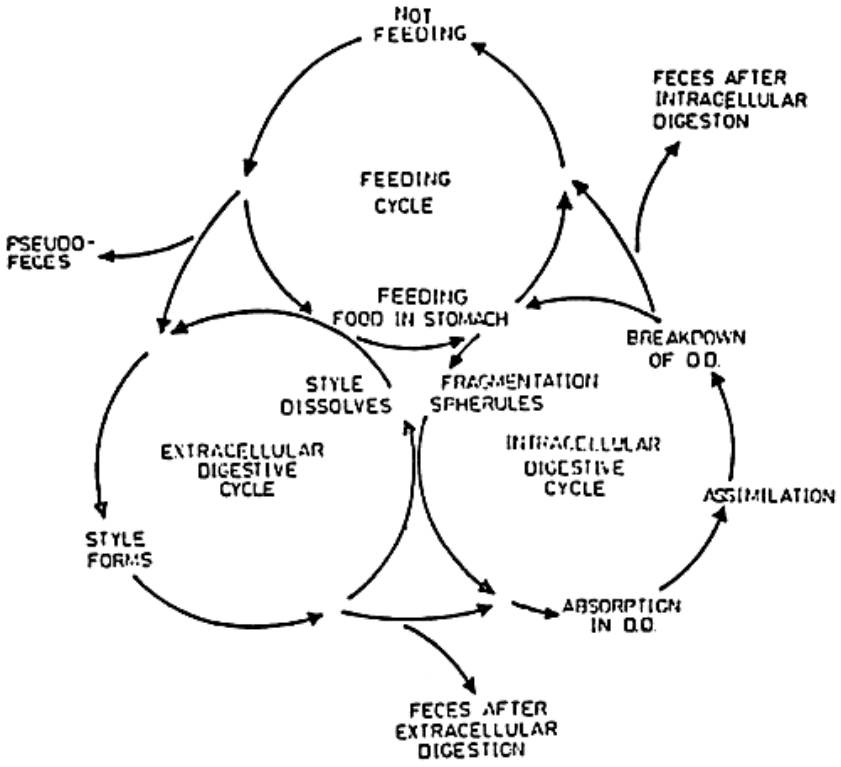


Figure 32 : The coordinated cycles of feeding and extra- and intracellular digestion of the bivalve. (from Morton, 1973)

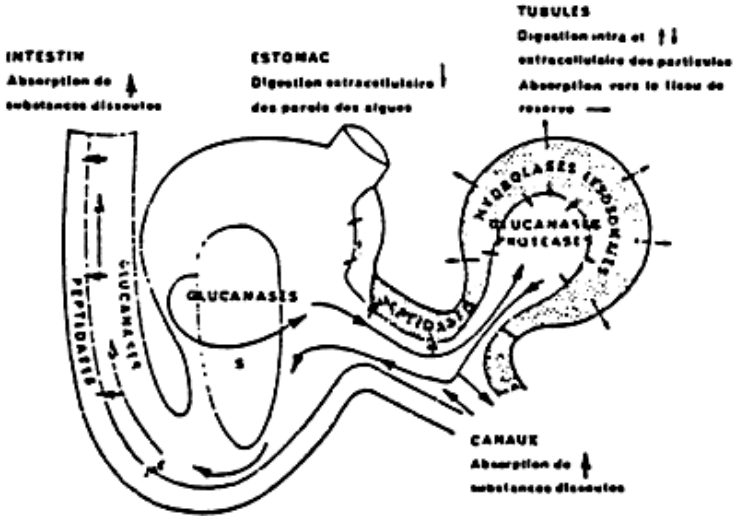


Figure 33 : Schematic representation of the digestion for Crassostrea gigas. (from Boucaud-Camou et al., 1986).

←		passage of the food
↑	,	transmembranous absorption
↑		absorption by pinocytose
↓		secretion of digestive enzymes
s	:	cristalin style

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THE USE OF RAW MATERIALS IN FISH FEEDING

F. GUILLAUME

1. INTRODUCTION

Long ago, the choice of raw materials was almost the only unique preoccupation in nutrition, before the scientific era came into being, nutrition dealt with nothing more than the choice of the appropriate food, and eventually the rations required. The study of food requirements, their digestive and metabolic utilization relagated the choice of raw materials to application level. This choice remains however very important for the success in the rearing results and it has even progressively entailed considerable scope in food chemistry research and is now included in animal food industry.

2. GENERALITIES

2.1. Definition

The definition of simple food or raw materials, intended for compound food manufacture is not unique. Some time ago, the principal trend insisted on the properties of food to stimulate or slow down the peristalsis of the digestive tract along with its action on the other physiological functions. Today, we tend to consider its as simple sources of nutriments (energetic, proteic, vitaminic, mineral); the composition tables employed for the formulation only includes these latter elements. In fact, food raw materials are not only sources of nutriments, but also of inert essential elements (especially cellulose or fibres in general), antinutriments, factors with a certain toxicity, substances with a pharmacodynamic effect, appetence or inappetence factors, etc... To these natural compounds must be added the more or less systematic contaminants such as living organisms (bacteria, fungis, acarida), chemical contaminants derived from human activity (heavy metals, organochlorine, organophosphorus, etc...) or of natural origin (mycotoxins). Finally, other very important characteristics must be taken into account : the physical properties of raw materials, for example the way in which they are easily mixed and agglomerated, etc..., in other words their technological properties.

2.2. The choice of raw materials

The choice of raw materials concerning feeding on general, is based on two axes : the empiric knowledge on one hand, and the sciences of nutrition, on the other. Empirism and tradition have led to the acquisition of appetizing products which are reliable or simply cheap or easy to find, much to the detriment of new food sources, suitable for advantageous combinations which can however be very complex and require treatment before hand. The actual scientific knowledge concerning the requirements of a species can be taken into account today, in less expensive formulation programmes. In theory, these programmes permit the selection of all the economical advantageous raw materials and to reject those which are two costly and even indicate for the latter the "interest price" at which their employment should become interesting.

In practice, these programmes very often only take into account the raw material tenor in nutriments or in a few other components such as cellulose or ash. For numerous properties such as appetence, technological characteristics, there are neither quantitative nor additive criteria at disposal which can be employed directly in linear programming. One can certainly take this into account indirectly through the technical constraints by imposing for example a minimum threshold or the complete opposite, a maximum threshold of such and such ingredient. But low costing formulation through linear programming is still greatly limited, especially in cases where the qualitative or quantitative requirements of the species are not well known and this is the case of the aquaculture species such as marine fish or even more so crustaceans.

In practice, the choice of raw materials must always be taken in two steps, in other words, a scientific confrontation with the results remarked in situ and an allowance made for the empiric data obtained through practice.

2.3. Classification

Raw materials can be classed in many different ways: in taking into account their origin (vegetable, animal, bicrobial -in general-, mineral, chemical), their main chemical characteristics (cellulosic food, amylaceous food, fat matter, protein sources, etc...). We shall adopt a combined classification which shall take into account the origin of the products, the species from which they were obtained along with the technological treatments which they were submitted to.

There are not many raw materials of animal origin (a few dozen), but those of vegetable origin most likely to be employed in aquaculture can be counted in hundreds. This report can only deal with a limited part of the above.

3. PRINCIPAL NUTRIMENTS REQUIRED

3.1. Relative importance

The principal elements which are taken into account in linear programming are , energy (expressed in digestable, metabolisable energy) proteins and essential amino-acids, lipids and essential fatty acids, phosphorus and calcium along with occasionally, raw cellulose, certain vitamins, carotenoids. We can impose for each one of these elements either a maximum or minimum or intermittant limits. In general, the most expensive among these elements are firstly energy and then proteins. We can remark that the priority for energy in comparison to protein, results partly from the establishment method of the diets we use, this method dating back to RUBNER, at the end of the 19th century. Indeed, the energy of the diet is that of all nutriments, including proteins which in this way, contribute in outclassing all the other sources of energy which afterwards will be counted one by one as sources of amino-acids. Whatever the case, the most expensive elements in a ration is energy and the principal plastic nutriments : protides. On the contrary, minor elements from a quantitative viewpoint, vitamins and mineral oligo-elements are very low costing at formulation level so that, for security reasons, vitamin and mineral premixes are nearly always added to the complete diet. In other words, it is cheaper to add synthetic vitamins to food than to divide into doses those of raw materials, while taking into account the requirements of the animals. Due to this, the vitamin in linear programming is very often omitted except in certain cases, so as to know the total inputs and eventual excesses. The essential fatty acids and phosphorus which are utilized, hold intermediary positions between those of energy or proteins and those of vitamins or oligo-elements.

In theory, it is important not only to consider the gross inputs of proteins, glucids or lipids but to take into account at the same time the precise nature of the basic components (amino acids, simple sugars, fatty acids, etc...) and their availability. This is a complex problem which has already been discussed by somebody else during this session. Let us however, recall a few of the most important points :

3.1.1. The digestibility of nutriments is still quite unknown for marine animals. The "digestible protein" taken from old tables (pepsic digestibility in vitro) is hardly ever employed. Only "raw" elements are at present utilizable in linear programming.

3.1.2. It is always preferable to replace the high class nutriments (proteins, glucids, lipids) by their components or better still to use both at the same time.

3.1.2.1. Essential amino-acids and principally those which are scarce in the food eaten by animals (lysine and methionin) weigh much more than the non-indispensable amino-acids. But this priority is not as evident in fish, which in any case always use a great quantity of amino-acids for energetic purposes.

3.1.2.2. There will be always great uncertainty regarding glucids, as long as only the nitrogen free extrative is maintained for the estimation of the "total glucids". Even for real glucids such as starch, great differences in digestibility can be remarked, depending on the nature of the molecule, that of the strarch grain, technological treatments that the raw material has been submitted to, etc…. To take all these parameters into consideration, is still difficult in fishculture feeding.

3.1.2.3. Among the lipid components, fatty acids at low melting point (unsaturated, or polyinsaturated are digested best by aquatic animals.

The criteria used at formulation level are therefore not simple and are not always accessible either : certainly the usual protein tenors in amino-acids are easy to find in tables, but the same does not apply for fatty acids, simple glucids, etc…. When there is no data available, the nutritionist must then make out his own tables or formulate judgements on such and such a raw material, according to the more or less subjective knowledge at his disposal.

4. A SUMMARY ON THE ANTINUTRITIONAL AND TOXIC SUBSTANCES FOUND PRESENT IN FOOD (Table I)

4.1. Distribution of these substances

On the whole, animal raw materials are poor in antinutritional factors, in spite of assertions made by supporters of vegetarian feeding. The principal examples known, are the presence of the chelating factor, biotin and of an antiproteasic activity in the white of a raw egg, along with the presence of a thiaminase in raw fish especially.

Certainly, animal meal (from blood, meat,.fish, ...) can sometimes be considerably toxic, when it are not properly stored or when the products employed are they themselves damaged before the food is manufactured ; but this toxicity caused by bacterial toxins is not part of the main characteristics of this food. The same applies for products which have been submitted to badly supervised technological treatments especially, the use of fatty acid peroxides which have a renown noxious characteristic, along with the presence of toxic preservatives (formol in fish meal).

Plants on the contrary contain, quite often, substances which can limit, either the appetence (unpalable substances) or the utilization of the nutriments by the animal (antinutritional substances), or interfere with its physiological functions (toxic poisonous substances). The latter have often no role in the physiology of the plant and appears as a simple means of defense of the plant against the animals; these means, are generally more dissuasive (toxic but not mortal) than deadly (real poisons). The principal families of this immence arsenal are as following: complex membranous glucids, polyphenolic substances, saponins, and other glucosids, alkaloids, enzymatic inhibitors, lectins. Other compounds can have an unfavourable and even a real toxic effect: phytic acid, fermentable glucids, "abnormal" fatty acids, amino-acids containing selenium, etc...

Let us now give a brief summary on each one.

4.2. Complex membranous glucids

These are widespead compounds in all vegetable diets and have an unquestionable physiological function on the plant : They are the basic structures of the

vegetable cell wall ; they can therefore form the constituant matter of whole tissues such as textile fibre cellulose or wood lignin.

These compounds, being very diversified, are always formed by polymerization of simple sugars (glucose, fructose, galactose, pentoses, etc...). They differ from reserve glucids by the nature of the bounds (instead of) and because of this, they are not hydrolysable by the enzyms of superior animals. Lignins differ from other compounds by the presence of numerous phenolic functions. These compounds form distinct groups in terrestrial superior plants (cellulose, pentosans, other hemicelluloses, pectins, lignin) and in algae algin, carragheen, agar agar. The precise dose of these diverse compounds being difficult and long to carry out, the WEENDE method is often employed instead, for the cellulose and raw fibre, which furnishes a rough and often debatable estimate of all these compounds. The VAN SOEST method which distinguishes hemicellulose and cellulose on one hand, lignin on the other, should be more suitable but is still not adapted to all the products.

Let us recall that if superior animals contain no compounds of this type, we can find in crustaceans, insects and some other invertebrates, a compound similar to cellulose : chitin.

Purified and added to diets in moderate doses, these compounds have often no effect at all or a benefical one (the ballest being favourable for the functioning of the digestive tract). In a natural state in the vegetable tissues, even when minced or mixed, they always limit the digestion of nutriments, first, of their own tissue and then of other food components. The same applies for certain polysaccharides which are added in high doses to fish food, such as alginates or guar gel.

The norms vary according to the species (herbivora are more tolerant due to the particular anatomy of their digestive tract and the bacterial flora which they accomodate) and these are not well known for fish. In practice, as the food contains less than 5 % of raw cellulose, it rarely creates nutritional problems ; at around 10 to 15 %, they must be incorporated with care into the diets of non herbivorous fish, at more the 20 % they must on general be rejected. In the case of herbivorous species such as mullet, supplementary research is required.

It must be remarked that certain invertebrates, in particular crustaceans, possess enzyms which hydrolize cellulose and chitin. The fresh water shrimp Macrobrachium rosenbergii in particular, seems capable of benefiting from high doses of food cellulose which for this animal is a real source of nutriments.

4.3. The phenolic compounds

These are very diverse compounds, which are widespread in vegetation. Although deriving from a same basic molecule (5 hydroquinic acid) they can have very different formulas and degrees of polymerization. There exist compounds with small molecules (coumaric acid, hydrolizable tannins) while others have real macromolecules (condensed tannins). Lignins, complex macromolecules are indirectly linked to this group. This complexity make global dosing difficult or even impossible : The diverse colourmetric methods proposed give very conflicting results. Tannins which are classed by nutritionists as the most important group of polyphenols do not form a well defined entity. They are more or less polymerized molecules which tan leather (from where they got their name), and precipitate gelatine and other proteins.

Phenols and polyphénols play an important physiological role for plants, they are sometimes linked to the colour of teguments and flowers, but their role is often obscur,

apart from their mechanisms of defense. Ingested by the animal, they have almost systematically a negative effect. Their role can reach interference stage, with the reproduction function of female mammels (oestrogenic role of the isoflavin by-products). Generally they reduce appetite due to their sourness, and have and astringent power on the digestive tract, they precipitate enzymatic proteins which decreases therefore digestibility. They can be absorbed, and release a mechanism of intoxication which will modify for example the methionin and cholin requirements. They can also influence the intestinal flora (superior vertebrates). For example, doses of around 0.5 % of tannic acid will suffice to reduce the growth of animals and cause mortalities in poultry. A decrease in the toxicity of tannins can be obtained by supplementing diets with methyl group donors. Tannins can also become more or less denatured due to heat, lime, etc... , but the best means of elimination remains, the selection of varieties which are poor in or completely devoid of these polyphenols (sorghum and leguminosae without tannins).

Let us remark that the study of these molecules is not well developed, and that only a very limited data is available on the raw material tenors in tannins or other phenols.

4.4. Saponins and other glucosides, alkaloids

Glucosides are molecules containing one simple or very little polymerized glucide which is linked to another molecule of varied nature called aglycone. Certain glucosides possess very active and even severe toxic pharmaceutic properties.

Glucosides, whose aglycone is either steroide or triterpenic are called saponines due to their hydrophilo-hydrophobic properties (affinity with water and lipids) which are similar to those of soap. They are found present in a great number of plants. Saponine doses are measured in specialized laboratories which carry out chemical methods and tests on insects. Saponines are toxic for superior vertebrates, principally because of their sequestrum properties with regards to cholesterol ; they are even more toxic for invertebrates, which do not synthesize cholesterol, and also for fish. For the latter, it seems that the toxicity of saponine is derived from its foaming capacity which perturbs the functioning of their gills.

Another example of glucosides of great practical importance is that of colza thioglucosides (vinylthio-oxazolidon), which have antagonizing effects on the thyroid hormones. These compounds can be inactived by thermic treatment. We shall also remark the presence of gossypol in the cotton seed, and which seems to play a multiple role: decrease in the digestion of proteins, slowing down of the proteic synthesis, anemia weaking power.

The aglycone of glucosides can consist in an alkaloid molecule, a group of compounds, characterized like glucosides themselves by the very remarkable properties at pharmaceutical level, which ranges from bitterness to acute toxicity. The solanine found in the potato is an example of a glucoside, with an alkaloid of little activity and found present is small quantities in common foostuff. On general, the presence alone of alkaloids in a vegetable makes it inedible. However, there exist certain cases where the alkaloid rich raw materials can be used after the correct treatment (bitter lupins) has be carried out.

Alkaloids are after very resistant to heat and difficult to destroy, but there also exists, as in the case saponines, important variations from one sort to the next, within the same species. The acquisition of different lupins without alkaloids is not new; the

production of colza, poor in thioglucosides and lucerne containing hardly any saponine, constitute another improvement stage of the food values of fodder.

4.5. Antitrypsic and other antienzym factors

These are compounds of proteic or peptidic nature which are very common in the seeds of certain plants, especially leguminosae, such as the soya bean. These factors inhibit the action of trypsin which also causes a hypersecretion of this enzym by the pancreas, which becomes hypertrophied. This effect is more or less remarkable depending on the dose ingested. Its effect is negligible at feeble doses but corresponds to proteic malnutrition at high doses and can cause the apparaition of cancer.

Most of the antitrypsic factors are thermolabile (this is the general case for leguminosae) and the appropriate thermic treatment will suffice so as to bring them down to an acceptable level.

In the same way, as certain vegetable proteins inhibit trypsin, others inhibit trypsin, others amylases. These last factors have been remarked especially in cereals. Their action is however much more moderated, and due to this fact, they are of less importance.

4.6. Lectines

Lectines are like antitrypsines, proteic molecules, found commonly in vegetable diets. They have a curious characteristic of sticking onto the glucidic residue of the membranes of certain animal cells, causing their death (ex. hemagglutinins settle on erythrocytes).

Lectines can be violent poisons (ricin aced lectines) or cause simple reductions in the digestive functioning through their destructive action on the intestinal cells.

Due to their proteic nature, lectines are inactivated by heat, but some of them are quite resistant and are a real danger to man and animal (seeds of certain bean species).

4.7. Diverse compounds

The most classical example of antinutriments is certainly phytic acid or hexaphosphoric inositol acid. This molecule, found commonly in the seeds and tubers, has as role the stocking of phosphore and metals in plants. It has multiple chemical properties (antioxidant and complexing especially) which give it a particular role in the digestive tube of animals : to begin, it can complex calcium and other metals, such as zinc copper, manganese, thus making them scarce. It can also form complexes with proteins or starch which makes them less digestible. It also constitutes a source of phosphorus, which is not greatly employed, as the animals have no phytase, and only a part of the phytic phosphorus is absorbed after hydrolysis by the bacterial enzyms.

Let us remark that for reasons which are still obscur, phytic acid is not toxic for japanese shrimp but its nocivity for other fresh water fish has been clearly established.

Let us mention some other elements or compounds, found present in plants, and which are likely to cause disorders in animals which consume them; erucic acid (unsaturated C 22 fatty acid), amino acid which contains selenium, instead of sulphur, oxalic acid, diverse antithyroids, toxic amino acids, enzyms such as the ascorbase of cucurbitacae seeds and the urease of soya, etc...

5. NATURAL AND ARTIFICIAL CONTAMINANTS

We have already stated the bacterial toxins which can confer to animal products an acute toxicity: similar properties can be found in vegetable raw material which are attached by microscopic fungus (mildew). This toxicity, which has been known for a long time, was attributed to substances which are being progressively identified, and whose activity is extraordinary (aflatoxin for example is 100 to 1 000 times more cancerigenic than the most powerful synthetic oncogenes known). Although present at trace stage, these substances can now, be measured in a certain number of laboratories. They are frequently found in raw materials which are not properly stocked and especially in those which have been stored in a hot humid atmosphere. Let us remark that the presence of a species of fungi secreting a mycotoxin is no proof of the presence of the latter. Save exception (destruction of aflatoxin by ammonic vapors) it is not possible to eliminate these toxins but only to separate damaged products.

Let us only recall to mind briefly the compounds commonly employed by man which are likely to contaminate animal foodstuff: weedkillers, fungicides, and moreover organo-chlorinated or organophosphorated insecticides, etc... The most active among these can be easily dosed. Heavy metals are also very toxic, in particular zinc and copper (which are necessary in feeble doses) lead cadmium and mercury especially. It must be remarked that they can also originate naturally. We shall only briefly recall to mind the possibility of the transmission of pathogenic germs by foodstuff : this is an extremely rare case.

6. QUALITY CONTROLS (cf. Tables 2 and 3)

if we are only concerned by the verification of the nutriments tenors, two attitudes can be adopted by the manufacturers; either the sole use of raw materials which have been correctly analysed or the complete trust in the mean composition of the product.

The second attitude is still more widespread, especially for manufacturers who have not a sufficient means for analysis at disposal. This method is also adopted by modern and well managed enterprises where at least certain raw materials have been verified, it is more economic to use rapidly a stock of raw materials which have be bought in confidence, while leaving a security margin in the formulation rather than to immobilize, analyse and to more or less adjust the formula to the real composition.

But this attitude, which has proved itself valid for products of very constant quality (cereals, soya cake) usual fatty matters is not acceptable for vegetable by-products which can have the rate of cellulose vary from 1 to 2, for animal meals whose ash tenor can not be guaranted, for vegetable species of unknown variety, which can contain one or more toxic substances. The resort to more or less numerous analyses has become more and more necessary as the foodstuff can vary naturally, thus it is badly known and adulterations have been suspected (fraud). But due to the number of analyses required theoretically, it is difficult to perform all of them. Due to their cost, we must therefore limit ourselves to the strict minimum necessary. It is also important to know how to interpretate them and especially to keep in mind the remaining unknowns. The WEENDE method of analysis remains valid although its limits have been underlined more than once (graph 1). It is therefore important to complete by a more detailed analysis: amino acids, fatty acids starch and sugar (Table 4) etc…. However, detailed these analyses may be, they only give a rough idea of the elements present, and not the digestible or available food. In practice, the measuring of certain antinutritional factors or of certain indirect criteria (red phenol test on soya) can however give indications on the

digestibility of nutriments. Chemical analysis is not the only method of control employed : Bacteriological or fungal research can also be carried out, along with the identification of raw materials under microscope and the use of market value criteria (density, aspect of cereals, etc...). Table 5 shows diverse criteria of the market value which is more or less employed.

7. THE PRINCIPAL RAW MATERIALS (Brief summary)

7.1. Animal raw materials

Although they represent a very feeble part of the food for terrestrial animals, raw materials of animal origin nearly always represent more than 50 % of the food for aquatic animals. This is due to two reasons : on one hand, aquatic animals (poikilothermic) require high quantities of protein when expressed in percentages of the total ration, on the other hand "carnivora" are much more numerous than "herbivora" among the fish species that man has chosen for aquaculture.

It was rapidly discovered that for nutriment inputs of around equal amounts, the carnivora species showed better growth when their diet basis contained animal meal than when vegetable products were employed. The reasons of this superiority are not always evident: It is true that their digestibility is always higher and the profile of their amino acids is generally quite similar to that of the ideal protein for the animal. But other factors can also play a capital part: appetence, richness in group A vitamins, the presence of "unknown growth factors" along with, for products of marine origin, the presence of essential fatty acids (longchain polyinsaturated fatty acids).

These products have also the advantage of being devoid of cellulosic type compounds along with the presence of nearly all the antinutritional factors, but they also entail notory disadvantages: high risks of bacterial contamination; limited time of conservation, poor binders (with the exception of blood meal). Amongst the specific disadvantages of the products, let us point out the excess calcium of bad quality in meat or fish meal, the excess of saturated fatty acids in meatmeal, the risk of peroxidation of unsaturated fatty acids of fish meal, the unbalance of certain proteins (blood and especially feather), etc...

The most useful controls, apart from those for proteins, lipids and ash, are the microscopic examination, the measuring of the peroxidation of fats, available lysin, heavy metals, eventually the histamin tenor. Adulteration of these products is feasible with urea or feather meal, etc...

7.2. Vegetable products (cf Table 6, 7, 8)

Very numerous less adapted to the requirements of marine fish (save some exceptions such as mullet and vegetarian shrimp), they can however be very useful:

- as sources of digestible glucides, they permit substantial saving of protein and even lipids,
- as sources of digestible or indigestible polysaccharides, they can be greas: binders (wheat and wheat by-products; purified starch, pregelatinized or processed starches, guar gum, alginates, etc...

Some of them are excellent sources of group B vitamins which do not leach easily as they are enclosed in vegetable cells (By-products of rice and wheat)

- Being generally cheaper than animal meals, they often permit to cut down greatly on the production coasts.

Nevertheless, these products are genrally less appetizing to fish than the preceding raw materials, their tenors in cellulosic products must be carefully surveyed along with the numerous antinutritional factors that they contain or may contain if not properly treated. From this viewpoint, it is essential to take the products one by one into consideration, by referring to the individual composition wnile not forgetting that all antinutritional factors are not completely known, especially as to concern inhabitual raw materials, and that their effect on fish is even less known. Certain vegetable products, especially those from tropical countries are also likely to contain very dangerous fungal toxins. The presence of pesticide residue is also a common occurence in these raw materials.

In comparison to unprocessed products, such as cereals and proteagineous leguminosae, industrial by-products are often standardized and their tenor in antinutritional factors is more often lowered. It is however necessary to verify the composition (cellulose) and the efficiency of the technological treatments carried out.

Table 7 shows the characteristics of some inhabitual Mediterranean products.

7.3. Microorganic products

Although these raw materials are scarce, they are relatively well employed in fishfeeding. The most re nown are yeasts, rich in group B vitamins, but they also contain other products (bacterial protein PRUTEEN).

The supply of growth factors and of antinutritional factors is still quite unknown for this food.

7.4. Other products

Salts (sources of essential minerals), pure amino-acids, binders, carotenoids also make up raw materials of which can not be taken into account in this report.

CONCLUSION

Raw materials, vast basic sources of nutriments from which the food manufacturer can make a precise and strict choice (if he only takes nutriments into account), are in reality a series of complex products. Their systematic control is far from being accessible for the manufacturer as it is very expensive, antinutritional factors are not all known, their effect on fish is even less known. Therefore, for the present, it is advisable for fish rearers to choose only sure products avoiding any surprises occurring at either analysis or employment levels. The technical and economical progress expected will only be feasible if we enlarge progressively the gamme of raw materials and for this, much theoretic and applied research, many initiatives and empiric tests, are necessary.

TABLE 1 : PRINCIPAL ANTINUTRITIONAL FACTORS OF SOME LEGUMINUSAE

(Non restrictive list)

Scientific Name (Latin)	Vernacular Name		Tan., Polyphen.	Alc. Gluc., - Sap.	Inhib. Tryps.	Lect.	Ac. Phyt.	Div. and Rq
	French	English						
Arachio hyrdges	Arachide	Puanut				+	++	Frequent
Cajanes cajan	Pois d'Angole	Pigeun pes			+		+	Aflatoxin
Canavalia meifornie	Pois sabre	Jack bean				++		
Coratonis eillius	Caroube	Carob	++					In pods
Cicer arlation (Lane asculmea)	Pois chiche	Chick pea	±	+	+		+	
Delichoe lablab	Dolique	Myscinth bean	++		+		+	
Ervue Lane	Lentille	Lentil			++	+	+	Thersolabile
Glyeine max	Soys, sojs	Suybean		+		+	++	Factors
Lathyrus eativus	Gesea	Chickling vetche		++			+	Difficult factors to be destroyed
Lathyrus sp.	Gesses	Chickling vetches		++			(+)	by heat
Levesane glauco	Ipil ipil	Ipil-ipil Lead-tree		++				In leaves
Lupiane altue	Lupin blanc	White lupin (e)	-	±, ++	-			
L. angustifolius. L. sp.	Lupin bleu, ...	Blue lupin (e)	-	±, ++	-			Varieties without
Medicago eativa	Luzerne	Lucerne, alfalfa		±, ++				alkaloids
Phaeoclus vulgaris	Naricot (ord.)	Bean, kidney bean navy bean	±	±	±, ++	++	±, ++	Very thernelabile factors
Phaecolus linatus	Naricot de Lims Pois du Cap	Lias bean	+		++	+	+	
Phaecolus op.	Naricots div.	Beans	(+)	?	(++)	(++)	(+)	
Plaun eativus	Pois (de jardin)	(Garden) pea	±		±	+	+	
P. avense	Pois fourrage	Field pea,	+		±	+		
Trigonella fomusroecun	Fenugrer	Fenugreek		++				
Vicia fabe	Fèe.	Field bean.	±		±		+	Varieties

	fèverole	fabs bean, etc...						without tannin
Victi sp.	Vesces	Vetches	(++ ?)	(++ ?)	{+ ?)	(+ ?)	(++ ?)	Not well known
Vigna mungo = *Fhsecolus*	Haricut mungo	Mung bean, black gram	+, ++		+		++	
Vigna radiats = *Phageoius iureue*	Amburique	Green gram	±, ++		+		+	
Vigna uncicuiata = *Vigna sinemsis*	Doligur mungette nicbe, yeus nuirs	Cuv pea. black vyes	±		±, ++		+	

TABLE 2 : AN EXAMPLE OF THE CONTROL OF RAW MATERIALS FOR FISH FOOD (BENAYAS BEVIA, 1986)

A. CHEMICAL CONTROL

- Proteins
  Digestible protein (pepsic digestion in vitro)
  Amino-acids
  Available lysine
- Lipids
  Linoleic and linolenic essential fatty acids
  Acidity of fatty material
  Peroxide index
- Minerals
  Tenor in oligo-elements = Fe, Cu, Zn, Mn, Co
  Tenor in toxic element = Pb, Hg and F
  Insoluble HCL
  Sodium chloride
- Glucids
  Monosaccharides : Glucose, fructose
  Disaccharides : saccharose (= sucrose)
  Starches
- Antinutritional elements
  Mycotoxins : aflatoxins, ochratoxins, zaralenone
  Nitrites and nitrates
  Gossypol, etc...

B. BACTERIAL CONTROL

- Lacteous products
  Genus Salmonella
  Species Escherichia coli
  Genus Staphyllococcus
- Animal meals
  Genus Salmonella
  Species Escherichia coli
  Genus Staphylococcus
  Mesophile aerobic bacteria
  Coliforms
  Fungi and yeasts
- Research of residues
  Insecticides
  Rodenticides
  Pesticides (sic)

TABLE 3 - An example of control analysis on raw material for fish food
(BENAYAS BEVIA, 1986)

	F Fish	F Meat	F Blood	F Feather	Vegetable products (in general)	cakes	Mineral substances	Vitamins	Fatty matter
H^2O	+	+	+	+	+	+	+	+	+
Prot.brutes (N × 6.25)	+	+	+	+	+	+	+		
Prot.dig. (in vitra)	+		+	+	+				
Lysino disponible	+	+	+			+			
A.A. soufrè				+					
N non pracoique			+						
Lipides brute	+								
Aciditè des lipides	+								+
Indice de pèroxyde	+								+
Ac.gras.									+
Sterols									+
Point Fusion									+
Anvioxydancs at autres conservateurs								+	+
Cellulose brute			+		+	+			
Glucides					+				
Cendres		+			+		+		
Nacl	+								
Na					+				
Insoluble Hcl					+		+		
P		+							
Fe			+						
Cu					+				
F							+		
Fb							+		
Cations correspondancs: No^-_2 No^-_3		*							
Aflacoxinos					+	+			
Rèeid. insoeticides					+				
Vitamines								+	
Salmonelles	+	+		+					

TABLE 3 (continuation)

An exemple of the norms retained for the control of fish food

(BENAYAS BEVIA, 1986)

	Optimum	Maximum	Remark
NaCl		1,2 %	Risk of water pollution
Ca	2.2 - 1,8	2,2 %	
P	1.5 - 0,9	1,5 %	
Fe		1250 ppm	
Zn		250 ppm	
Mn		250 ppm	
Cu		50 ppm	
Co		10 ppm	
F		15 ppm	
Pb		1 ppm	
Hg		1 ppm	
Digestibilieé pepsique des pretéines	80 - 90	80	
Lysine *	D 3,1 - C 2,8		
Methionine + cystine *	13 - 1,7		
Aflatoxine		5 ppb	
		5 ppm	
Gossypol		20 ppm	

	Excellent	Optimal	Normal	Abnormal
Acidity of the fatty matter (%) **	8	15	15 – 25	30
Peroxide index (meq o2 kg^{-1})		8	12	20

* Trout food - D : demarrage, C : growth

** Expressed in oleic acid

TABLE 4 : PRINCIPAL ANALYSISES COMPLETING THE ANALYSIS BY WEENDE

	Dosed Elements	Methods	Remarks
Ash	P	Colorimetry	Usually employed
	Ca, Na, K, Mg	Spectrophotometry	Only Ca employed
	Fe, Cu, Zn, Mn, Co	(atomic absorption)	
Proteins	Amino acids	Liquid phase chromatography, HPLC	Quite usually employed, Use of tables possible
	Available lysin	Colourimeter	Debatable
Lipids	Lipid categories	Solvant separation	
	Fatty acids	Gassy phase chromatography	Quite usually Employed
Cellulose	Acid detergent extracts		Easy, should be generalized but the method to be adopted to particular cases
	Neuter detergent extracts		
Glucids	Starch	Acid hydrolysis Enzymatic methods	Numerous methods only to be chosen in accordance to the nature of the product
	Glucose	Reducting power Enzymatic methods	
	Other sugars	Numerous methods	

TABLE 5 : Different types of criteria for the quality control of raw materials

Traditional "sale value" (Cereal especially)

- Specific weight
- (Small blender, broken, grains, ...) aspect of the grains
- Colour of the grains
- Odour
- Contamination (mildew, insects)
- Presence of impurities
  - Other grains
  - Soil, debris

Other biological criteria

- Degree of ripeness
- Varietal purity

Microbiological examinations (animal meal, cakes)

- Bacteria
- Fungi spores

Microscopic examination of raw materials (grounded or minced products)

- Micrographic analysis

Chemical analysis

- by WEENDE
- Supplementary analysis of the constituents
- Research of contaminants
- Indirect tests

Biological tests

- Toxicity
- Digestibility

TABLE 6 : PRINCIPAL CEREALS

Scientific Name	Vernacular name		
	French	English	
Triticum aeştivum	Blé	wheat	Cereal of reference - Antinutritional factor negligeable with the exception of phytic acid. Acid very suitable for fish.
Zea mays	Maïs	maize(corn)	Less proteins and certain vitamins more energetic
Hordeum sativum	Orge	barley	Less digestible glucids - rich in cellulose and gum - Not well utilized by fish - Some tannic products
Avena sativa	Avoine	oats	High cellulose content - It has less digestible glucids - Much less energetic hardly utilized by fish
Oryza sativa	Riz	rice	Whole cereal (paddy) very rich in cellulose, nethertheless it is employed in aquaculture food. (especially by-products)
Sorghum vulgare	sorgho	milo	A cereal similar to corm by its composition, although more variable - Some varieties are very rich in tannins (and in phytic acid).
Triticale x (Triticum x Secale)	triticale	triticale	A new cereal interesting due to its composition
Polygonum tataricum P. fagopyrum	sarrazin	Buck wheat	A rather similar composition to that of graminacae, contains a photosensitivizing alkaloid.

TABLE 7 : PRINCIPAL CAKES

Scientific Name	Vernacular name		
	French	English	
Glycine maz	soya	soybean	Excellent source of protein if well cooked (methionine deficiency)
Arachis hypogea	arachide	pea nut	High protein content, but lacking lysine and methionine - Can present aflatoxins.
Sesamum indicium	sesame	sesame	Low lysine content -
Gossypum sp.	coton	cotton	Rather high cellulose content. - not a good energetic source - Presence of gossypol
Elaeis guineensis	palmiste		High cellulose content - Not a good energetic source, rather low protein content of average quality - Can present aflatoxins.
Cocos nucifera	copra	coconut	
Helianthus annuus	tournesol	sun flowèr	Protein lacking lysine - Difficulty in hulling this product correctly (excess cellulose)
Brassica napus 3. compestris	colza	rapeseed	Average protein content of good quality -Rather high cellulose content - Presence of great quantities of glucosides (Vynil- thioxa-zolidones) except when treated or of special varieties.

TABLE 8 : COMPOSITION OF SOME INHABITUAL RAW MATERIALS WHICH ARE EMPLOYED IN ANIMAL FEEDING IN MEDITERRANEAN REGIONS

	French	English	Prot. brut.	Cell. Brut.	E.N.A.	Extr. eth.	Min. tot.
Rare Cereals							
Phalaris canarensis	mils, millets	millets, panics	13,0	5,8	55,8	7,4	6,6
Setaria italica			10,6	4,1	58,7	10,8	3,8
Panicum miliaceun (décortiqué)			13,2	3,8	70,0	2,0	1,5
Andropogon durra			10,3	3,5	72,4	1,6	2,0
Rare cakes							
Guizotia abyssinica	niger	niger	33,1	6,5	22,2	18,6	8,8
Citrus sp. (graines)	citrus	citrus	28,3	8,0	33,0	18,0	4,0
Amygdalus communis	amande	almond	40,8	16,5	18,9	9,2	4,3
Orbynia speciosa	babassu	babasou	24,5	0,8	40,7	18,0	6,0
Camelina sativa	cameline	dotter seed	33,0	9,7	29,1	11,2	6,5
Cannabis sativa	chanvre	hemp seed	30,5	9,8	19,2	20,5	8,0
Cucurbita sp. (graines)	courge, citrouille	cucurbit	50,5	12,0	6,6	15,0	7,9
Papaver sp.	pavot oeilleté	Poppy seed	36,0	1.0	24.6	15,1	11,5
Diverse graines							
Ceratonia siliqua	caroube	carob	16	1,7	58,2	6,8	3,0
Castanea sativa (décortiquée)	chataigne	chest nut	5,7	4,0	79,0	1,5	2,3
Quercus sp.	gland	acorn	6,4	4,0	69,2	4,9	2,5
Diverse vegetable products							
Phoenix dactylifera (pulpe)	datte	dattes	5,2	0,3	62,5	5,5	2,5
Laminaria sp.	algues	sea weeds	9,2	1,5	58,5	6,2	15,2
Rare animal products							
Bombryx mori	ver à soie (chrysalides)	silk worm	61,6	5,6	7,6	8,0	5,2

GRAPH N° 1 : DOSAGE ACCORDING TO THE WEENDE METHOD

	Fresh material	
	H_2O	
Oven 110°	Dry material (1)	Difference (6)
Oven 550°	Ash (2) (mineral)	Non azotized extract = glucids "utilizable"
N Kjeldahl	N x 6.25 (3) (Raw proteins)	
Delipidation	Ether extract (4) (Raw lipids)	
Separation of the fibres	Raw cellulose (5) = Raw fibre	

Sources of error

(1) Peroxidation
(2) Oxidation of metal, loss of ponds possible
(3) Factor 6.25 - N non proteic
(4) Extraction adjusted to inferior or superior unit limits
(5) Underestimated
(6) Accumulation of previous errors
No data recording on the chemical nature of glucids

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FISH SILAGE : PREPARATION AND USES

Irineu BATISTA

1. INTRODUCTION

Silage is the product of the process of preserving and storing wet fodder and its utilization in agriculture has been known for a long time. Analogous products prepared with whole fish or parts of fish have been called fish silage.

Fish silage production was initiated in the 1920s when A.I. VIRTANEN used sulfuric acid/hydrochloric acid to preserve green fodder[1]. This method was adopted by EDIN[2] in the 1930s to preserve fish waste. Acid fish silage is commercially manufactured in DENMARK, NORWAY and POLAND with lesser amounts produced in other European Countries. Its production is being introduced also in Southeast Asian Countries using waste, by-catch, and surplus fish.

2. PRODUCTION PRINCIPLES

Fish silage can be prepared by the following methods :

i) Addition of acid, inorganic and/or organic acid which lowers the pH sufficiently to prevent microbial spoilage. The liquefaction of fish tissue is made by enzymes naturally present in the raw material.

ii) Bacterial fermentation with lactic acid bacteria which are naturally present in the fish but it may be advisable, however, to add a starter culture of proper lactic acid bacteria. To favor the growth of these bacteria, it is essential to add a fermentable sugar because fish contain little free sugar. The lactic acid bacteria produce lactic acid and antibiotic which together destroy competing spoilage bacteria and the reserve of fermentable sugar suppress amino-acid degradation.

3. ACID-PRESERVED FISH SILAGE

In the production of acid-preserved fish silage, inorganic or organic acids or mixtures of these can be used. If inorganic acids are used the pH of silage is lowered to 2, or below in order to obtain a fully preserved product[2]. The quantity of acid required to lower the pH to 2 depends on the content of protein and ash in the raw material and before this type of fish silage can be fed to animals it must be neutralized. However, the high ash level of the neutralized product is undesirable in nutrition and neutralization is an additional laborious operation. Handling of mineral acids is also very hazardous and corrosion problem; are more severe at the low pH of these fish silages.

Formic, acetic, and propionic acids are the organic acids usually utilized in the preparation of fish silage. If formic acid is used, the silage is stable at pH 3.5 - 4.0[3-6] and 4.5 with propionic acid[7]. These organic acids are more expensive than mineral acids, but a previous neutralization is not necessary before feeding the animals.

The antibacterial effect of weak organic acids is associated with undissociated molecules. Cell membranes are in general impermeable to the anion of the weak acid and to hydrogen ions (H^+), but non-charged and undissociated molecules can freely pass through cell membranes. At low pH, a certain percentage of molecules of organic acid is undissociated which pass through cell membranes and inside the cell, pH is neutral and the organic molecule will dissociate, giving a hydrogen ion and an anion which will be trapped there. pH in cytoplasm, therefore, gradually decreases and the anion of the organic acid accumulates which contributes to the antimicrobial activity of these acids. In some instances the anion may be metabolized by the cell counteracting in this way its toxicity. This probably happens with acetate whose antimicrobial activity is considerably lower than propionate and formate. According to this theory of the mode of action of weak organic acids, their antimicrobial activity should increase with falling pH

and it should be very high when pH is lower than the pKa of the weak organic acid. More than 50 % of the propionic acid (pKa = 4.86) will exist in its undissociated form at pH values below 4.86. In the case of formic acid (pKa = 3.75) the pH must be below 3.75 in order to obtain its maximum anti-microbial activity.

The percentage of formic acid required for adequate preservation depends on the ash content of fish and OLSSON[4] derived the formula :

$$L = 0.25 + 0.3 \times \% \text{ Ash}$$

where L is the volume of the formic acid (90 %) in liters required to treat 100 kg of fish. However, for reasons of safety, a reduction in the amount of acid appears inadvisable whilst for reasons of simplicity recommendation of different amounts of acid for various materials was undesirable, and although 3.5 % of acid is known to be too much for some materials, it appears to be adequate for most fish or waste fish likely to be treated.

On account of the high price of organic acids it can be recommended to use a mixture of inorganic and organic acids. The inorganic acid lowers the pH sufficiently for the organic acid to become antimicrobial. The use of these mixtures has been referred to by many authors and such experiments have been carried out mainly with formic acid and sulfuric acid. Fig. 1 shows the results obtained with different mixtures of formic acid (85 %) and sulfuric acid (50 %) In this diagram the upper region corresponds to mixtures of acids which give silages of good conservation quality.[8]

4. PREPARATION OF ACID FISH SILAGE

The process steps in the production of acid fish silage are (1) mincing or chopping the raw material ; (2) addition of acid and homogenization (3) autolysis and lipid separation ; and, (4) storage. De-oiling is not necessary if the fish silage has an oil content of less than about 2 % of the wet weight.[9]

The production of fish silage starts with the mincing of fish and mixing the mince of this substance with the acid. However, when fresh fish is used, the muscle components become rubber-like after the addition of acid and the mince tends to form closed pockets in which the acid do not enter quickly enough to prevent spoilage. This problem can be overcome by cutting or chopping the fish into pieces before the addition of acid. If the raw material is not fresh, thawed fish for example, the mixture of minced fish with acid is more easily done and it is vital that the acid and fish be well mixed because pockets of untreated material will putrify[10].

4.1. Liquefaction of fish silage

During storage of silage there is a gradual liquefaction due to the degrading enzymes present in fish, mainly in guts. The increased liquefaction (antolysis) of silage can be measured by a fall in viscosity or by an increase in the volume of the aqueous phase after centrifugation. In Fig. 2, we can observe the liquefaction of blue whiting silage prepared with formic acid (3 %)[11]. Viscosity after two weeks of storage is relatively low, but in the case of snipe fish (a fatty species) the viscosity values are higher which means that the fat content is the main contribution to the final viscosity.

Protein breakdown can be measured by determining total nitrogen[12] or trichloroacetic acid (TCA) soluble non protein nitrogen (NPN)[13] present in the aqueous phase after centrifugation or filtration.

The rate of liquefaction depends on the type of raw materials, its freshness, the activity of digestive enzymes in the fish, the physiological condition of the fish at the time

it was caught, the pH, the temperatures and the nature of the preservative acid [10,11]. Changes in low molecular nitrogenous substances depending on catching season and storage temperatures can be remarked in fig. 3[14]. Prior freezing of the raw material did not influence the rate of autolysis (fig 4)[15].

Proteases are the main enzymes responsible for autolysis which have an optimum in the pH range 2 to 4, when assayed with hemoglobin as substrate[10]. This activity decreases sharply above 4. The pH curve of autolysis of different tissue components does not coincide with that of a standard protein assay because the proteases have different pH optima according to the tissue components. The percentage of soluble material as a function of pH is plotted in fig. 5[16] showing a maximum solubilization near pH 3.5.

4.2. Stability of amino-acids

It has been reported that amino-acids are very stable in an acid fish silage. The percentages of amino nitrogen released as ammonia are small but this may imply a significant reduction of the nutritional value of the silage if the ammonia is derived from essential amino-acids. In fact, there are a few reports referring to the decomposition of tryptophan, methionine, cystine and histidine. Tryptophan which degrades at low pH when it is free[17] and its rate of degradation increases markedly with temperature[12].

Low levels of methionine and cystine in liquid herring protein were detected, but this fact was not observed in fish silage prepared from white fish which had similar levels of isoleucine, threonine, cystine + methionine and lysine to the levels in white fish meal[18].

Histidine is quickly destroyed by spoilage bacteria and may be thé limiting amino acid in fish silage prepared from spoiled fish[19].

4.3. Changes in the oil

During the storage of fish silage, the fish oil present becomes rancid and oxidized. Glycerides in the oil are decomposed by lipases in free fatty acids which increase regularly during storage. Fig. 6 shows the free fatty acid content of a snipe fish silage stored at room temperature[15].

Unsaturated fatty acids in lipids react also with oxygen to form hydroperoxides as the initial products. These hydroperoxides in foods or feed decompose readily through a free radical chain giving stable secondary products. The rate of decomposition of hydroperoxides accelerates as its level increases whereas the rate of formation decreases as more fatty acid become oxidized. This means that the level of hydroperoxides in foods and feed attains a maximum during storage which is reached in a few days or months depending on the temperature of storage, available oxygen, the presence of pro and anti-oxydants, reactivity of lipids, etc...

Fig. 7 shows the evolution of the peroxide value during the storage of a fish silage prepared with snipe-fish[20]

The presence of oxidized fat in animal rations causes loss of appetite, decreasing ability to gain weight, and death when the peroxide value exceeds 100 milli equivalents per kilogram of diet[21-22].

The breakdown of hydroperoxides can be increased by heat, light, metal ions, and metalo-proteins and since oxidized lipids are responsible for the poor nutritional quality of fish quality, different ways has been tried so as to avoid its formation or to accelerate the decomposition[23]. Anti-oxidants like ethoxyquin or BHT has been used

successfully to prevent the formation of hydroperoxides (see fig. 7) and boiling of fish[24] is also effective in the reduction of its formation in the oil.

The secondary products of lipid oxidation may react with proteins causing some reduction of the nutritional value[23]. These oxidized products may be also responsible for carcass taint.

4.4. Vitamin degradation

Only a few papers dealing with vitamin degradation in fish silage have been published. However special attention has been given to vitamin B_1 (thiamine).

The tissue of clupeid fish (herring, sprats, anchovy, etc ...), carps and fresh-water fish have frequently been shown to contain an enzyme which will destroy vitamin B_1. This enzyme, thiaminase, can induce a deficiency of thiamine caracterized by damage to the central nervous system. These deficiency symptoms of thiamine were observed in salmon and rainbow trout fed on herring for extended periods.

According to the results obtained by ANGLESA and JACKSON[25], the ensilation of fish containing thiaminase does not inactivate the enzyme immediately, but after extended storage the thiaminase activity decreases below levels which can be reliably estimated. The presence of this enzyme in silage may not be a problem if the silage is then mixed with a dry meal to form moist pellets. If the dry meal contained a thiamine supplement, this would therefore remain intact until the feed was eaten by the fish.

Thiaminase becomes completely inactivated after 5 min. at 82° C[26].

Thiamine is also decomposed by physical/chemical conditions such as intensive light, heavy metals, sulphite and some carbohydrates. It is heat stable under acid conditions but labile under neutral and alkaline[27-28].

The deficiency of vitamine E observed in chicken fed on a silage/cassava meal diet was attributed to the decomposition of this vitamin by oxidized lipids[10], but the same results were not obtained in another case[11].

Riboflavine, pyridoxine and niacin seem to be stable in the acid silage[29] and also vitamine B_{12} which has a half life of 2 months at 10° C[30].

5. FEEDING TRIALS WITH FISH

Fish silage has been used in fish feeds with varying success depending upon the species of fish, the type of acid used in the ensiling process, and the method of processing.

Silage based moist pellets have been proven excellent, diets for salmonid fish in Norway and a few papers have been published on the utilization of fish silage in feeds for these species.

RUNGRUANGSAK and UTM[31] used acified feeds treated with hydrochloric, formic or sulfuric acid (2.5 % w/w) to feed rainbow trout to test the effects on protease activities, growth and feed utilization. Hydrochloric acid had no apparent effect on growth or proteolytic activities in any part of the digestive tract. Formic acid at all levels tested seemed to have a depressive effect on both growth and proteolytic activities. Sulfuric acid showed similar effects, with the exception that the protease activity in the stomach was not depressed.

The results obtained by HARDY et al.,[32] indicate that the lenght of storage of fish silage affects its nutritional value, suggesting that if silage is allowed to liquefy and is

stored for a long period before being dried and used in trout diets, total replacement of fish meal by silage should not be attempted. Stopping the liquefaction process before completion by heat treatment may allow higher levels of dried fish silage to be used in the diet without affecting fish growth. This study showed also that fish silage made with sulphuric acid can be neutralized with $Ca(OH)_2$ without reducing the whole body levels of zinc in the fish.

According to AUSTRENG and ASGARD[29], silages containing propionic acid do not seem to be accepted by salmon, while silages preserved by formic acid alone or in combination with sulphuric acid have given good results. Rainbow trout seems to be more tolerant to different acids than salmon. For both species, silage from trash fish, fish offal, blood and casein have been used with success.

TORRISSEN et al.[33] demonstrated that astaxanthin is stable in an acid silage of shrimp processing waste. The digestion by rainbow trout of the astaxanthin present in this waste material was improved by ensiling to about 71 % as compared to 45 % in the corresponding fresh or dried material. Also the rate of accumulation of the pigment in the fish muscle was markedly higher in fish fed the silage diet than those given fresh or dried shrimp waste.

Identical results ware obtained by CHEM and MEYERS[34] in the acid ensilage of crawfish waste in which the astaxanthin pigment present was stabilized.

In diets for carps, fish silage has been shown to be equally good as a source of protein[35].

6. FERMENTED FISH SILAGE

The utilization of lactic bacteria to preserve fish was invented by CARL[36], who obtained a fermented product with good keeping quality and no unpleasant fish odour. On media containing sugars, lactic acid bacteria produce large amount of lactic acid which decreases the pH (fig. 8)[37] and thus renders the medium unsuitable for the growth of most microorganisms. These bacteria preserve food or feeds, preventing its microbial spoilage, add flavour to the products and may also prevent rancidification and other undesirable chemical reactions[38-40].

Fish has a very low level of free sugars available for fermentation by bacteria. While spoilage bacteria utilize amino-acids as a source of energy, lactic acid bacteria have limited ability to decompose them, but the presence of fermentable sugars like glucose, fructose, and ribose, enhance its growth even when the environment is rich in amino-acids. If the environment is anaerobic, spoilage bacteria ferment also glucose producing acids and lactic acid bacteria become predominant.

At the early stages of fermentation the presence of hetero fermentative bacteria can occur, producing CO_2, acetic acid and ethanol in addition to lactic acid. These kind of bacteria must be avoided and may be suppressed by adding 5 % sodium chloride. This can be also accomplished adding small quantities of organic acids and boiling the fish prior to inoculating with a suitable starter culture.

Fermented fish silage have an acceptable hygienic quality and it appears that coliforms, typhoid bacteria and coagulase positive staphylococci are destroyed along with spores of Clostridium botulinum[40]. If the silage is exposed to air, the growth of yeast and fungi[41] may occur.

As referred to before, a relatively high percentage of carbohydrates must be added to the fish in order to ensure a successfull preservation. A few sources of

fermentable sugar have been used including mixtures of malt meal and oats meal, mollases, cassava meal, and whey powder. The quantities of carbohydrates used are quite variable but it seems that at least a 10 % addition of mollases is required to produce a stable silage[9].

As with acid fish silage there is also in fermented fish silage, a protein digestion to soluble compounds but it seems that is significantly lower than for acid silage. Fig. 942 shows the results obtained in a silage prepared with snipe fish and cassava meal as the carbohydrate source.

The production of volatile bases and ammonia in a fermented fish silage is considerably higher than in an acid fish silage. The evolution of total volatile bases in both types of silage from capelin is represented in fig. 10[37]. Nevertheless, the ammonia or volatile bases are not derived necessarly from essential amino acids since the nutritional value of fermented silage is not affected[43].

Lactic acid bacteria are naturally present in the fish in very low numbers when compared to potential spoilage bacteria[44]. To enhance the preservation process, it is advisable to add suitable bacteria unless the carbohydrate additives carry such bacteria as natural microflora. Starter culture must, of course, be used when the fish is boiled prior to preserving[41]. The inoculation of fish/carbohydrate can be done by the addition of pure starter cultures produced commercially, or by reinoculation from a previous silage of acceptable quality. Another method is to mix it with a "sauerkraut"[45-46] which can be produced by mixing sliced cabbage with vinegar, sodium chloride, cassava meal and some sugar.

7. FISH SILAGE VERSUS FISH MEAL

According to RAA and GILDBERG[9], fish silage is a means of utilizing waste fish in situations where conventional fish meal production is inappropriate or unavailable. Such situations are characterized by scattered and irregular landing of fish or when the fish quantities are too small for viable operation of a fish meal plant. Fish silage has some advantages on fish meal :

1) Acid-preserved fish silage does not putrefy, retaining a fresh acidic smell even after storage for weeks at tropical temperatures. As well, there are not the same environmental problems with silage as with fish meal manufactures.

2) A fish silage is almost sterile and pathogens like Salmonella are efficiently killed in it.

3) The scale of production of fish silage can be varied at will without the economy of the process being greatly affected. The capital investment in equipment may be anything from a homemade drum with a chopper to a sophiscated plant designed for the de-oiling of large quantities of fish silage.

4) The energy requirements of silage production are very low compared with fish meal.

5) Mixtures of acid-preserved fish silage and carbohydrate fillers can be dried in open trays tropical conditions without fly infestation because flies are repelled by the evaporating acids.

On the other hand, fish meal is less bulky and thus cheaper to transport.

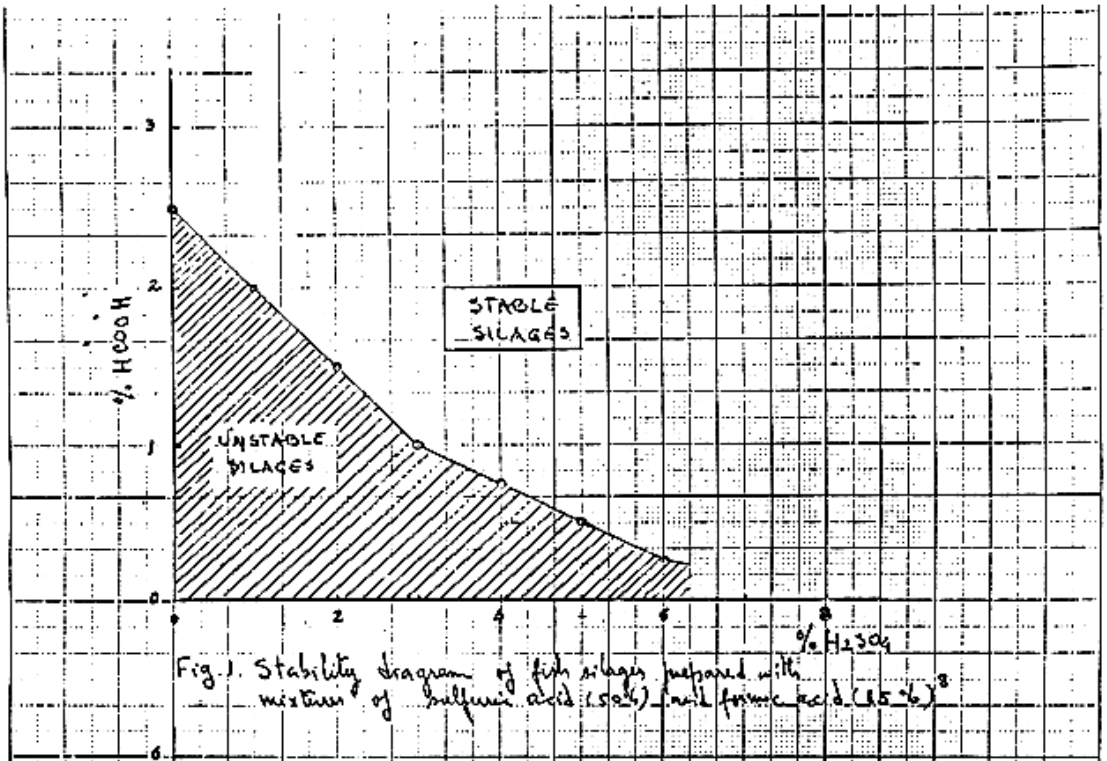

Fig. 1. Stability diagram of fish silages prepared with mixtures of sulfuric acid (50%) and formic acid (85%)[3]

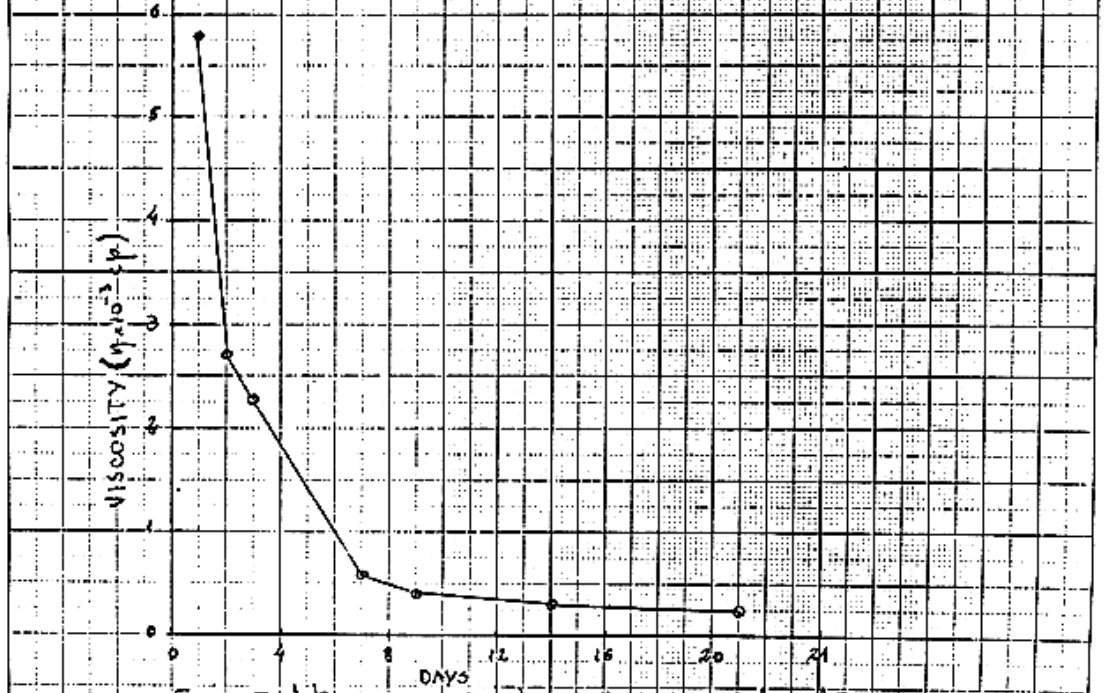

Fig. 2 Evolution of viscosity during storage of a blue whiting silage prepared with 3% of formic acid[1]

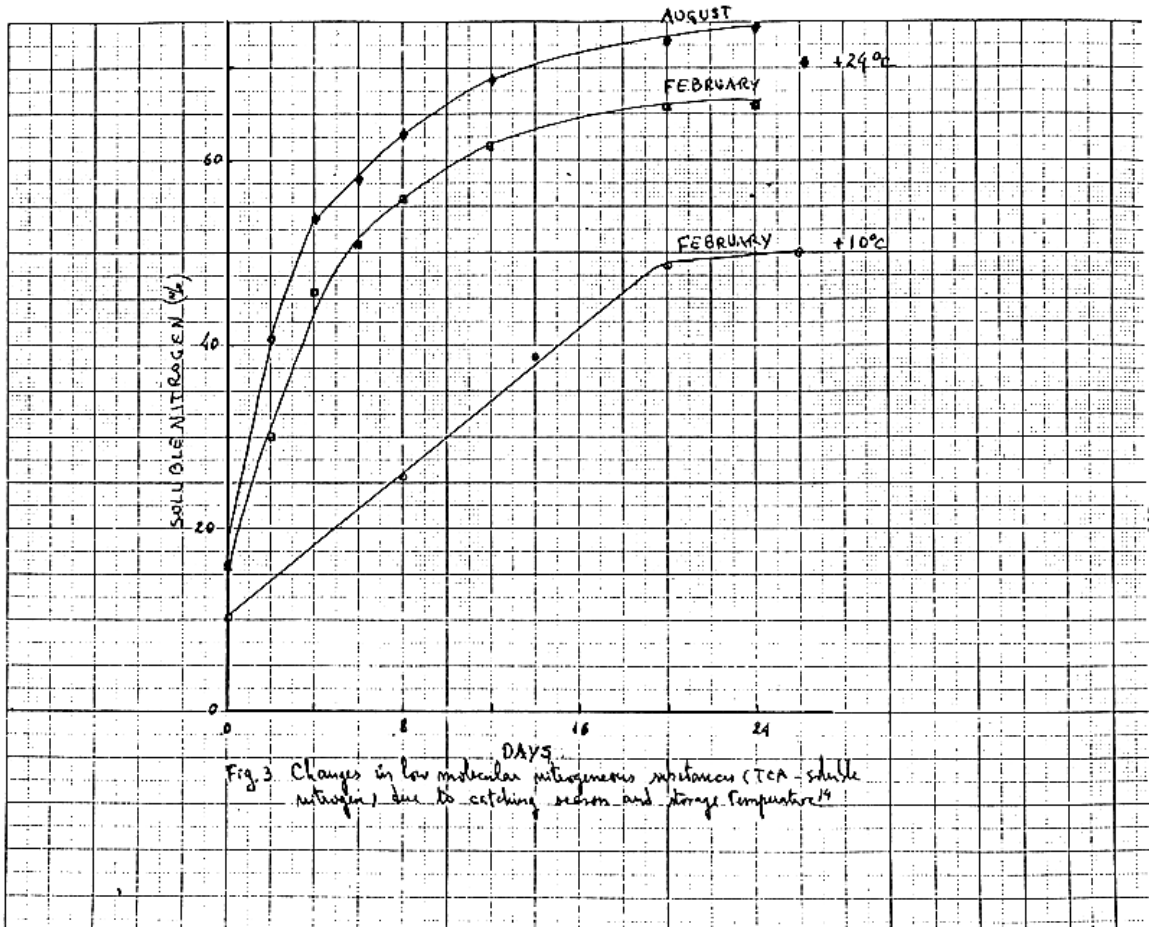

Fig. 3. Changes in low molecular nitrogenous substances (TCA - soluble nitrogen) due to catching season and storage temperature[4]

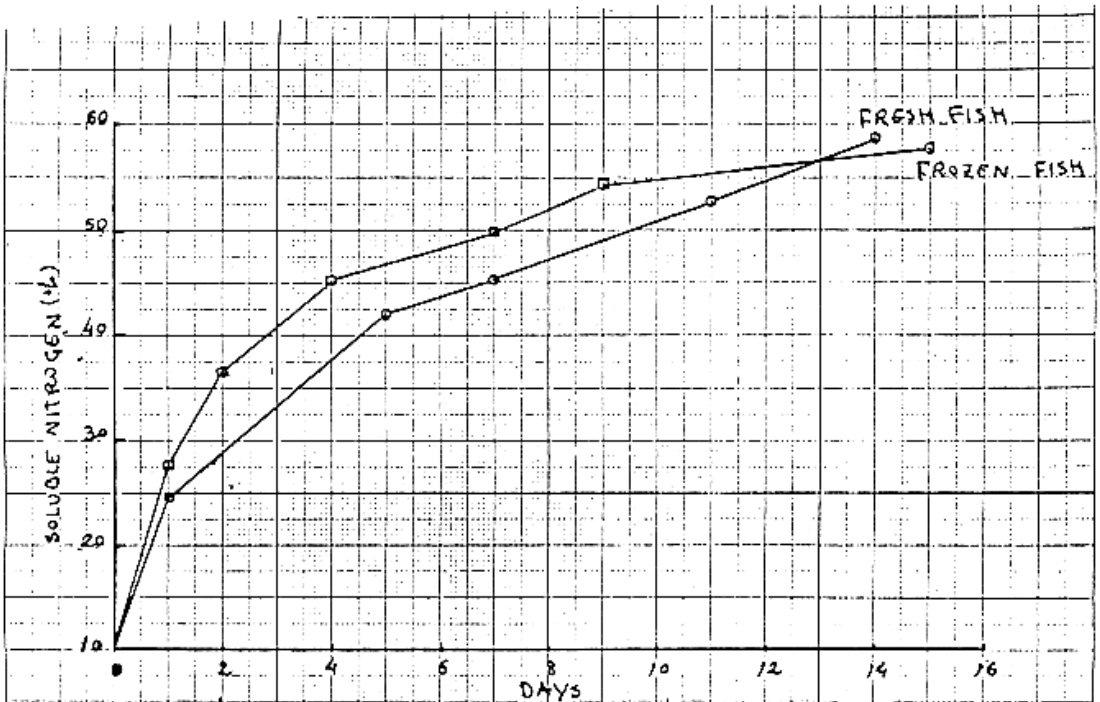

Fig 4. Soluble nitrogen as a percentage of total nitrogen during storage of mackerel-fish stage —● Fresh fish o—o Frozen fish 15

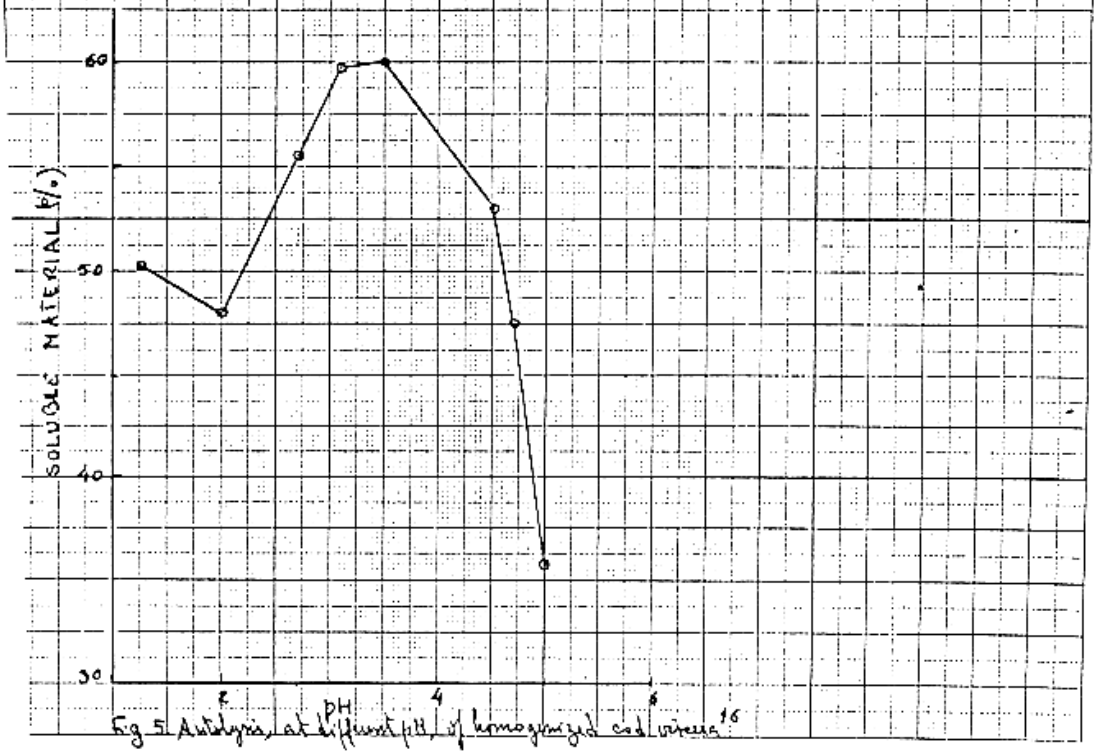

Fig 5. Autolysis at different pH of homogenized cod viscera 16

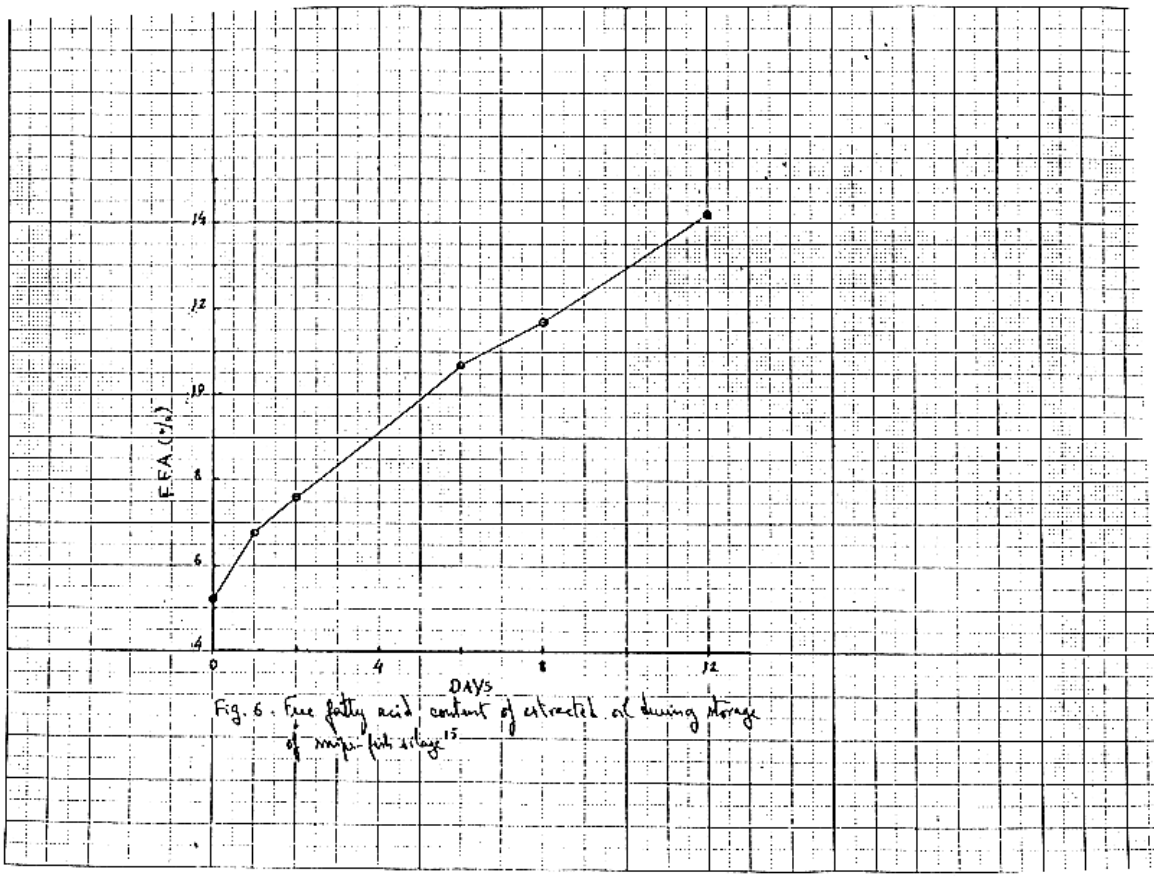

Fig. 6. Free fatty acid content of extracted oil during storage of mixed fish silage[15]

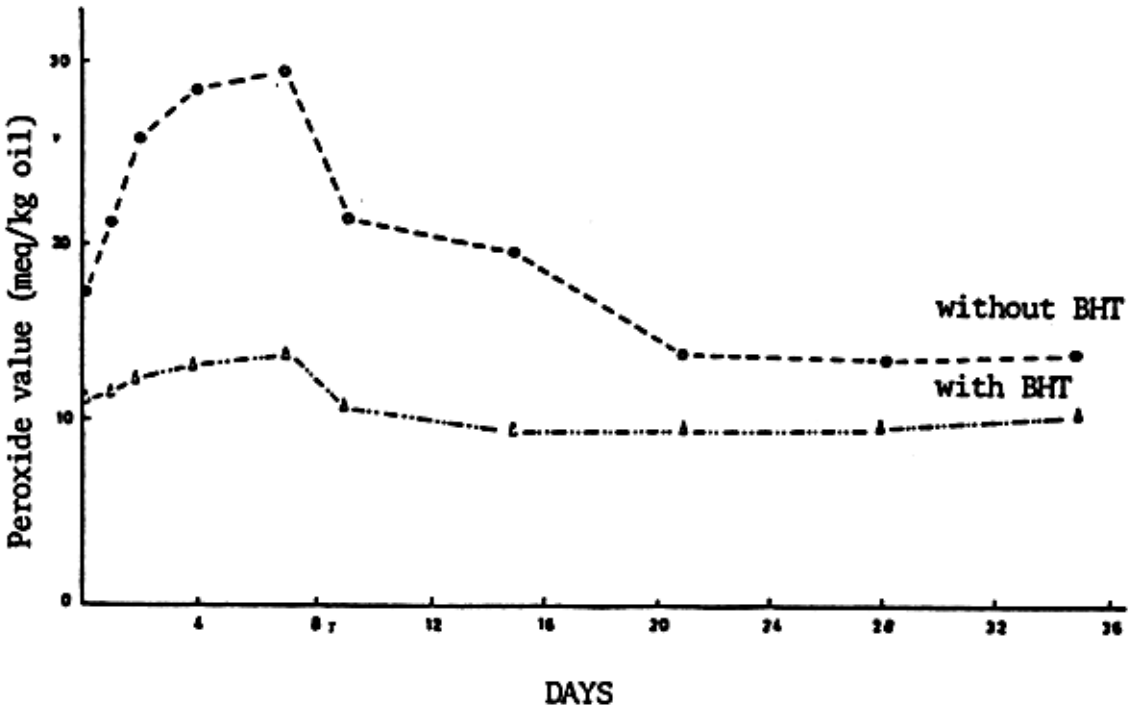

FIG. 7 - Peroxide value of extracted oil during storage of snipe-fish silage.[20]

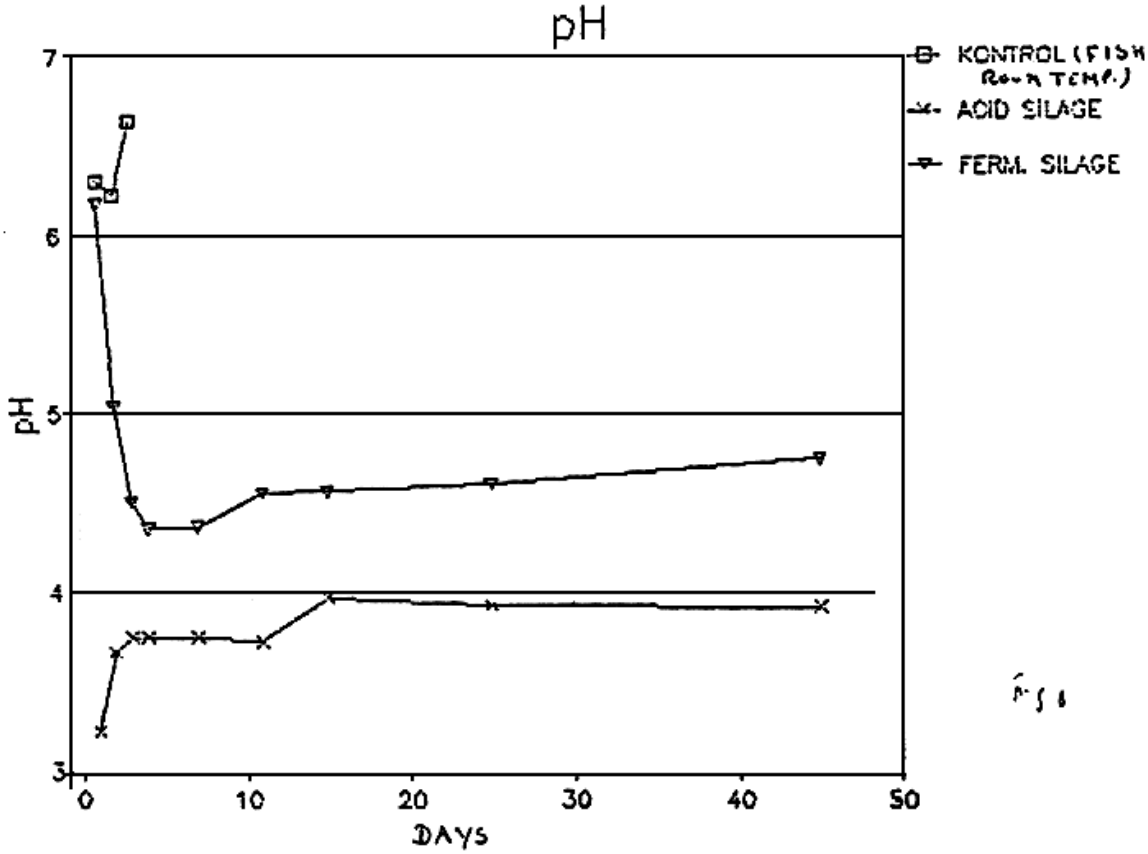

FIG. 8 - Evolution of pH in a fermented silage and in an acid silage.[37]

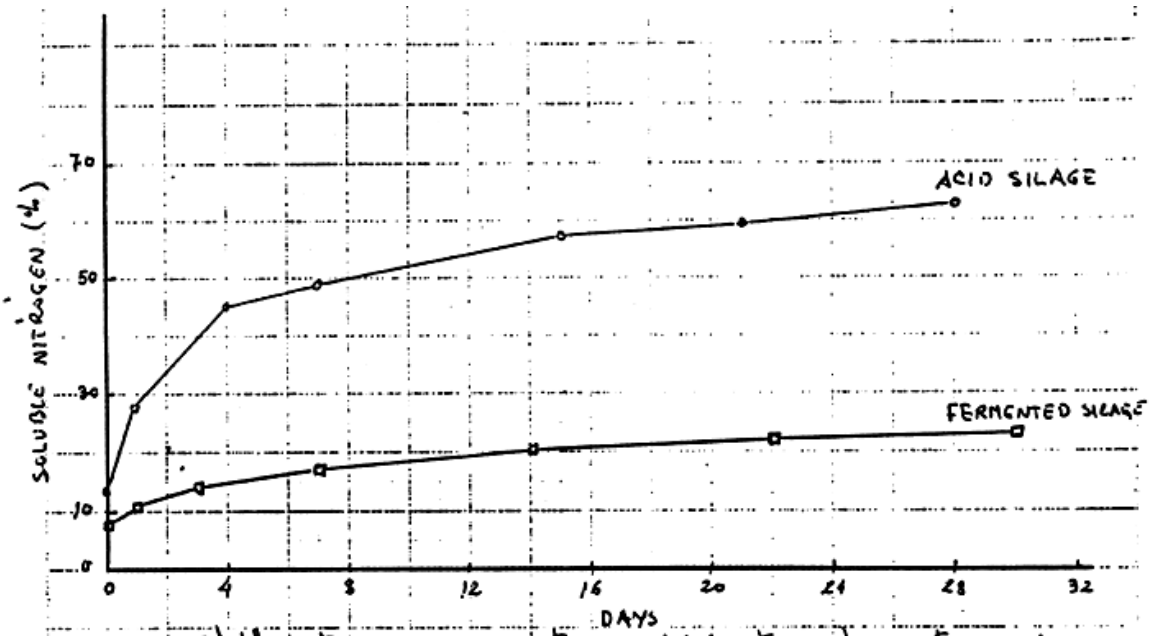

Fig. 9 Soluble nitrogen as a percentage of total nitrogen during storage of swipe-fish-silage. □—□ Fermented-silage o—o Acid silage. 420

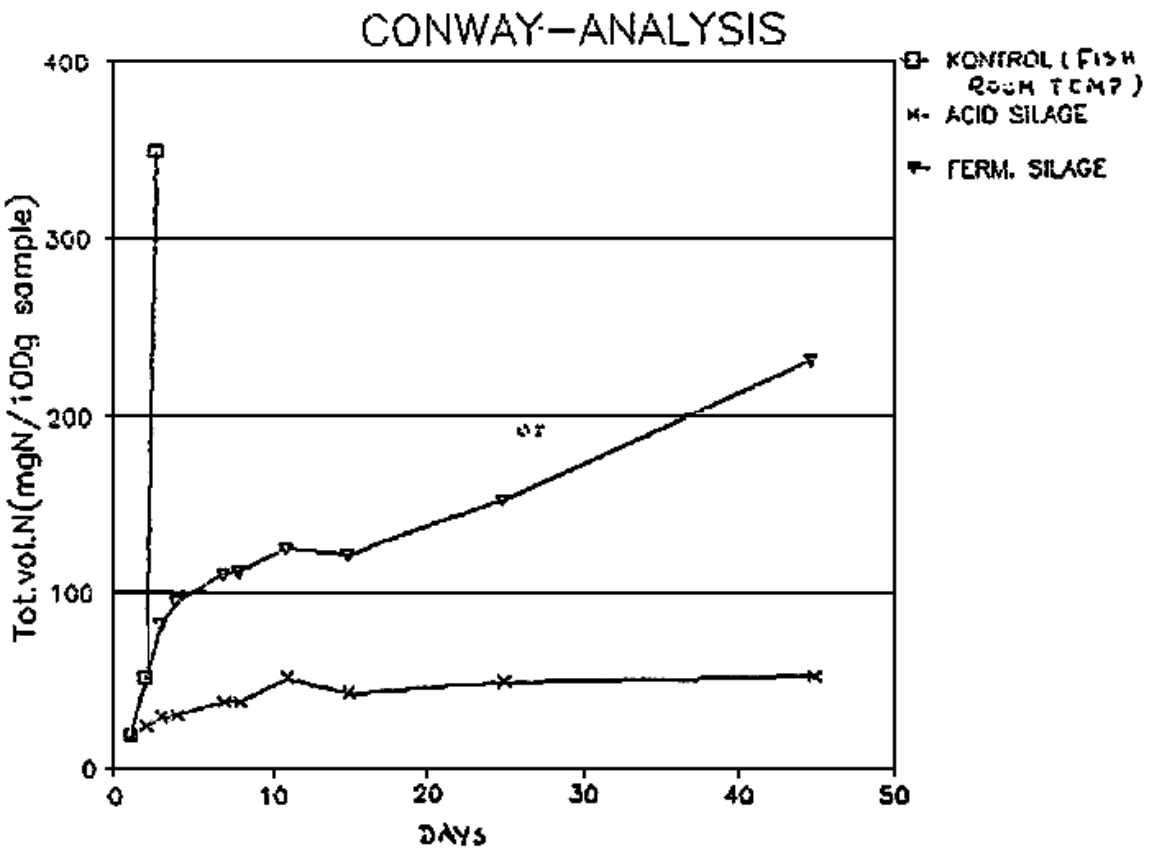

FIG. 10 - Evolution of TVN in a fermented silage and in an acid silage.[37]

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FISH FEEDS PROCESSING AND TECHNOLOGY

J.P. MELCION

1. GENERAL FLOW-SHEET (fig. 1)

2. GRINDING

2.1. Definitions

The purpose of grinding is to reduce the raw materials into small particles in order :

a) to facilitate the mixing operation,

b) to improve their nutritionnal utilization.

The yield of a grinder is the output (t/h) or the specific production (t/Kwh); the particle size analysis describes the quality of the ground product: it can be measured by sieving.

The most commonly used equipments in grinding materials are hammermills, because of their rusticity and flexibility.

2.2. Hammermill description (fig. 2)

Hammermill consists of stationnary or swinging hammers mounted on a rotor driven at speed usually from 1 500 to 3 000 rpm (50 - 100 m/sec). The materials are first contacted by hammers, until the sizes of particles would be near the desired size. A negative draw of air through the hammermill will assist in moving the sized particle toward the screen.

2.3. Grinding parameters

- related to the grinder (tab. 1)

- related to the materials :

a) moisture : the specific production is reduced from 12 to 25 % when the moisture increases from 12-14 to 17-18 ‰.

b) type of raw materials (fig. 3)

The particle sizes will be different with each type of material within the same grinding conditions.

A very small particle size is important for fish feeds (0.5 mm).

3. MIXING

3.1. Definitions

Blending consists to associate ground and proportionned raw materials, in order to distribute them homogeneously within the mixture.

Mixing quality is homogeneity: it can be measured with tracers.

The word "homogeneous" means every element of a mixture must be present in a Feed sample, as small as the sample will be.

For a given ingredient, there is a relationship between the number and the size ratio of the particules.

3.2. Mixing equipments (fig- 4)

The horizontal ribbons mixer is the most commonly used equipment for mixing dry materials (until 18 % moisture).

Running conditions :

Degree of filling: 50 - 100 % of the total volume

- mixing time : 4 - 5 mn
- full bottom discharge
- Use of a premix for inclusion under 0.5 to 1 ‰.

3.3. Demixing

Particle segregation may occur during transportation, conveying and delivery of the feed when the sizes and densities of the ingredients particles are quite different.

4. "DRY" PELLETING

4.1. Definitions

Pelleting can be defined, as the agglomeration of small particles into a larger solid with a given shape and texture, by means of a mechanical process in combination with moisture, heat and pressure.

The quality of pellets is expressed as hardness, durability, water stability, sinkability, rehydration. Water stability is to be considered because of the leaching effect of water : loss of nutritive elements (soluble nitrogen, vitamins), and ponds eutrophisation.

4.2. Principle and equipment (fig. 5 and 6)

The pellet mill :

The rolls act to compress the material and to extrude it through the holes of the die. Steam is eventually added to the meal with a conditionner.

4.3. Factors affecting pelleting

The main factors are (tab. 2)

- Ingredients caracteristics (fat, starch).
- Moisturizing or steaming of the meal prior to pelleting (1 - 3 %)
- Diameter (2 - 4 mm) and die thickness (compression ratio 12) : the higher the die thickness for the same diameter, the harder are the pellets, but the lower is the specific production.
- Binders : the Feeds may disintegrate into water unless ingredients such as wheat gluten or pre-gelatinized starches are included to help form a continuous matrix producing adhesion between particles. Their use is optional.

4.4. Further operations (fig- 7)

4.4.1. Cooling

The temperature of the pellets leaving the pellet mill is high (60 to 80° C) : they must be cooled prior other operation or delivery.

Equipment : vertical or horizontal coolers.

4.4.2. Crumbling (fig. 8)

Crumble rolls are used to take relatively small pellets and break them into smaller particles for special feeding applications, especially for fingerlings and young fishes (d = 0,025 x fish length).

4.4.3. Screening

Fines from cooled pellets are usually removed prior to delivery. Particles from the reground pellets are separated by screening into various sizes according to the fingerlings sizes.

4.4.4. Coating

Some dry pellets or expanded products (salmon) are coated with fat in order to include to the diet essential fatty acids or liposolubles vitamins, and to Form an hydrophobic layer out of the product. Liquid fat is applied by spraying a mist of liquid over the product as it enters a rotating drum or screw.

5. EXTRUSION-COOKING

5.1. Principle

Extrusion cooking can be defined as to squeeze a mealy product through a small hole, the die, under action of very high pressure (30 to 120 bars) with the aid of one or two screws, which compress and shear the material before it passes through the die. A part of mechanical energy is converted into heat, generated by friction: the product is cooked at variable intensities, depending from the degree of treatment and the additional heating (80 - 250° C). The water, which is under pressure in the meal, evaporates on leaving the die, creating a very low density and expanded structure. On emergence, the product is cut by a rotating knife, cooled and dried to approximatively 10 to 12 % moisture for safe storage.

5.2. Extruders (fig- 9)

- mono-screw and twin-screws
- 3 sections : feeding, compression, melting.

The quality of the extruded feeds is expressed by :

- Geometry : expansion ratio, density,
- Fonctionnal properties: water stability, rehydration, floatability,
- Biochemical modifications : starch, available amino-acids.

5.3. Extrusion variables

Extrusion-cooking is a process difficult to control because of numerous parameters. Its mechanisms are practically unknown.

5.4. Application to feeds for marine animals (appendix 1)

6. WET GRANULATION (or PELLETIZING) (fig. 10)

Diets are normally prepared by mixing the ingredients into a dough like mass (25 to 40 % moisture), extrusion of the dough through a mincer plate or similar forming device, at low pressure and temperature (30 - 60° C).

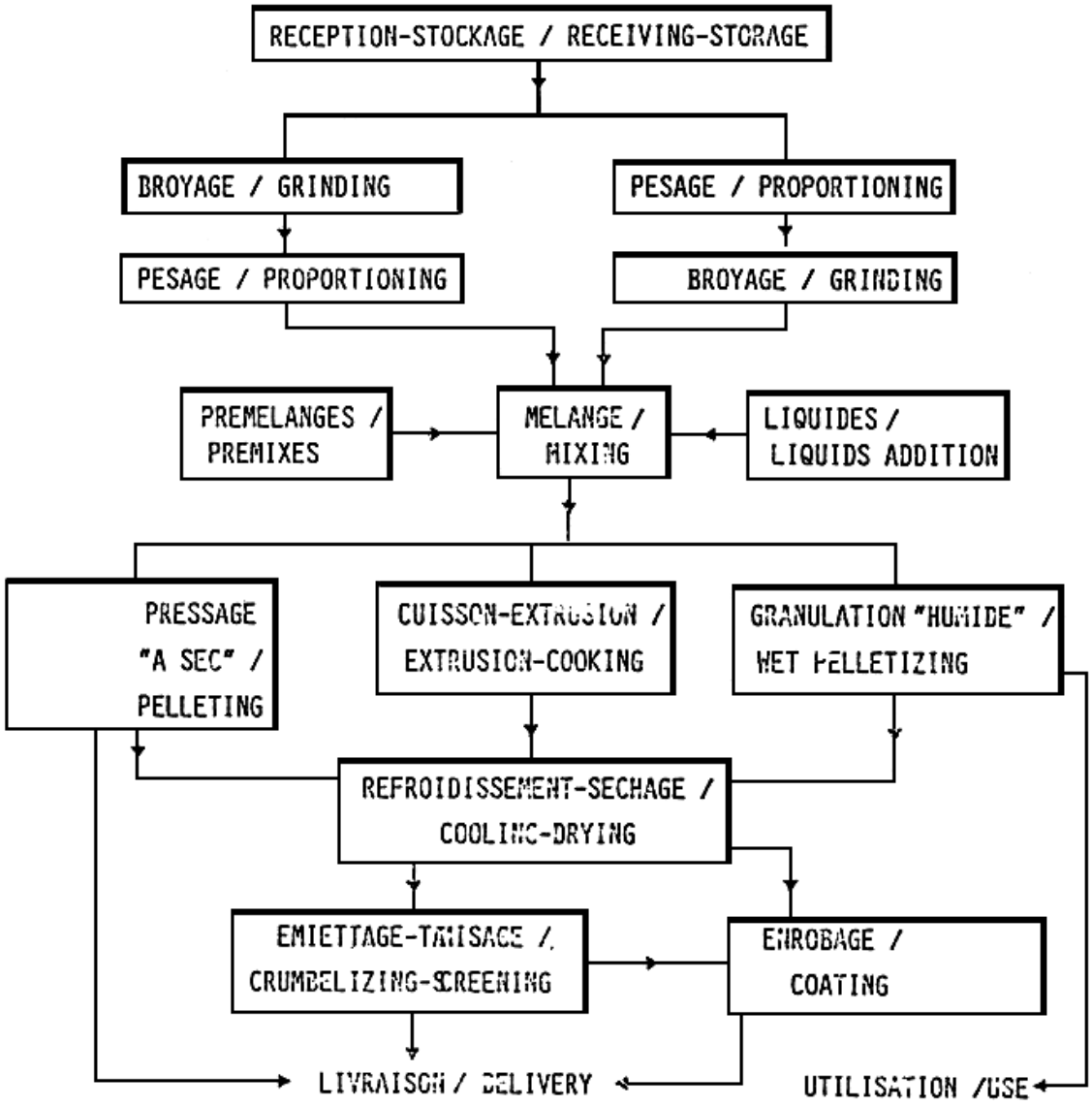
Ingredients are :

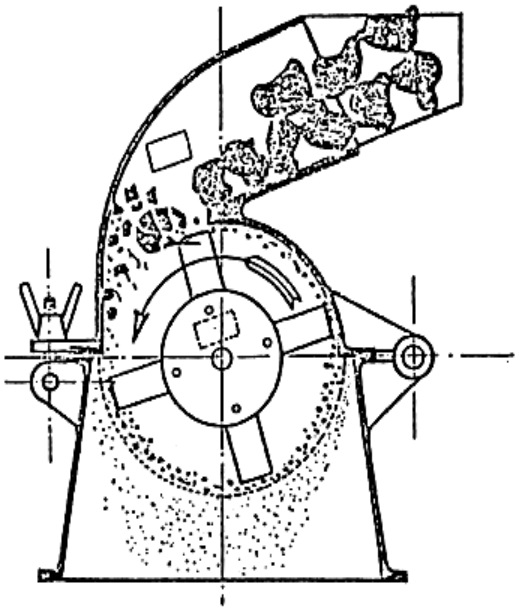
1) Fresh or refrigerated by-products and fish offals, blended with oilseed cakes or fish meals, extruded into moist strands and refrigerated until required. This type of feed obliges to dispose of fresh meat and to be produced on the farm. It is suitable for sea-trout, turbot, sole.

2) Meals from meat, fish, oilseeds and cereals, mixed with a solution of binders ‘wheat gluten § - 8 %, alginates associated with Ca++ to produce a firm water insoluble gel. Products must be dried, or added with additives (2 - 4 % propylen-glycol) to keep them from microorganism spoilage if stored and use as a wet form. Binders may be useful tools for preparation of experimental shrimp feeds, mainly at larval stages.

FIGURE 1

DIAGRAMME GENERAL / GENERAL FLOW-SHEET





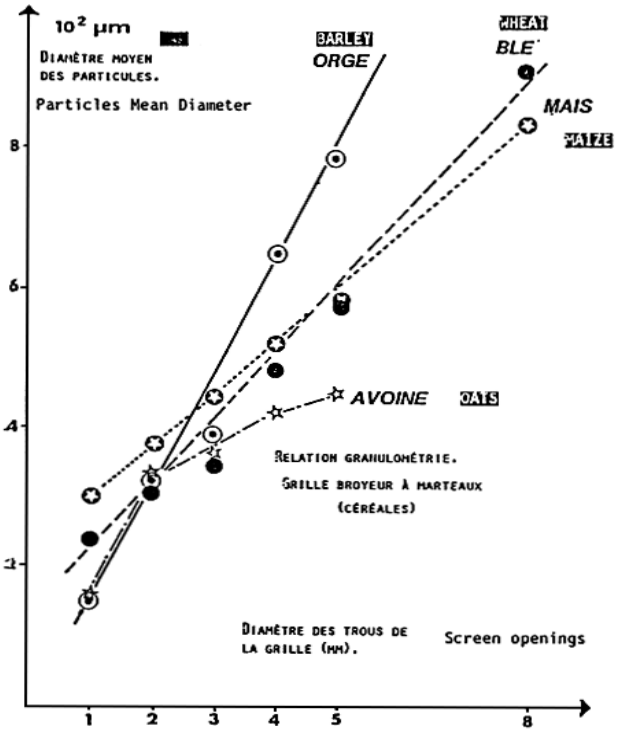


FIGURE 2
BROYEUR AA MARTEAUX
HAMMERMILL

TABLEAU 1 / TABLE 1

Paramètres	*Rendement (débit horaire)*	*Qualité (finesse de la farine)*
Mcrteaux		
vitesse	+++	+++
nombre	-	+
largeur	+	-
usure	--	0
aspiration	+	-
grille		
diamètre	+++	---
surf. ouverte	+	-
dpaisseur	-	+
usure	--	++

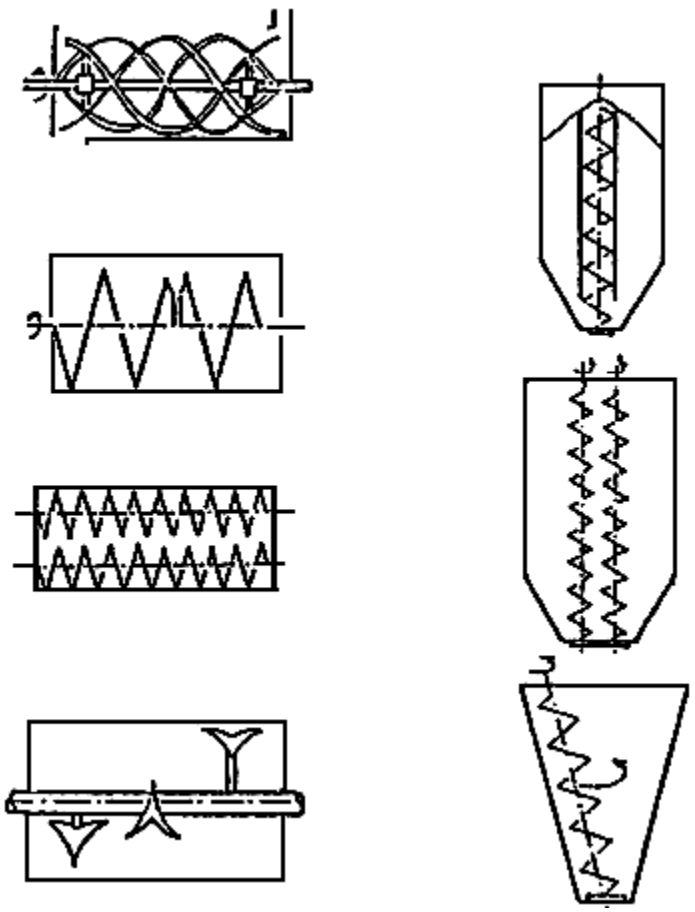


FIGURE 4

MELANGEURS A CUVE FIXE ET MOBILE D'AGITATION
MECHANICAL MIXERS

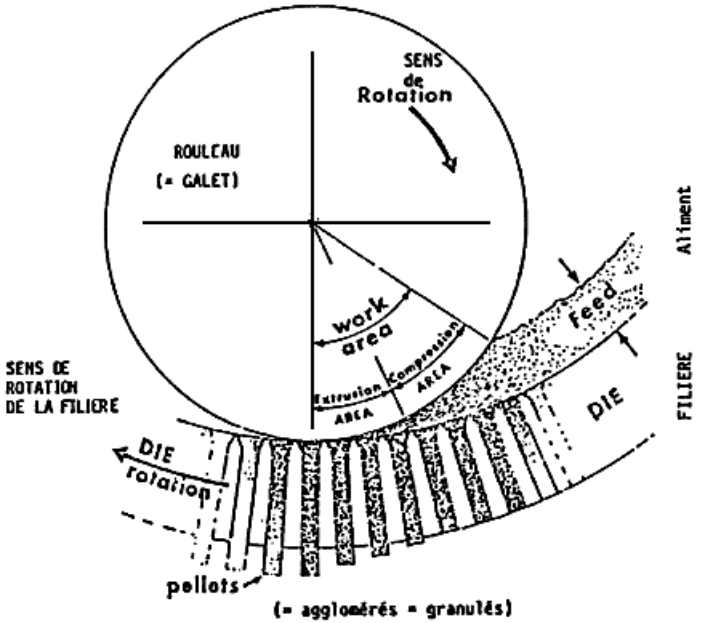


FIGURE 5 : THE DIE AND ROLLER ASSEMBLY
ENSEMBLE FILIERE & ROULEAU DE PRESSE

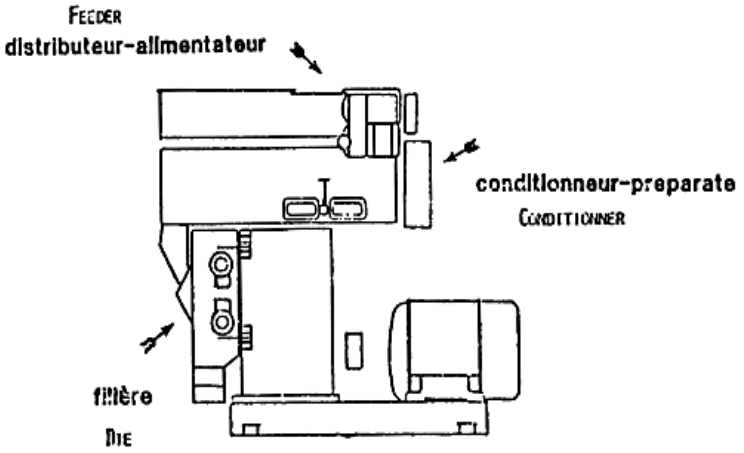


FIGURE 6 : PRESSE A AGGLOMERER
PELLET MILL

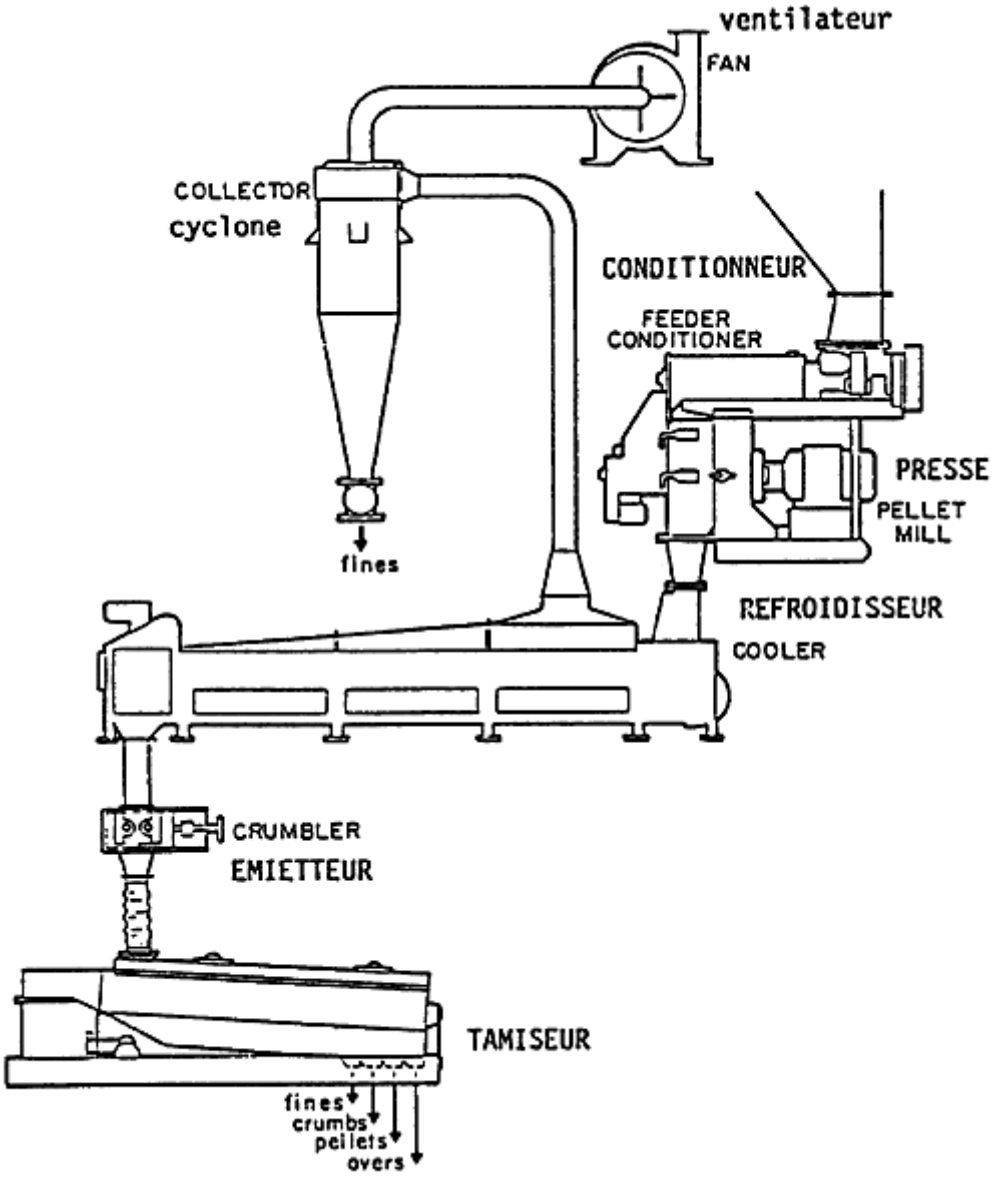


FIGURE 7

TYPICAL FLOW DIAGRAM . EXEMPLE TYPIQUE DE DIAGRAMME

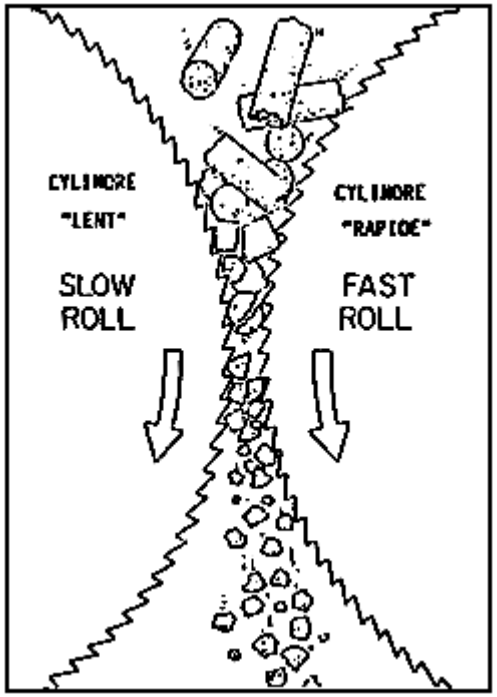


FIGURE 8 CRUMBLE ROLLS / CYLINDRES EMIETTEURS

TABLEAU 2 / TABLE 2

Stability of pelleted fish feeds in water	
Process Variables	Water stability. % of pellet retained after 10 mins in running water
Unground, no steam, thin die	21.5
Unground, no steam, thick die	24.3
Unground, added steam, thin die	31.3
Unground, added steam, thick die	78.9
Ground, no steam, thin die	65.8
Ground, no steam, thick die	74.5
Ground, added steam, thin die	84.9
Ground, added steam, thick die	88.0

Notes.

1. Unground feed particle size range 0.2-2.0mm, 40% of particles less than 0.6mm dia.
2. Ground feed particle size range 0.15-1.7mm. 70% of particles less than 0.6mm dia
3. Chemical analysis of diet: Moisture 9.5%, fat 9.0%, crude protein 30.2%, crude fibre 10.0%, ash 10.4%
4. Data adapted from Hastings[3].

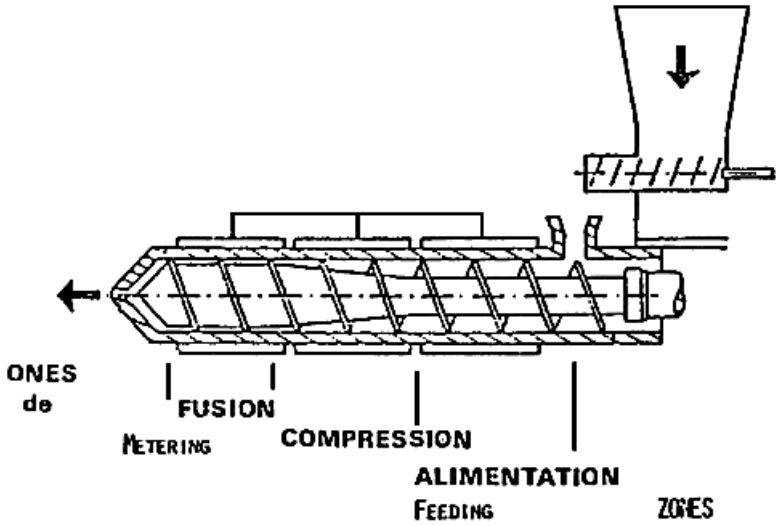


FIGURE 9a

COUPE EXTRUDEUR MONO-VIS

VIEW OF A SINGLE - SCREW EXTRUDER

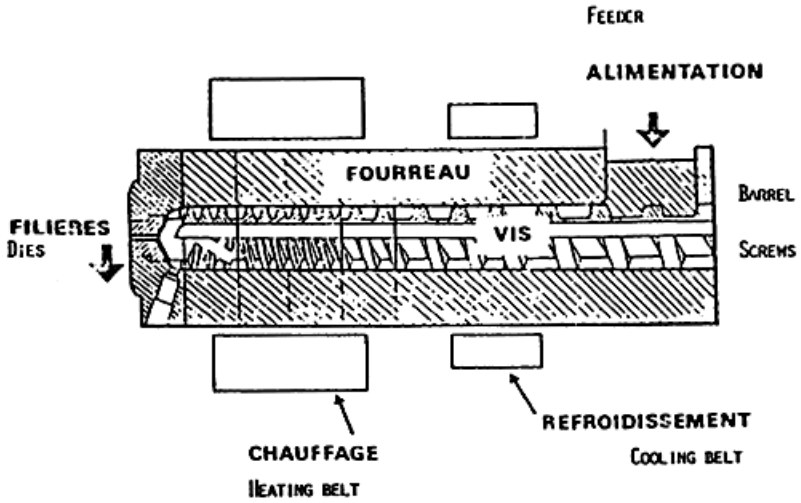
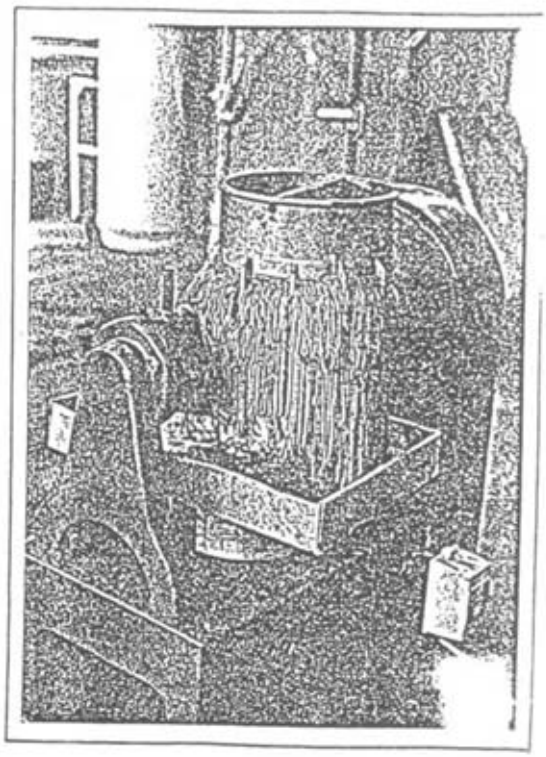


FIGURE 9b

COUPE EXTRUDEUR BI_VIS

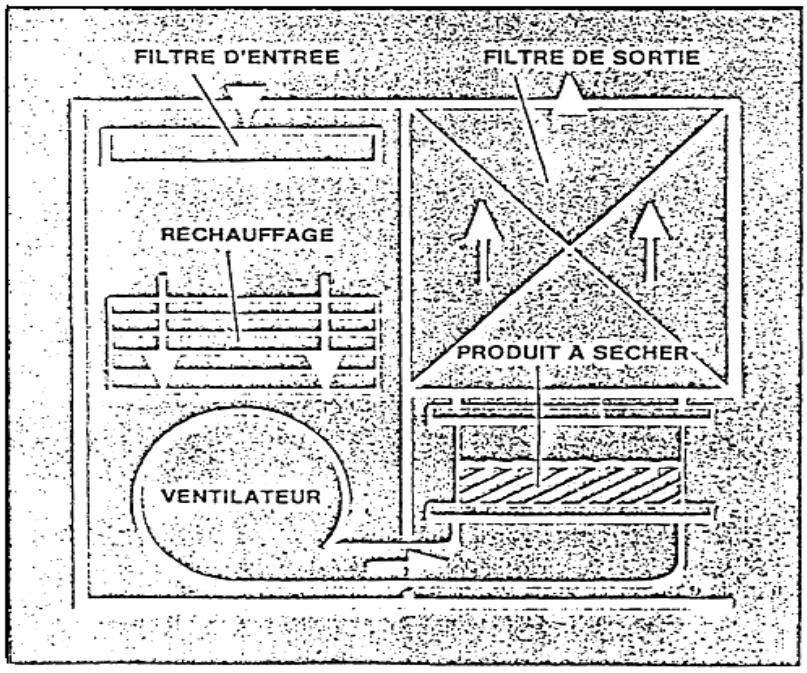
AVEC CONTRE-FILET EN POSITION TERMINALE

VIEW OF A TWIN SCREWS EXTRUDER (WITH REVERSE FLIGHT SCREW IN TERMNAL POSITION)



MELANGEUR et
GRANULATEUR
par voie humide

MIXER and WET
PELLETIZER
<u>FIGURE 10</u>



SECHOIR en lit
fluidisé
Fluidised bed
DRYER

APPENDIX 1

PREPARATION BY EXTRUSION-COOKING OF IMPROVED FEEDS FOR MARINE ANIMALS

The feeding behaviours of marine animals are quite different and often badly known. Extrusion-cooking is a way to obtain unusual physical caracteristics of feeds measured by the rehydration, the water stability, the consistency and the floating or sinking ability.

Extrusion-cooking at moderate temperatures (80 - 90° C) and water additions (22 p. 100) of a gluten containing meal gives favourable water stability (76 - 89 p. 100) to shrimp feeds.

On the contrary, salmon and turbot feeds need expansion to increase water and/or oil, absorption.

Expansion would be given by extrusion at fairly high temperature (180° C) of a meal added with pregelatinized starches. Rehydration capacity of the product is between 250-330 p. 100.

A good floatability is usual with such a feed, well adapted to salmon and trout behaviour, but the problem of the sinking ability of a turbot feed is difficult to solve.

Compared with other techniques, extrusion-cooking is expensive and has a tremendous effect on the vitamins of the feed, but it seems to be actually well adapted to produce feeds for salmonids and flat fishes.

CARACTERISTIQUES PHYSIQUES RECHERCHEES DES ALIMENTS POUR NIMAUX AQUATIQUES

(Requested physical properties of feeds for aquatic species)

	"TRUITE DE MER" ET SAUMON ("sea-trout "and salmon)(a)	TURBOT (Turbot) (b)	BAR ET DAURADES (Sea bass and sea breams) (c)	SOLE (Sole) (d)	CREVETTE JAPONAISE (Japanese shrimp (e)
REHYDRATATION (Rehydration)	++	+		(+)	
STABILITE A L'EAU (Water stability)	+	++	+	+++	+++
APTITUDE A FLOTTER (Floating ability)	++	--	+	--	--
CONSISTANCE (Consistency)	-	-		-	

(a) Salmo Gairdneri, Onchorynchus Kisutch, Salmo salar, (b) Scophtalmis maximus, (c) Dicentrarchus labrax, Sparus sp., Chrysophrys sp., (d) Solea solea, (e) Penaeus Japonicus.

	MAIN FACTORS	*ADDED WATER [%]*	*TEMPERATURE [deg.C]*
Rehydration Floatability	*Pre-cooked starches*	*20-22*	*180*
Rehydration Sinkability	*starches*	*17-18*	*120*
No rehydration Sinkability	*gluten*	*22-25*	*80*

Annexe 2

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Aliscope (français)

Aqua-revue (français)

Kraftfutter (deutsch)

Die Muhle (deutsch)

Feedstuffs (english)

Aquaculture (english)

Progressive Fish Culturist (english)

Animal Feed Science and Technology (english)

Milling and Feed Fertilizer (english)

Annexe 3

CONSTRUCTEURS DE MATERIELS

Liste non limitative

A. BROYEURS, MELANGEURS, PRESSES

FRANCE

PROMiLL - BP 109 - F - 28 104 - DREUX – Cedex

Tél. (37) 43 20 74 - Tlx 760 732 F

STOLZ SA. Division Rousselle - Wailly Beaucamp - F. 62 170 - MONTREUIL SUR MER

Tél. (21) 81 26 11 - Tlx 820 728 F

SUISSE

Pelleting Service International (PSI), CH - 9 402 - MORSCHWIL

Tél. (071) 96 18 88 - Tlx 71 299 PSI CH

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FEEDING STIMULANTS FOR FISH - APPLICATIONS IN MARICULTURE

A.M. MACKIE

FEEDING STIMULANTS FOR FISH - APPLICATIONS IN MARICULTURE

A.M. MACKIE

The aim of the fish farmer is the efficient conversion of compounded diet into live fish, with the minimum of waste, and clearly any way of increasing the acceptability or palatability of fish diet would be advantageous. Fish vary considerably in their willingness to accept compounded diet. Salmonids in general readily accept fishmeal-based diets while Dover sole require the addition of scallop or Nephrops waste. This is due, in part at least, to the fact that salmonids are visual feeders while Dover sole use their chemical senses, smell and taste, in the detection of food.

To be acceptable, a diet must satisfy several criteria -

1. Appearance : size, shape, colour
2. Smell : which in the case of aquatic animals, should be termed long-range chemical attraction, since such animals can use both smell and taste to detect food at a distance.
3. Feel : is the material hard or soft, most or dry, rough or smooth?
4. Taste : taste buds in the mouth monitor the taste of the material.

Which are the most important features of a food depends on whether the particular fish is a visual or chemosensory feeder. The chemical activators can be divided into three groups. Attractants guide the fish towards the food, incitants invoke biting and tasting, and feeding stimulants induce the fish to swallow the food.

It is the nature of the gustatory feeding stimulants that has been studied at the Institute of Marine Biochemistry (IMB). The test diet was based on casein (Table 1) and the unflavoured diet was unacceptable to most fish. However, when a mixture of chemicals, based on an analysis of squid mantle tissue (synthetic squid mixture, Table 2) was added at a level of 1-2%, the fish readily ate the diet. The feeding stimulant could then be identified by adding various components of the complete mixture to the casein diet and measuring the quantity of test diet eaten.

Table 3 gives a summary of the results obtained at IMB and elsewhere, along with the major prey animals of the fish. Tabulation of the fish under Order and Family indicated that if there is any taxonomic relationship with the feeding stimulants it exists at or below the Family level.

Another possible factor in the "choice" of feeding stimulant is the feeding habits of the wild fish under natural conditions. Free L-amino acids, the feeding stimulants for sea bass (Mackie & Mitchell, 1982), European eel (Mackie & Mitchell, 1983) and Japanese eel (Takeda et al., 1984), are present in all animal tissues, both vertebrate and invertebrate. Different amino acid mixtures acted as feeding stimulants for the various species, and in all cases the corresponding D-amino acids were ineffective.

Inosine and inosine 5'-monophosphate are the specific feeding stimulants for the turbot (Mackie & Adron, 1978) and the brill (Mitchell, unpublished results), and the prey of juveniles and adults of both species all contain this chemical. Inosine 5'-monophosphate also acts as a feeding stimulant for juvenile yellowtail (Hosokawa et al., cited by Takeda et al., 1984).

The red sea bream, Dover sole and puffer eat worms, molluscs and Crustacea which all contain glycine betaine and free L-amino acids, the feeding stimulants. It is generally considered that teleost fish contain little or no glycine betaine (Love, 1970), while invertebrates and elasmobranchs are rich in this chemical. However, Carr et al. (1977) has shown that in the pigfish, which eats small fish in addition to invertebrates, the feeding stimulant is again a mixture of glycine betaine plus free amino acids, and these authors also reported fairly high levels of glycine betaine in mullet tissue.

A much wider range of fish species will have to be investigated before any definite conclusions can be drawn, and before any predictions can be made regarding the chemical nature of the feeding stimulant of an untested species. However, fish-eating species most probably have amino acid mixtures or inosine 5'-monophosphate as feeding stimulants and these eating invertebrates, glycine betaine plus anino acids.

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Table 1. Composition de la nourriture d'essai

(Composition of test diet)

	g/2kg
Caséine (sans vitamines) (vitamin-free casein)	1100
dextrine	200
α-cellulose	252
D-glucose	100
huile de poisson (fish oil)	180
agglomérant (CM-cellulose, Na) (binder)	100
mélange des vitamins (vitamin mixture)	56
mélange des mineraux (mineral mixture)	10
matière colorante (Sunset Yellow) (food colour)	2

100 g nourriture sec mélangé avec 160 ml solution aqueuse

(100 g dry diet mixed with 160 ml aqueous solution)

Table 2. Composition of du mélange "synthetic squid"
(composition of synthetic squid mixture)

		% composition	
L-aspartic acid	0.31	L-proline	24.69
L-threonine	0.73	glycine	15.03
L-serine	0.55	L-alanine	4.60
L-glutamic acid	0.89	L-arginine	3.84
L-valine	0.61		
L-methionine	0.61		
L-isoleucine	0.49	glycine betaine HCl	15.34
L-leucine	0.92	trimethylamine oxide HCl	19.17
L-tyrosine	0.37	trimethylamine HCl	1.53
L-phenylalanine	0.49	hypoxanthine	0.80
L-lysine HCl	0.49	Inosine	0.43
L-histidine HCl	0.24	adenosine 5'-monophosphate	0.67
taurine	5.67	L-(+)-lactic acid	1.53

Basé sur l'analyse de la chair de l'encornet, Loligo forbesi (Mackie, 1973).

Mélange dissous dans l'eau distillé et pH ajusté à 6.5.

Table 3. Survey of food organisms and feeding stimulants

Ordre	Proie	Phagostimulant
Anguilliformes		
Anguille européene, Anguilla anguilla	Crustacés, mollusques, vers, poissons	Mélange des acides aminés de serie L (Mackie & Mitchell, 1983)
Anguille japonaise, A. japonica	Crustacés, mollusques, vers, poissons	Mélange des acides aminés de serie L (Takeda et al., 1984)
Perciformes Famille Serranidae		
Bar, Dicentrarchus labrax	Juvéniles: crustacés, poissons Adults : poissons	Mélange des acides aminés de series L (Muckie & Mitchell, 1982)
Famille Carangidae		
Seriolle, Seriola quinqueradiata	Juvéniles: cephalopods, poissons	Inosine 5'-monophosphate (avec les acides aminés). (Hosokawa et al., cité par Takeda et al., 19
Famille Pomadasyidae		
Orthopristis chrysopterus	Invertebrés divers, poissons	Glycine betaine avec les acides aminés (Carr et al., 1977)
Famille Sparidae		
Daurade royale, Chrysophyrs Major	Crustacés, vers	(Glycine betaine avec les acides aminés (Coh & Tamura 1980)
Pleuronectiformes		
Famille Bothidae		
Turbot, Scophthalmus maximus	Juvéniles: mollusques, vers Adults : poissons	Inosine ou inosine 5'-monophosphate (Mackie & Adron, 1978)
Barbue, S. rhombus	Juvéniles: mollusques, vers Adults : poissons, cephalopods,	Inosine ou inosine 5'-monophosphate (Mitchell, oeuvre non publié)
Famille Pleuronectidae		
Plie, Pleuronectes platessa	Vers, mollusques, crustaces	Mélange complex des produits chimiques (Mackie, 1982)
Famille Soleidae		
Sole. Solea solea	Vers, mollusques	Glycine betaine avec les acides

	crustaces	amines (Mackie et al., 1980)
Tetraodontiformes		
Fugu pardalis	Vers, mollusques, crustaces	Glycine betaine aves les acides aminés (Ohsugi et al., 1978)

THE USE OF MICROPARTICLES IN AQUACULTURE

F.J. GATESOUPE

INTRODUCTION

The first successes in Marine Aquaculture are linked with the more or less local opportunity to collect spat, fry or post larvae shrimp. The development of this activity is connected with the design of the hatcheries which must be capable of ensuring the production of juveniles, while not relying on the natural environment. All the species employed, begin to feed soon after hatching, although the locomotive, sensorial and digestive faculties of the larvae are just beginning to form. Thus, it is obligatory that the hatcheries furnish a food which is as similar as possible to the plankton consumed in a natural environment. It is often possible to collect plankton, but this is too hazardess, thus hatcheries generally cultivate live prey from isolated strains. These annex cultures are expensive, and from 1970 onwards, numerous tests were carried out so as to replace them by a complete artificial diet, presented in the form of microparticles. Confident with the results obtained in the rearing of the rainbow trout, where the fry accept, without any difficulty, the pellets from the first feeding onwards, the precursors believed that the microparticle was but a smaller sized pellet than that used in fattening. Unfortunatly, when trout fry start feeding they are no longer larvae and have already a functional stomach: It was necessary to try out more elaborated formulas immediatly, which would hinder the particles from dropping to the bottom and from disintograting too quickly, while at the same time ensuring that the above could be adapted to the locomotive behaviour of the larvae, and that the addition of a colour solution and appetizers would stimulate their sensorial faculties. But the fundamental question remaining unanswered is that of the choice of nutritive componants adapted to the larvae.

The advantage of employing microparticles in aquaculture is not only limited to larvae: They can be employed to feed the live prey which will afterwards be fed to the larvae. Their use for filterer molluscs after their metamorphosis has taken place, can also permit the acquirement of a better knowledge of the diet of these species, whose feeding is ensured by a natural primary production.

Besides compound microparticles, there also exists natural ones: eggs or bacteria, for example. In addition, it is possible to modify the composition of live prey through their culture conditions or feeding. FONTAINE and REVERA (1980) have even termed as "microcapsulation" the forcible feeding of rotifers with nutritive particles. On the other hand, the organisms employed as live prey are also an important source of raw material used in the composition of microparticles. Indeed, between the plankton collected and the compound microparticle of purified raw materials, there exists a great variety of intermediary solutions with live or inert prey whose composition is more or less monitored (Diagram 1). Thus, it is all the feeding techniques employed that will be of interest to us, by making a list of the food at disposal, and the results obtained in the larval rearing. We do not need to go into detail since nearly all hatcheries develop their own techniques, but instead to emphasize the tendancies and establish the different choices possible.

1. THE "NATURAL" MICROPARTICLES

Whether live or dried, numerous organisms make up the "natural" micro-particles which can be directly employed to feed live prey or larvae.

1.1. Unicellular organisms

The great advantage of these organisms lies in the quality and quantity of proteins that they contain: Generally, they represent 50 to 80 % of the dry matter, having

an amino-acid balance much the same as that of muscular proteins. They are also rich in nucleic acids and in certain vitamins (LITCHFIELD, 1983). In addition to the industrial products, it is possible to cultivate specified isolated marine strains (Diagram 2).

1.1.1. Bacteria

The industrial production of bacteria is advancing rapidly and is the most promising source for natural microparticles. Some of these products have already been tried out in aquaculture, as is the case of Methylophilus methylotrophus which is used successfully on oysters in fattening (NELL and WISELY, 1983). The marine bacteria are, a priori, the most interesting for aquaculture but if production techniques do exist (YAMAMOTO et al., 1978), there, to our knowledge, exists no commercialization. In addition, even marine bacteria do not seem to contain the essential fatty acids; eicosapentaenoïc and docosahexaenoïc (ORO et al., 1967; ANDREE et al., 1979).

1.1.2. Yeasts

There exists a great variety of Saccaromyces cerevisiae, of commercial source, either in atomized form or live form (baker's yeast or brewers yeast, etc...) One of these which is especially adapted for aquaculture has been developed in Japan ("w-yeast" - IMADA et al., 1979): Its culture medium containing squid oil gives it a high tenor in essential fatty acids. Candida utilis yeast has been introduced into the feeding of juvenile oysters (URBAN and LANGTON, 1984). Finally, there exists culture techniques of marine yeasts (KAWANO and KAMEL, 1980) but as in the case of bacteria they contain very few or no essential fatty acids (HIGASHIHARA et al., 1983 a).

1.1.3. Algae

Mass culture of algae permits commercialization. This especially concerns fresh water chlorella, in Taïwan (CHEN, 1977) and spiruline in Mexico (which are indeed pluricellular algae - CIFERRI, 1983). These algae are available in the atomized form, principally intended for human consumption, and so very expensive. However, there exist mass productions of diatoma and marine chlorella which are meant especially for molluscs (GOLDMAN, 1979; CLAUS et al., 1980).

The controlled pure culture of algae is performed in most hatcheries (De PAUW, 1981; LE BORGNE, 1986). This, to a certain extent, permits to modulate the food quality, by diversifying the culture conditions: for example, the quantity of nitrate has an effect on the protein rates (MOSTERT and GROBBELAAR, 1981); The digestibility of proteins increases with the age of the culture (KANDATSU and KAWAGUCHI, 1979); the salinity increases the tenor in essential fatty acids, contrary to the temperature (SETO et al., 1984).

1.2. Animal live prey

If we leave out Artemia cysts which are a natural resource, the animal live prey must be cultivated from isolated strains: Then, there is no question of an alternative with other industrial products such as compound micro-particles.

1.2.1. Copepoda

Marine calanoïds belong to the plankton which is normally consumed in a natural environment : Thus, they are prey which are very well adapted for larval rearing, moreover as, they have an important essential fatty acid tenor (WATANABE et al., 1983). Unfortunatly, around 20 days is required before adults can be obtained and rearings such as these run very expensive on hatcheries ((STOTTRUP et al., 1986). The detrivorous and benthic harpacticoïds copepoda have the same disadvantage but

they seem to feed more easily on artificial food (for example, if Tigriopus japonicus and Acartia clausi are compared - KITAJIMA, 1973).

1.2.2. Brachionus plicatilis

The performances of the parthenogenetic reproduction of this rotifer adapted to a marine environment, along with its resistance to environmental changes and its facility to eat, permits it to be reared in all hatcheries (POURRIOT, 1986). The most reknown and reliable technique is the culture with baker'yeast starting from an inoculum cultivated with the algae (HIRATA, 1979). The marine yeast cultures and to a lesser degree bacteria, can replace the algae culture for rotifers (HIGASHIHARA et al.,1983 b). In the same way, a compound diet consisting of atomized unicellular organisms and additives (oil, vitamins, raw starch) could be effective (GATESOUPE and LUQUET, 1981). Compound feeding permits not only to improve the ensurance of these nutritional needs of the rotifer - for example, by adding vitamin B 12 and fish oil to the baker 's yeast (HIRAYAMA and FUNAMOTO, 1983) but also its food quality. For the latter, only the effect of a fish oil supplementation is sure (WATANABE et al., 1983). There exists other ways of supplying a strong tenor in essential fatty acids to rotifers: we have already stated the special yeast of IMADA et al., 1979; Enrichments through baths for time lengths varying between 30 minutes and 24 hours is also carried out just before the rotifers are distributed to the larvae: For thus, the rotifers are placed into a concentrated suspension of algae, emulsion (WATANABE et al., 1983) or compound food (GATESOUPE and LUQUET, 1981).

Microcapsules have even been tested (TESHIMA et al., 1981). A priori, it seems surprising to search for a capsuled particle for a rotifer which is capable of feeding on soluble substances (SCOTT, 1983) and bacteria; however, the flora associated with rotifers is difficult to control (COVES et al., 1986) and microcapsules could help limiting the bacterial proliferations caused by uneaten food. We have, also, employed microcapsules for the supply of antibiotics to turbot larvae by forcibly feeding the rotifers, thus avoiding the need of employing bath treatment techniques (RUBIO RINCON, 1986).

1.2.3. Artemia

Artemia cysts permit obtaining nauplii which can be given directly to the larvae, so long as the Artemia strain is of "marine type" (WATANABE et al., 1983), in other words, that the nauplii contain an important quantity of essential fatty acids. If not so, it is necessary to proceed with a diet rich in fish oil while taking into account that nauplii dont feed until several hours have passed, after hatching (URBANI, 1959). There is a lot of data on this subject and the techniques don't differ greatly from those employed for rotifers (Diagram 3); we shall refer to the more recent documents (SORGELOOS and al., 1986; LEGER et al., 1986).

1.3. Fish eggs

There exists other natural microparticles: Let us take for example fish eggs which constitute and interesting food for sea-bass Micropterus salmoïdes (BRANDENBURG et al., 1979) or shrimp Macrobrachium rosenbergii (MANZI and MADDOX, 1980). Unfortunatly, the availability of these eggs is often not compatible with the regular supply from a hatchery.

2. COMPOUND MICROPARTICLES

We must distinguish here, two types of consumers which dictate different constraints for the utilization of microparticles: the filterers and the predators (Diagram 4).

The filterers injest large volumes of water and find a considerable part of their food in the dissolved substances (STEPHENS, 1982) and bacteria. The microparticles can then either be filtered directly or disintegrated and increase the concentration of bacteria and dissolved substances, provided however that the filterers resist the environmental alterations that this entails. With shrimp or mollusc larvae, it is advisable to use stable microparticles even if this should require the necessity of supplying separatly certain bacteria or dissolved substances. We have remarked that on the contrary, live prey can feed on compound food which has not been treated in any particular way so as to avoid its desintegration. In all cases, microparticles must have a slightly higher density than that of sea-water so that the disturbance caused by the aeration will ensure that they are kept in suspension.

Fish larvae and shrimp post-larvae have a predator behaviour. This behaviour must be stimulated by the microparticles, to which is added appetizing ingredients and colour solutions (red or orange more often). This results in a new constraint: a same microparticle must contain a complete and balanced food, if not, the preference for one type of particle in the case of a mixture, would entail, and unbalance in the feeding. This would mean a great difference with regard to filterers who can receive separatly an emulsion, solid particles, and dissolved substances. Finally, its water stability and its maintenance in suspension must be ensured for as long as possible. Several technological treatments permit the ensurance of these requirements (Diagram 5).

2.1. Pressure cooking methods

Its stability in the water is obtained through the action of vapor pressure compaction. Cooking also permits the better digestibility of starch and the destruction of certain undesirable substances such as avidine found in the hen's egg. Unfortunatly, certain vitamins can be destroyed on parallel (ascorbic acid and thiamine for example).

2.1.1. Flakes

Compaction is carried out at more than 100° C on a rotative drum which permits the obtention of flakes. This particular form facilitates their maintenance in suspension in the water (MAYERS and BRAND, 1975).

2.1.2. Cooking extrusion

A die is employed for compaction. Stable food is obtained with temperatures of below 100° C, but the main advantage of this method lies in the expansion provoked at higher temperatures, which permits to rehydrate the particles just before their distribution with an emulsion containing labile substances (Vitamins, appetizers, oil - METAILLER et al., 1983).

2.2. Gels

Certain food gels are very effective and permit to obtain very stable microparticles without the need of any specialized equipment: the small scale manufacture of microparticles is thus possible at hatchery level. The most widely used gels are agar, kappa-carragheen (TESHIMA et al., 1982) which are insoluble in cold water and need to be heaten to 80° C, and the, alginate of sodium which is soluble when

cold and precipated by the calcium ions (L'HERROUX et al., 1977) : This last technique is most interesting for the preservation of thermolabile substances.

2.3. Microencapsulation (by coacervation)

There are numerous methods in which to obtain particles with a protecting wall covering. We will give here a description of two: Microencapsulation by coacervation which consists in precipitating a polymer in liquid phase around the microparticles or the micella and the microcoating where wall is obtained by the evaporation of the external phase. This classification is not conventional as on general a spray is employed for microencapsulation. Our objective is to insist on the essential difference between the two methods when they are employed for compound food. Indeed the external liquid phase necessary for the coacervation -either water or an organic solvent- causes the loss of soluble substances: It is therefore difficult to obtain a complete food (JONES and GANNOTT, 1976). On the contrary, this is the only technique through which will be obtained graded particles of a little micron dimension without the use of specialized equipment. TESHIMA et al., (1982) give a list of the principal methods employed: nylon protein capsules, gelatine gum arabic, chitosan, zein. LANGDON (1983) proposed a technique using emulsion to cover hydrosoluble substances in a coating which contains fish oil ; It is unfortunatly impossible to isolate these capsules from the external liquid phase : This is however a method which could be very useful so as to ensure the soluble ingredients for filterers while not having to dissolve great quantities.

2.4. Microcoating

With this technique, the external phase is evaporated, which hinders all losses of soluble elements during its manufacture and permits the incorporation of appetizing substances in the coating (GATESOUPE and LUQUET, 1977). The use for particles of some hundreds of microns does not require specialized equipment : the coating then consists of zein or of a cholesterol mixture - lecithin (TESHIMA et al., 1982). The spray (atomization, etc...) permits obtaining much finer particles (BALASSA and FANGER, 1971).

3. THE CHOICE OF MICROPARTICLES DEPENDING ON THE SPECIES

Feeding tests, using compound microparticles have been tried out on nearly all the species employed in aquaculture. However, few of these tests have been applied on large scale in hatcheries: Most of the tests were promising, but the growth performances and survival have rarely been as good as that obtained with live prey. An economy can be made through compound microparticles if there is a prolonged larval rearing duration, moreover as a low growth is a sign of a bad nutritional state which will irremediably endanger the ulterior growth performances. This is why we can have but a rather restrictive vision of the use of microparticles in the actuel state of affairs.

3.1. Live prey

We have seen that there exists a large possible variety of food. The determinant factor for these prey will be their food value. When the climatic conditions permit so, the production of selected unicellular algae in outdoor large volumes is the most rational solution to adopt. Moreover as it is always possible to complete the ration with baker's yeast for example. In the cold regions, baker's yeast is the safest basic food to employ, while ensuring a minimum quantity of live algae regularly and that the prey are enriched with a fish oil emulsion before being distributed to the larvae.

3.2. Filterer molluscs

The larval and post-larval stages of these species being particularly sensitive to the physiochemical and microbiological quality of their environment, it is preferable to feed them on algae cultivated in controlled conditions. There is but one link in the food chain here, thus the cost of an algae room is quite acceptable for a mollusc hatchery. The use of microparticles is however promising for the future (TESHIMA et al., 1982).

It is necessary to carry out the fatteneing of these species in an open environment. We shall thus limit the use of compound microparticles to the experimentation in nutrition: except in particular cases, intensive rearing having a food supply has little chance of being more profitable than extensive rearing.

3.3. Shrimp

Alginated microparticles are already widely used in hatcheries of Macro brachium rosenbergii (AQUACOP, 1983). Likewise, particles linked with carragheen seem to answer the requirements of Penaeus japonicus when Zoe stage is reached TESHIMA and KANAZAWA, 1983) along with those of tropical peneids (GALGANI-TURIN, 1986). The preservation of ther molabile substances is an important factor for success (TESHIMA and KANAZAWA, 1983): the employment of fresh raw materials is thus strongly advisable, especially as squid flesh contains a growth factor (CRUZ and GUILLAUME, 1983).

3.4. Fish

If the consumption of compound microparticles by fish larvae as their first food is frequently observed, the results for growth and survival are extremely disappointing. For certain species, it seems possible to obtain good results 10 days after hatching: this applies for japanese red sea-bream (TESHIMA et al., 1982) and for sole (GATESOUPE, 1983). On general, weaning is carried out at the end of the first month, and research is still necessary so as to reduce the quantities of Artemia consumed, by advancing the date for weaning. This seems possible for sea-bass for example (PERSON - LE RUYET, 1986).

The most current reason put forward for this difficulty in rearing fish larvae, with compound food is the inadaptation of the stomachless digestive system. The absorption of proteins and their intercellular digestion seems to be of considerable importance, even with the trout fry which has a stomach (GEORGO-POULOU et al., 1986). It is most probable that the quality of the macromolecules present in the food plays an essential part in this absorption-digestion. On the other hand, the distribution of proteic fractions is very different in live prey (HAYASHI et al., 1985), and in the muscle of fish (HASHIMOTO et al., 1979) (Diagram 6) : the proportion of free amino-acids, peptides and soluble proteins is indeed much greater in the former. These are evidently the most difficult fractions to be retained in compound microparticles. The method developed by PIGOTT et al., (1982) conferring to the proteins of fish bindery proporties through hydrolyses could be an effective way to risolve this problem.

CONCLUSION

A lot remains undone for the generalization of the employment of compound microparticles in hatcheries. This is however an inevitable evolution, at first for economic reasons: the Artemia cysts constitute a limited resource and it is preferable not to waste them, if we wish to increase the production of juveniles. Microparticles should permit to avoid a too considerable increase of the demand for Artemia, but it must not be hoped that they themselves constitute a low costing food. Indeed, the quality requirements of raw materials - sometimes mean for human consumption such as squid-

and the use of expensive machinery risk to maintain high prices, moreover as the delays of conservation do not permit the manufacture, at real industrial scale, of a product of limited demand. Secondly, compound microparticles should permit the improvement of the nutritional state of juveniles, with regard to what is obtained with Brachionus plicatilis and Artemia which when even enriched are not necessarily the ideal food. The first results are already available: CHEN et al., (1986) have obtained better growth of peneid post-larvae by distributing simultaneously Artemia and compound food.

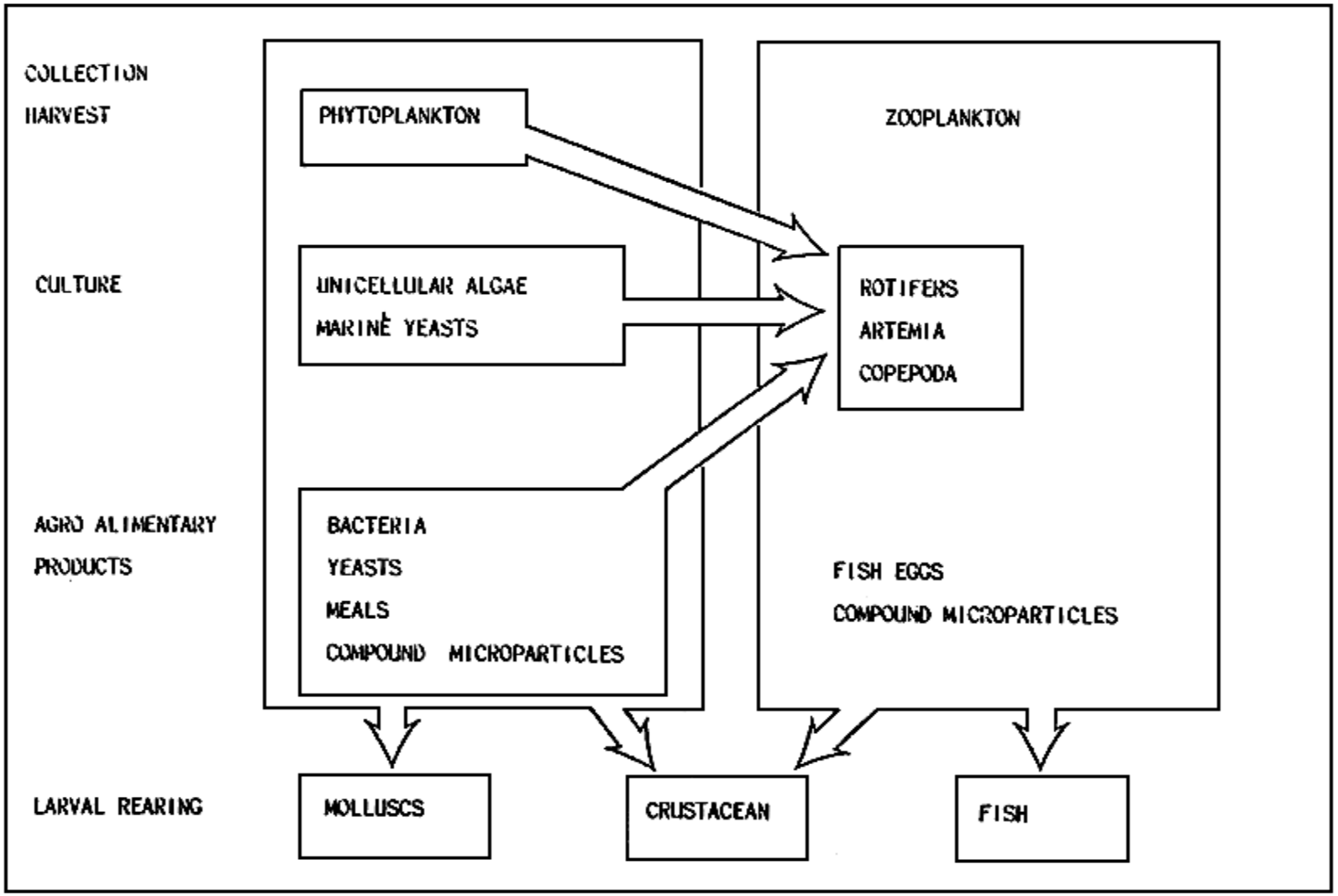

Diagram 1 - The food chains employed in hatcheries (the arrows indicate the trophic relations

	Industrial Products	Special çultures of marine strains
BACTERIA	Methylophilus methylotrophus	Cultures in methanol
YEAST	Saccharomyces cerevisice Candida utilis	Saccharomyces sp. Candida spp. Torulopsis inconspicua
ALGAE	Chlorella spp. (eau douce) Spirulina maxima	- Mass cultures: Chlorella spp. Diatoma - Monitored cultures for hatcheries

Diagram 2 - The unicellular organisms used in marine aquaculture

	REQUIREMENT OF A FOOD TREATMENT	FOOD EMPLOYED IN FATTENING	ENRICHMENT IN ESSENTIAL FATTY ACIDS	MARINE ALGAE EMULSION COMPOUND FOOD
ROTIFERS	YES	LIVE UNICELLULAR :	OFTEN UNNECESSARY	
		- MARINE ALGAE		
		- MARINE YEAST	OFTEN NECESSARY	
ARTEMIA	NO	BAKERS YEAST	NECESSARY	
		- NORMAL		
- OF MARINE TYPE		- SPECIAL	UNNECESSARY	
		ATOMIZED UNICELLULARS	NECESSARY	
- FRESHWATER TYPE	YES	MICROCAPSULE COMPOUND FOOD	UNNECESSARY	
		RICE TRAN AND OTHER DRY PRODUCTS	NECESSARY	

Diagram 3 - Food For Rotifers and Artemia according to their nutritive quality

CONSUMERS	LIVE PREY	FILTERERS		PREDATORS	
		SHRIMP (Larvae)	BIVALVES	FISH (Larvae)	SHRIMP (Post-larvae)
QUALITIES REQUIRED FOR THE COMPOUND MICROPARTICLES	[——— ADAPTED SIZE] ———				
	[——— COMPOSITION ANSERING THE REQUIREMENTS] ———				
	(SLIGHTLY HIGHER DENSITY THAN THAT OF SEA-WATER ———]				
	[——— STABILITY IN THE WATER ———]				
	[COMPLETE FOOD IN EACH PARTICLE]				
	[——— APPETIZER ———]				
	[——— COLOURING ———]				

Diagram 4 : Quality required for the compound food

TYPES OF MICROPARTICLES	SPECIALIZED MACHINE	NECESSARY TEMPERATURE (degre C)	PRINCIPAL ADVANTAGE
FLAKES	YES	130-190	MAINTENANCE IN SUSPENSION
EXTRUDED CRUMBS	YES	80-90 140-190	REHYDRATABLE TEMPERATURE
GELS: AGAR & CARRAGHEEN ALGINATE	 NO NO	80-100 AMBIANT (Vacuum drying)	EASY SMALL SCALE MANUFACTURE
MICROENCAPSULATION	NOT NECESSARY	5-80	PARTICLES OF SOME POSSIBLE MICRONS
MICROCOATING	NOT NECESSARY	AMBIANT (Vacuum drying)	THE COATING CAN CONTAIN APPETIZING SUBSTANCES

Diagram 5 - The different types of compound microparticles

	Hayashi & ol (1985)			Hashimoto & ol (1979)
FRACTION (%)	BRACHIONUS PLICATILIS	TIGRIOPUS JAPONICUS	ACARTIA CLAUSI	WHITE MUSCLE OF THE SARDINE
SOLUBLE TCA	28	55	48	12
SARCOPLASMIC	52	17	6	26
MYOFIBRILLARY	9	2	1	55
SOLUBLE ALKALI	2	12	32	6
STROMA	9	14	13	2

Diagram 6 - Distribution of the proteic fractions in live prey, compared with that of the sardine muscle.

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EXPERIMENTATION IN NUTRITION

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PLAN

1. What is meant by experimentation in Nutrition
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     - Filleting yield
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   - 6.9. Histology of the different organs
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1. WHAT IS MEANT BY EXPERIMENTATION IN NUTRITION ?

It is group of elements linked together and organized in the following way :

- A question on nutrition is asked, objectives are defined
- An experimental protocol is elaborated
- An experiment is implemented, it begins proceeds and ends
- The results are analysed
- The conclusions obtained answer the question asked :
  - Yes. In this case, there is no need to bring up this problem again
  - Yes partially : the protocol must be changed, part of the experimental plan must be re-examined
  - No : The protocol must be entirely reviewed ; a new experimental plan must be scheduled

These different points will be the main objective of the present work. In order to avoid going into too much detail in this report, we shall only envisage the problem from a fish viewpoint leaving in the hands of those concerned by the rearing of other zoological groups, the task of carrying out the necessary transpositions and additions.

2. STARTING POINT : A QUESTION IS ASKED, OBJECTIVES ARE DEFINED

There exists a multitude of questions of variable complexity which a nutritionist or a group of scientists may have to answer on a given context. They result from diverse motivations of zoological, nutritional, economical, pathological type. We shall not try to list them here when faced with a question, two possible attitudes may be adopted :

- An attitude of research (explicative, analytical), which will lead us to study a small number of factors which will vary by placing "everything equal elsewhere".

- A practical attitude (synthetic) which applies more to field work : here, we shall not study one or more factors, but compare the systems, the technical or factoral combinations without obligatory placing "everything equal elsewhere" .

Indeed, the "border-line" between these two attitudes is not always evident, as they coexist within the same experiment. It is therefore of particular importance, from the beginning, depending on which attitude has been adopted, to formulate the question correctly, to situate the problem accordingly and to define properly, in distinct terms for everybody, the limits of the objectives which are to be set, the experimental protocol will depend on this along with the processing, the capacity and the generalization of the results.

3. EXPERIMENTAL PROTOCOL*

The objectives being well established, the nutritionist, with the means available, will conceive a plan, an experimental protocol which will lead to the implementation of an experiment. Indeed, the elaboration of a protocol requires a team of workers of at least 3 people :

- The person in charge of the protocol
- The research worker who will be in charge of the adaptation of this protocol "in situ"

– A person who is well versed in statistics.

It is certaintly difficult to give a typical model of the experimental protocol as each problem will in itself be a particular case, it is however possible to acquire a certain number of recommendations :

– The protocol must situate the question asked in regards with what is known on the subject.

– It must be precise and written in clear terms

– It must be complete and take into account the question asked on the whole, and not only one particular point.

– It must be simple, it is advisable to avoid going into to much detail, concerning the different parameters

– It must enable an inventory being made and take into account all the real constraints linked with a given problem (the rearing enclosures, water quality, the animals, the food ingredients, raw material, the distribution of food, lighting...)

* Read “The elaboration of a trail protocol”, published by ITCF, 8 avenue du Président Wilson, 75116 – PARIS, by which the author was greatly inspired.

– It must enable the inventory being made of the measures to take and the means required to perform them.

– It must enable the distribution of different tasks

– It must enable the establishment of an agenda

– it must allow the scheduling of the way in which the results will be processed and interpreted

– It must eventually allow the estimation of the costs

– It must be logical and clearly comprehensive. Why should one try to compare uncomparable "things" ?

When a protocol is formulated, the authors must verify that the experiment scheduled has every chance of correctly answering the question asked. They must also ensure the no deviation could likely compromise the interpretation of the results or be misleading.

Tables 1a, 1b, 1c and 2a, 2b, 2c, 2,d have but one objective, that is to visualize, as an example, the possible evolution of the experimental choices, in confrontation with a given problem : the testing of the different raw materials or the study of the protein requirements of a given species.

In all cases, it should be kept in mind that the formulation of an experimental protocol corresponds to a choice among the different possible options. The most important point is to define the consequences of this choice with regards to the question asked and the results expected.

4. IMPLEMENTATION OF AN EXPERIMENTATION IN NUTRITION

Indeed, this concerns the practical application, "in situ", of everything which has been thought and written in the experimental protocol.

4.1. Rearing enclosures - Environment, Experimental system

It is very difficult to carry out experiments in production units. On one hand, they are too big and require a great number of animals and a large quantity of food ; due to this fact, the experiment is naturally limited. On the other hand, it must be remembered that all experiments must receive a close follow up and more care must be taken than in ordinary routine rearing ; the personnel working in the production stations cannot always ensure this follow up correctly. Finally, the obligations demanded in production do not always comply with the experiment, (the animals must show good growth, bargain sales, etc...)

It is therefore advisable to have extra rearing structures installated for the experiment alone and specifically designated staff to operate them. The size of the "system" employed doesn't really matter; it can consist of a set of 5 1 to 500 1 capacity tanks, a floating cage raft of 30 m^3 or a series of ponds of 1 hectare. The essential point to remember is that there must be a clear distinction made between experiment and production.

The number of experimental enclosures is often a limiting factor when developing an experimental protocol, more so, as the results can vary greatly and it is not possible to test a given parameter in only one structure. It is absolutely necessary to carry out replications. The minimum number of replications necessary can be theoretically calculated in accordance with the variablility of the parameters measured, by the difference that we wish to emphasize and by the threshold chosen. In practice, a minimum of three replications is generally necessary for each experimental group with all the consequences that this entails, in numbers of animals, quantity of food, upkeep time.

Only when the experimentation itself has this as objective, the environment for the animals must be as similar as possible for all the groups : flow, quality, temperature, chemical composition of the water, dissolved oxygen, lighting, form, size, colour and position of the experimental enclosures, phonic environment and more so than ever this is necessary when the objective is axed on research.

So as to eliminate a certain number of badly controlled factors, inevitably linked with a given rearing structure, it is necessary to divide up, at random, the different experimental groups (and even better still, the different replicas) in the rearing unit (Diag. 1). This distribution can be done at random or by means of a table of numbers which are also picked at random. In this case, we resort to the most simple (from a statistic viewpoint) experimental system : this is a totally randomized system.

It is also highly advisable to regularly permute (when weighing especially) the experimental groups, but this implies having at disposal a certain number of free supplementary rearing enclosures (Diag. 2).

It is also possible when the variation of a factor is inexorable but defined (ex. Natural light gradient in the rearing room or tidal current in the floating cage raft) to use more or less complex statistic models, permitting to take into account these factors at implementation level of the experimental plan. Some of these plans (block systems) are highly recommended even when the gradient of the secondary factor has not been clearly defined.

Another point, which is often forgotten, is that of the experimental unit. From a statistic viewpoint, the different animals in an enclosure are rarely correct uncertain variables. On the contrary, the weight, length averages are much more reliable experimental units ; this remark reinforces the necessity of replicas.

4.2. The animals

They must be healthy and of the same origin; densities should be adapted to the rearing enclosures and defined in such a way so as to ensure good conditions for the animal throughout the experiment. It is necessary to schedule eventually the animals for sampling during the experiment. A histogramme of the population must be carried out; the distribution must be normal and as coordinate as possible so as to favour the apparition of significant differences between the groups and so as to minimize competition between the animals.

It must be remarked that the above recommendations are valid if research is the prime objective, (Chap. 2) and requires a homogenous population responding to the problem studied. On the contrary, in practice, there will be a tendency to employ a representative group of the population studied which will then be well defined in the protocol.

4.3. Experimental food

Experimental food differs from the classical food normally employed, by a certain number of criteria. It must be simple, in other words, it must contain many raw materials. To establish standard diets or to study food requirements, the raw materials must be obligatory known, of very good quality, and have a good intrinsic value (from year to year and during one year). This is one of the reasons why casein and gelatine are employed in purified food.

However important or insignificant the experiment scheduled may be, the manufacture of the food (whether 1 kg or 1 ton) will always be difficult to perform by means of the general routine convey or belt production system; therefore, it is necessary to have at disposal an apparatus adopted to the dimension of the experiment (mixers, meat mincers, press, dryer, crushing mill, sieve,…)

For a given experimentation, which lasts not more than 3 to 4 months, on general, it is advisable to schedule the manufacture of all the food at the same time and to ensure good processing and slocking throughout the whole period of experimentation. When the food is prepared daily, the same unique stock of raw materials shall be employed throughout the whole period.

A precaution very often neglected while very useful consists in the biochemical analysis (very summarily : humidity, total proteins and lipids, ashes) of the food before starting the experiment so as to verify that it is conform to the standards demanded. In all cases, the humidity rates should be regularly determined for the calculation of the ration distributed.

When manufacturing the food, especially if there is a great difference between the different diet formulations, a homogeneity in the food size along with its consistancy and stability must be respected, so as not to induce any errors in the experimentation. The use of diverse binders permits avoiding this type of artefact.

The distribution of the food is an important point to determine as the choice made (fixed ration or ad libitum feeding) has consequential effects on the experiment itself and on the interpretation of the results.

The distribution of fixed rations (for example 1 % of the biomass per day) is easy (food doses ready for manual or automatic distribution) but requires a regular follow up of the fish stock so as to adjust the quantity distributed according to the growth of the fish. This method of feeding will be employed especially when one wishes to distribute

isoproteic or isoenergetic rations... It implies on the contrary the regulation of the food ration which will be less consumed.

This risk causing certain groups being "underfed" and thus limits the differences in growth at the end of the experiment. Finally, it must be remarked that loss of appetite will cause slight differences in the quantity of food really ingested by the animals.

Ad libitum distribution will be adopted when the quantity of food ingested is the objective of the experimental result. This can however cause difficulties in the interpretation. The distribution while employing automatic devices (self feeders) limits human intervention. Manual distribution is more time demanding while permitting at the same time to give a better appraisement of the behaviour of the population.

4.4. Duration period of the experiment

This has been defined in the protocol, but different arrangements can be envisaged in accordance with the evolution of the population.

- For animals weighing less than one gram (wearing) 3 weeks to one month can be sufficient for the experiment: the stoppage in mortality is often chosen as a criterion to define the end of the trial.

- For animals weighing a few grams, 2 or 3 months must be scheduled for the experiment and 3 to 4 months for animals weighing more than one hundred grams. In these cases, it is the doubling or tripling in weight which is scheduled. In fact, if we have opted for a regular follow up of the stock, it is possible to stop the experiment as soon as significant differences of the factor in question have been established.

- In certain cases, longer duration periods are necessary, for example so as to observe the deficiency symptoms for which the multiplication by 10 of the initial weight is sometimes necessary.

5. BEGINNING, FOLLOW UP AND END OF THE EXPERIMENT

5.1. Notes on the rearing

It is absolutly necessary to keep daily notes on the rearing in a copy-book (and not on loose pages) where all the data may be clearly and precisely found, concerning the experiment from the general outline principles to the smallest details.

5.2. How to acquire experimental groups

They must be taken at random, from a graded stock of animals (§ 4.2), which are perfectly adapted to this environment. For salmonids for example, the acquirement of groups must be avoided when they are at sea. So as to obtain a good homogeneity in the different experimental groups, they must not be collected in one go, but little by little (Diag. 3). This operation, although it may seem simple, should be well prepared in advance and great care taken so as to avoid errors being made in the count.

The establishment of the initial weight of the animals (from day zero -beginning of the experiment) can be carried out as soon as the groups have been obtained while applying the method chosen, perhaps with a delay in time so as to avoid supplementary stress being caused to the animals.

In all cases, the animals should be weighed while fasting (24 or 48 hours) so that the true weight of animals may be correctly estimated.

- If the distribution is perfectly homogenous and if there is a sufficient number of animals available, it will be possible to schedule a supplementary group, which shall

receive the same treatment as the others and killed so as to define the initial average weight and the initial biochemical composition (for all the groups).

- It is also possible to weigh the different groups. This can be done by weighing individually the animals ; in this case, this operation also serves to verify the number of fish. Each group alone can also be weighed while counting them or not. Finally a sample can be taken from each group as long as a sufficient number of animals have been scheduled at the beginning. The samples are killed, weighed and kept for analysis.

As in all rearings, solutions causing the less stress possible are researched.

5.3. Upkeep of the stock group

So as to avoid all parasite parameters which have an influence on the experiment, the upkeep of the animals will be greatly taken into account (Cleanliness of the rearing tanks, food distribution, collection and deduction of dead animals).

Throughout the diverse manipulations, it should be advisable to schedule a light anesthetic for the animals so as to avoid causing repeated and great stress.

- 1 mg/l of quinaldine (1 ppm)
- 0.2 ml/l of phenoxyethanol (200 ppm) Ethyleneglycol monophenylic ether.

These doses are given only as indications and should be adapted according to the species, its age, and the anesthetic degree desired.

It is also advisable during each intervention, to carry out antiseptic treatments:

- Furazolidone : a 20 mn bath in a solution of 20 ppm.
- Quaternary ammonium : a 20 mn bath in Ca solution of 1 ppm of the active principle (which is 5 mg/l of CETAVLON at 20 %, for example). The doses indicated must be followed for these products.

5.4. Regular weighing

This is of assistance so as to follow the evolution of the different groups and necessary if the food distribution method employed is fixed rations. It must also be remarked that the attainment of a growth curve permits a better estimation of the initial and final weight.

Live animals can be weighed dry (or by means of a damp cloth or in a container containing water. As for all manipulations, the animals should receive a light anesthetic. Taking into account the local conditions, the solution causing the less stress will be chosen.

As has been stated in § 5.2, the animals may be weighed individually, the whole group together or a sample taken from each group and weighed, associated or not with the deduction of the animal. It must be remarked that the replacement of the samples back into the container must be avoided as this does not comply with the conditions imposed by the statistic calculations.

The weighing of a doomed sample presents many advantages, on the contrary

- Authentification of the statistic treatment
- Study of the homogeneity of the population
- Limited stress for the animals kept in the rearing

- Possibility of biochemical analysis.

It is difficult to define outside a given context, the dimension of the sample. This can only be defined through experience and by means of an appropriate statistical investigation ; Let us however remark that samples, counting around 30 animals at a time, is a good basis to begin with. Evidently, a sample must be taken correctly, which is not always an easy task and may call for a test being performed beforehand. It is necessary to hem in the population into one corner of the tank, pond or cage before taking the sample.

The precision of weighing itself must neither be taken too seriously nor too carelessly but a just medium must be found. Even with the use of a mg electronic scales, it is not reasonable to employ such precision for an aquatic animal (even dry) which weighs no more than a fews tens of gm. Even a 50 g precision balance must not be employed to weigh one kilo only.

When the weighing is carried out on land, the research scientist can choose his own method of precision. When the weighing is carried out at sea (boat, raft, floating cage) a problem will arise whatever the precision method chosen may be, due to the movement caused by the water. If the doomed sample solution (weighed on land) can not be adopted, a method employing several distinct samples must be utilized.

5.5. Sample taking of animals for analysis

"Weight" alone can not allow the judgement of the real effect of the treatments. A ponderal increase has indeed a completely different signification depending on whether it corresponds to an increase in tissue proteins or in fat deposit. Thus, in every experiment, it is necessary to take an initial and final sample so as to carry out the biochemical and eventually the clinical and histological analysises which will allow a better comparison of the different groups. The intermediary samples (weighing of doomed samples § 5.4), can be used so as to follow up the state of health of the animals during the period of the experiment.

As for the samples taken for the biochemical analysis, the best compromise to adopt between :

- the necessity of having to carry out a number of individual analysises so as to compensate for the heterogeneity of the composition of the animals,
- and the difficulty in carrying out numerous analysises (time, price, personnel), seems to initiate the division, into experimental groups of four pools of around 10 animals with an average group weight. The animals in each pool will be minced and homogenized. Only one aliquot pert will be conserved per analysis (deep freezing, lyophilization).

Before the sample is taken, the animals are not fed for 24 hours, or even 48 hours beforehand, so that the digestive content will have no effect on the analysis scheduled. However long the animals are kept fasting, it is essential that this period be the same for all the samples taken in the same experiment.

Let us point out that depending on the analysis method chosen a few tens of g (live weight) will suffice to carry out the obligatory elementary analysis : rates of humidity, proteins, lipids and ashes.

6. RESULTS

They will be treated in accordance with the demand :

- mean weight gain

– Final average weight (FAW) - Initial average weight (IAW).

The fact of using the average weight instead of the total weight allows the use of the notion "gain" even when there exists a certain mortality, provided however that there is a feeble rate.

Multiplier coefficient :

$$\frac{FAW}{IAW}$$

Not employed much by scientists, the multiplier coefficient is sometimes employed by aquaculturists :

* Relative growth

$$\frac{FAW - IAW}{IAW} \times 100$$

This parameter is hardly ever employed

* Specific growth rate (SGR) or daily increase percentage

$$\frac{LN\ FAW\ - LN\ IAW}{\text{Duration period of experiment}} \times 100$$

Or $$10\left| \left(\frac{FAW}{IAW}\right) \frac{1}{duration_{-1}} \right|$$

SGR is greatly employed. It permits the comparison between the results of the different experiments not having the same time lengths as one another. This in fact is slope B of the exponential aquation which characterizes the growth of the animals

$Y = ae^{Bx}$

where Y is the weight of the animals at time (X)

and a the initial weight.

6.2. Condition factor K - Weighing and measuring of the animals.

$$\frac{P}{L^3}$$

where P is expressed in g and L in cm.

It will be necessary to define if L corresponds to the total fork or standard lenght. This parameter permits to somehow visualize the state of "good health" of the animals. It is often linked with the fattening state of the animals.

6.3. Estimation of the quantity of food ingested

* Conversion rate CT (or sometimes known as feed conversion)

$$\frac{\text{Quantity of food (dry matter) ingested}}{\text{Weight gain (wet matter)}}$$

The conversion rate is largely employed ; it permits having a global idea of how the food is used. one mustn't be alarmed to obtain for certain species values of less than 1, this can appear aberrant at first glance for heterotrophic species. it is due to the fact

that the numerator is counted in dry matter weight, while the denominator is counted in wet or raw material weight.

Feed efficiency

$$\frac{\text{Weight gain (wet matter)}}{\text{Quantity of food (dry matter) ingested}}$$

This parameter is less employed than the preceeding one.

6.4. Dissection of the animals and weighing of the different organs

Hepatosomatic index RHS

$$\frac{\text{Weight of the liver (wet matter)}}{\text{Total weight of the animal (wet matter)}} \times 100$$

This index is often employed (easily worked out) to estimate the good state of the liver which is one of the keys organs for the metabolism of food.

Percentage of perivisceral fats

$$\frac{\text{Perivisceral fats (wet matter)}}{\text{Total weight of the animal (wet matter)}} \times 100$$

This will enable to define the state of the animals from a fattening viewpoint but it is often difficult to establish, as depending on the species, perivisceral fat is often more or less attached to the digestive tract:

* Carcass yield

$$\frac{\text{Total weight - Visceral weight}}{\text{Total weight}} \times 100$$

Filleting yield

$$\frac{\text{Filleting weight}}{\text{Total weight}} \times 100$$

The two latter practical notions permit the economic estimates depending on how the product is commercialized.

6.5. Protein composition of the food

Protein efficiency ration (PER)

$$\frac{\text{Weight gain (wet matter)}}{\text{Proteins ingested (dry matter)}}$$

This permits a global estimation of the transformation of food proteins.

6.6. Biochemical composition of the animals (whole) at the beginning and end of experiment

This concerns pools of animals (§ 5.5)

* Protein utilization coefficient (PUC)

$$\frac{\text{Fixed proteins}}{\text{ingested Proteins}} \times 100$$

with

Fixed proteins	% animal nitrogen whole at the end of the experiment	1 x 6.25 x FAW	- % animal nitrogen whole at the beginning of the experiement	x 6.25 x IAW

This allows the precise appreciation of the transformation efficiency of food proteins.

* Total lipids

The use of total lipids allows an objective global appreciation of the fattening state of the animals and its evolution throughout the experiment.

6.7. Biochemical analysis of the different organs

- Hepatic lipids
- Carcass lipids
- Muscular lipids
- Visceral lipids
- Hepatic glycogen

All this data will permit a better comprehension of the phenomena remarked.

6.8. Blood tests

- Hematocrit : gives a global idea of the eventual state of anemia of the animals.
- Circulating blood sugar
- Circulating free amino-acids

6.9. Histology of the different organs : liver - digestive tract

This permits the appreciation of the impact at cell level of the different treatments.

6.10 Digestibility measures by incorporating a trace element in the food and collecting the faeces

* Apparent digestibility coefficient

$$100 - \left(100 \times \frac{\%\text{ of the trace element in the food}}{\%\text{ of trace element in the faeces}} \times \frac{\%\text{ of nutriment in the faeces}}{\%\text{ of nutriment in the food}}\right)$$

This enables to define the digestibility of different nutriments.

7. DATA PROCESSING

When the data has be obtained in the appropriate way (correct sampling : § 5.4) and sufficiently repeated), it is necessary to do an adequate statistic processing of this. By means of a varience analysis, it will be possible to verify if the results obtained differ in a significant way on the whole. If this is so, a test of multiple comparisons must be carried out and this will permit the comparison of the different results with one another. The Newman Keuls test is particularly suited to problems which are frequently encountered in nutrition. A example of calculation is given in the chapter on "Statistic exploitation from experimental plan to a factor studied with repetitions.

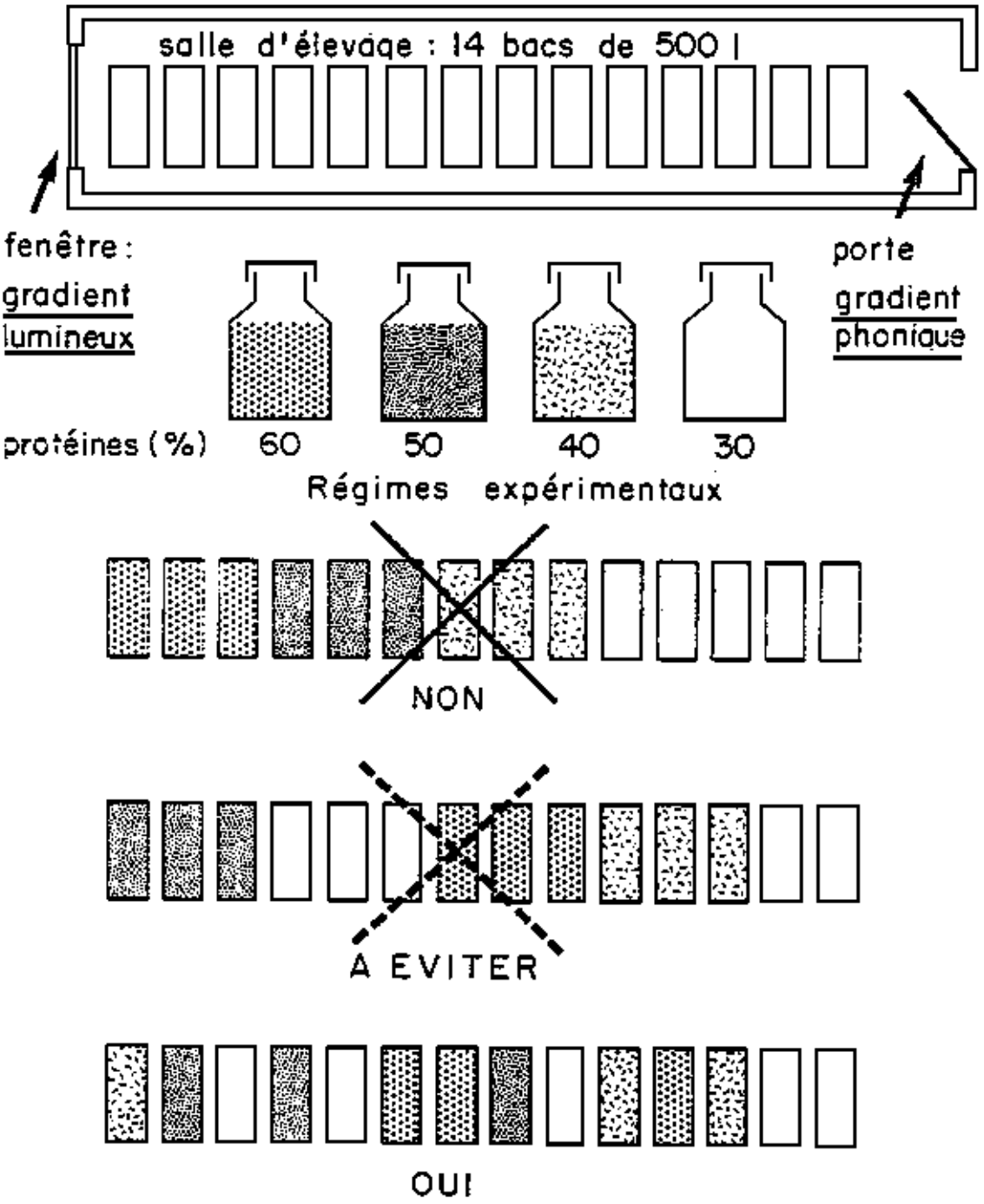


Figure 1 - Répartition des lots expérimentaux

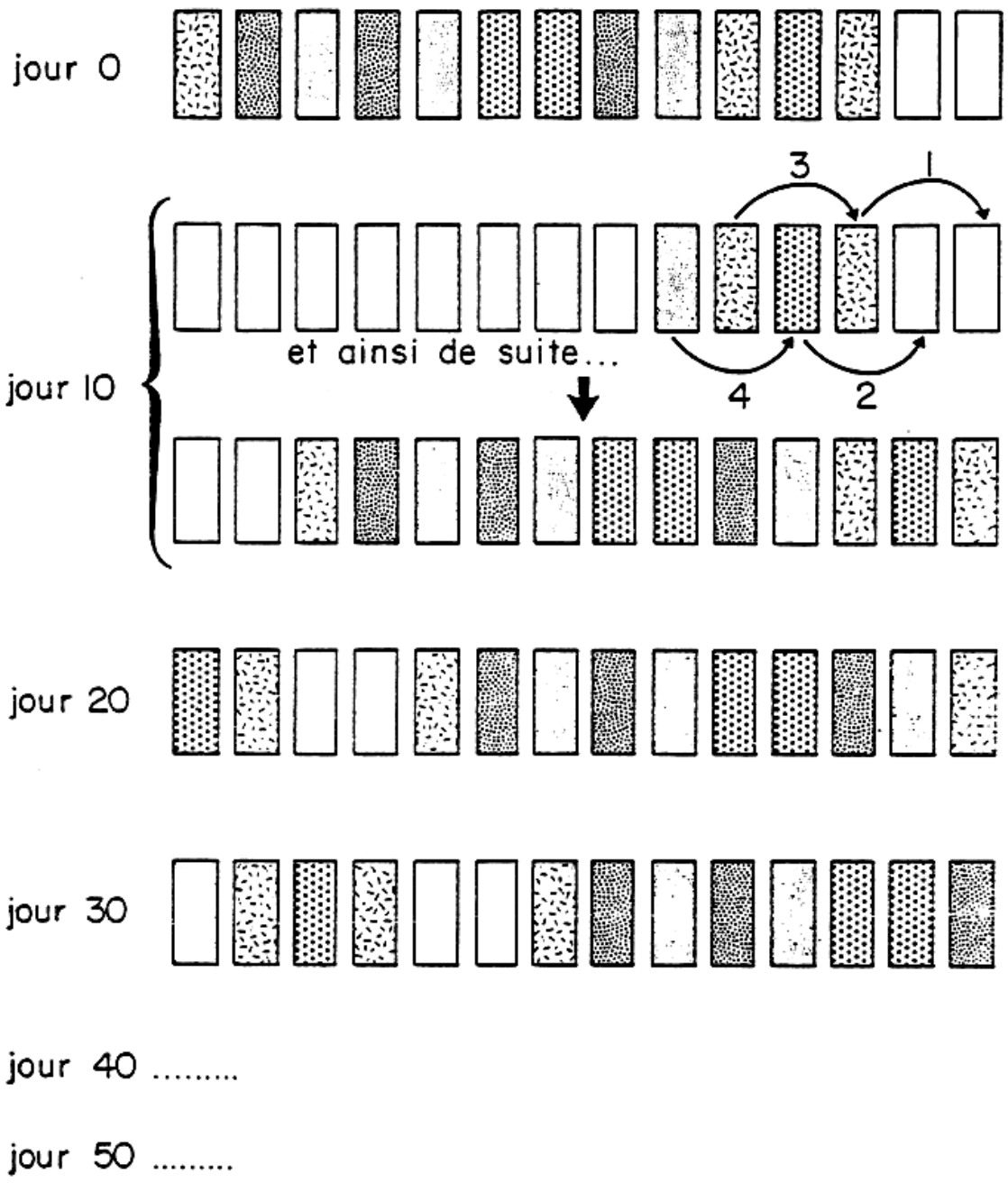


Figure 2 - Permutation régulière des lots expérimentaux

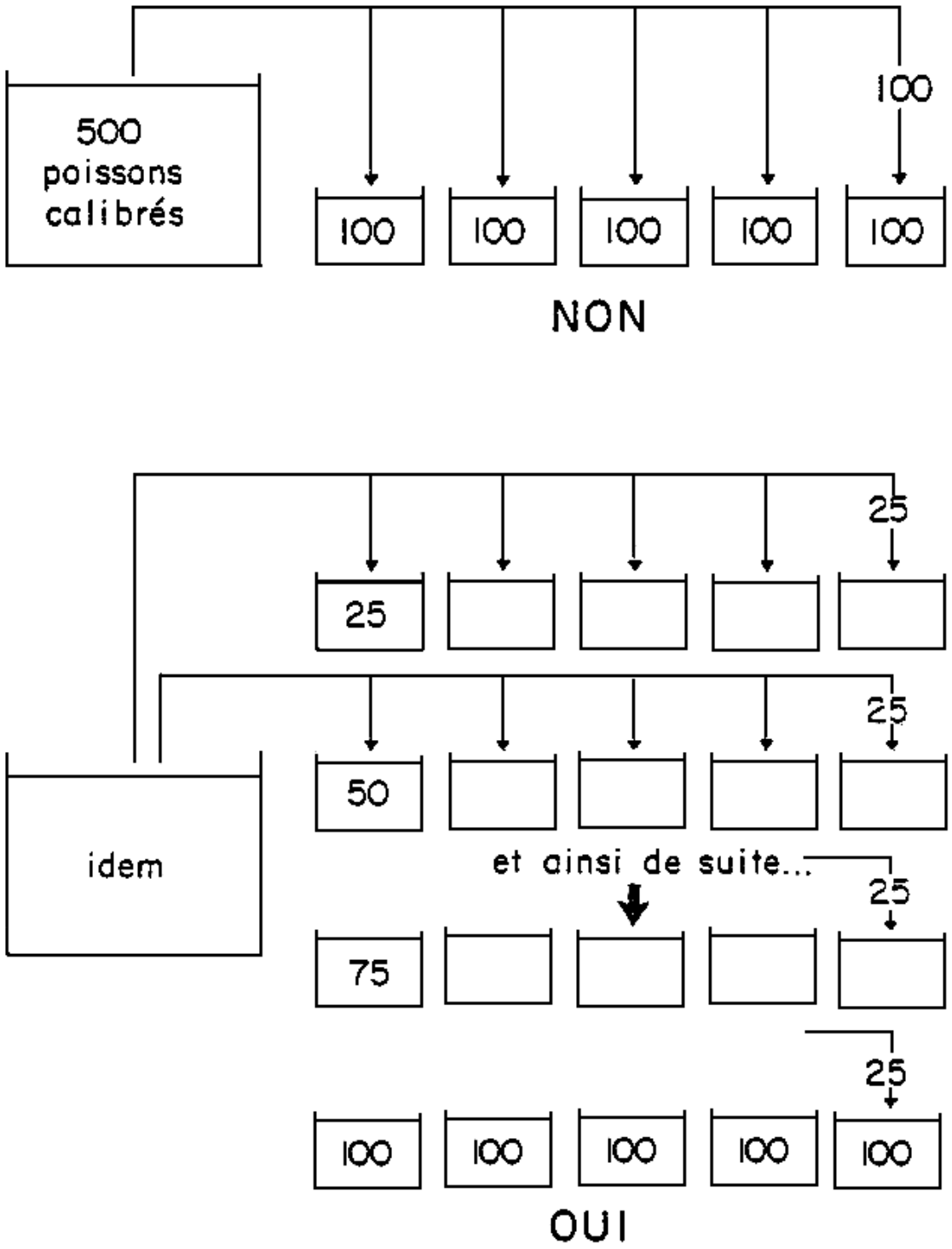


Figure 3 - Constitution des lots expérimentaux

REGIMES INGREDIENTS	A	B	C	D	E
Caséine	38	18	18	18	18
Dextrine	28	28	28	28	28
Gélatine	12	12	12	12	12
Huile de foie de morue	9	9	9	9	9
Cellulose	8	8	8	8	8
Prémélange minéral	4	4	4	4	4
Prémélange vitaminique	1	1	1	1	1
Farine de poisson	-	20	-	-	-
Levure de bière	-	-	20	-	-
Tourteau de soja	-	-	-	20	-
Blé	-	-	-	-	20
Protéines	43	41	36	36	29
Lipides	10	11	9	10	10

Tableau 1a : Testage de différentes matières premières. Composition (%) des régimes expérimentaux.

Substitution de 20 % de diverses matières premières à une quantité équivalente de caséine. De ce fait les compositions en protéines, lipides, . . . sont différentes pour chaque aliment. Les différences éventuelles que l'on peut mettre en évidence en sont pas interprétables au plan nutritionnel.

REGIMES INGREDIENTS	A	B	C	D	E
Caséine	38	21	27	26,6	35
Dextrine	28	28	24	24,4	19,4
Gélatine	12	12	12	12	12
Huile de foie de morue	9	7,3	9	9	8,6
Cellulose	8	6,7	3	8	-
Prémélange minéral	4	4	4	4	4
Prémélange vitaminique	1	1	1	1	1
Farine de poisson	-	20	-	-	-
Levure de bière	-	-	20	-	-
Tourteau de soja	-	-	-	20	-
Blé	-	-	-	-	20
Protéines	43	43	43	43	43
Lipides	10	10	10	10	10

Tableau 1b : Testage de différentes matières premières. Composition (%) des régimes expérimentaux isoprotéiques et isolipidiques).

Dans ce cas, il est tenu compte de la composition en protéines, lipides, ... des diverses matières premières lors de la composition des aliments. La substitution se fait au niveau protéique, lipidique, glucidique, ... ; elle fait donc intervenir la caséine, l'huile de foie de morue, la dextrine et la cellulose (qui sert de ballast).

Ainsi, c'est bien l'effet "matières premières" qui est étudié; cependant chaque matière première n'étant testée qu'à un seul taux d'incorporation, il est nécessaire d'être prudent dans l'interprétation et la généralisation des resultants.

REGIMES INGREDIENTS	C0	C5	C10	C15	C20
Levure de bière	-	5	10	15	20
Caséine	38	35,25	32,5	29,75	27
Dextrine	28	27	26	25	24
Gélatine	12	12	12	12	12
Huile de foie de morue	9	9	9	9	9
Cellulose	8	6,75	5,5	4,25	3
Prémélange minéral	4	4	4	4	4
Prémélange vitaminique	1	1	1	1	1
Protéines	43	43	43	43	43
Lipides	10	10	10	10	10

Tableau 1c : Testage d'une matière première (levure de bière : gradient d'incorporation). Composition (%) des régimes expérimentaux.

Chaque matière première est testée indépendamment. Il est possible ainsi d'étudier son efficacité réelle et son taux optimal d'incorporation pour la formule de base considérée.

REGIMES INGREDIENTS	A	B	C	D
Farine de poisson	50	61,1	72,2	83,3
Amidon cuit	2	2	2	2
Cellulose	36	26	16	6
Huile de poisson	7	5,9	4,8	3,7
Prémélange minéral	1	1	1	1
Prémélange vitaminique	1	1	1	1
Liant	3	3	3	3
Protéines	36	44	52	60
Lipides	12	12	12	12

Tableau 2a : Besoin protéique du bar. Composition (%) des regimes expérimentaux : gradient de farine de poisson centre gradient inverse de cellulose.

L'expérience envisagée est tout à fait valable ; cependant les régimes ainsi composés sont peu réalistes au plan pratique et même théorique (taux de cellulose excessif).

REGIMES	A	B	C	D
INGREDIENTS				
Farine de poisson	50	61,1	72,2	83,3
Amidon cuit	26	18	10	2
Cellulose	12	10	8	6
Huile de poisson	7	5,9	4,8	3,7
Prémélange minéral	1	1	1	1
Prémélange vitaminique	1	1	1	1
Liant	3	3	3	3
Protéines	36	44	52	60
Lipides	12	12	12	12

Tableau 2b : Besoin protéique du bar. Composition (%) des régimes expérimentaux : gradient de protéines contre gradient d'amidon.

Les régimes envisagés ici sont plus proches des régimes pratiques mois il n'est pas tenu compte de leur énergie, ce qui entraîne un bicis dans l'expérimentation.

REGIMES	A	B	C	D
INGREDIENTS				
Farine de poisson	50	61,1	72,2	83,3
Amidon cuit	37,7	25,8	13,9	2,0
Cellulose	0,3	2,2	4,1	6,0
Huile de poisson	7	5,9	4,8	3,7
Prémélange minéral	1	1	1	1
Prémélange vitaminique	1	1	1	1
Liant	3	3	3	3
Protéines	36	44	52	60
Lipides	12	12	12	12
Energie digestible (Kcal/100g régime)	388	388	388	388

Tableau 2c : Besoin protéique du bar. Composition (%) des régimes expérimentaux : protéines contre amidon, régimes isoénergétigues
(énergie digestible des protéines : 4,76 Kcal/g ;
de l'amidon : 3,2 Kcal/g ;
des lipides : 8 Kcal/g).

L'objection précédente (cf tableau 2b) est supprimée; cependant à chaque taux de protéines ne correspond qu'un taux de glucides : it faut donc être très prudent lors de l'interprétation des résultats.

REGIMES	B1	B2	B3	C2	C3	D3
INGREDIENTS						
Farine de poisson	61,1	61,1	61,1	72,2	72,2	83,3
Amidon cuit	2	13,9	25,8	2	13,9	2
Cellulose	26	14,1	2,2	16	4,1	6
Huile de poisson	5,9	5,9	5,9	4,8	4,8	3,7
Prémélange minéral	1	1	1	1	1	1
Prémélange vitaminique	1	1	1	1	1	1
Liant	3	3	3	3	3	3
Protéines	44	44	36	52	52	60
Lipides	12	12	12	12	12	12
Energie digestible (Kcal/100g régime)	312	350	388	350	388	388

Tableau 2d : Besoin protéique et glucidique du bar. Composition (%) des régimes expérimentaux : gradient de protéines et d'amidon.

Pour chaque taux de protéines, plusieurs taux de glucides sont testés. Les régimes indexés des mêmes chiffres sont isoénergétiques. Par souci de clarté, seuls les régimes B, C et D du tableau précédent sont envisagés.

FEEDING IN MARINE AQUACULTURE BY MEREA*

C. de la POMELIE

*** MEREA : Mediterranean Research Team in Aquaculture**

Man has been concerned by aquaculture for thousands of years, but often these traditional rearings feed at the expense of the natural production of the environment. At present, man knows how to master the biological cycle of numerous animal species in rearing while employing intensive methods. He must feed them.

Whatever the species may be -sea-bass, gilthead sea-bream, shrimp ... whatever the stage of the biological cycle, the aquaculturist's main objective is to obtain the best profit performance from the biomass in rearing, in other words, the maximum individual growth of the animals with a minimum mortality rate. For this, food plays a primary role, concerning both the quality and the quantities distributed, and the feeding methods employed are fundamental.

Aquaculturists are often classed as hatchery or fattening specialists. Indeed, the specialities and requirements of the hatchery -broodstock, larvae juveniles which must be weaned and those in nurseries or fattening are quite different; especially concerning feeding.

1. HATCHERY PHASE

1.1. Larval rearing

One of the special characters of this first phase is the use of live organisms, algae, rotifers and especially Artemia to feed many of the species in rearing.

- Larvae are very active and do not support fasting. Therefore, a sufficient density of algae or prey must be ensured so that they do not become exhausted in their search for food, especially at the beginning stage.

- On the contrary, prey are live organisms which are not only expensive to produce, but they also modify the quality of the rearing environment by the excretion of ammonia and the consumption of oxygen.

It is in keeping in mind these three fundamental points that the research team at IFREMER and especially those at the PALAVAS station have succeded, after ten years of scientific research, in perfecting the feeding sequences which are presented here in the form of figures,

- Figure 1 : Feeding sequence of P. japonicus (shrimp) in larval rearing : The algae distributed, Chaetoceros calcitrans or Phaeodactylum tricornitum are employed to feed zoea stage of course but also so as to avoid either a slump in the nutritional quality of the Artemia nauplii distributed and or abrupt peaks in ammonia.

- Table 1 : Feeding sequence of D. Labrax (sea-bass), at larval rearing stage.

In larval rearing, a great part of the production cost of the animals is caused by feeding, as the algae, rotifers, artemia nauplii or metanauplii are very expensive to produce (Table 2 and 6). Let us underline in particular that for sea-bass larval rearing (Table 7), feeding represents 96 % of the production cost, not counting manual labour for the 45 day old larvae and that for Japanese shrimp (Table 8). This is represented by 49 % of the P 3.

From these tables (7 and 8), it is evident that the most significant economy concerning the production costs are expected from feeding.

Important remark : All the economic approaches stated in this document have be calculated on unitary prices (m^3 of water, m^3 of pressurized air, etc...) with regards to the facility conditions of the PALAVAS IFREMER station.

1.2. Weaning and nursery

- Weaning or the change over from live feed to inert feed causes a great upset to juveniles in their feeding habits and also in their behaviour.

- The impact that feeding has during this phase of rearing is still important so as to estimate the production cost of the consumable animal. Along with this, feeding will also have as objective the safeguard of the animals who have begun weaning as these have now already reached a considerable production cost.

- As for the Japanese shrimp, the feeding sequence employed (Fig. 2) permits to estimate the importance of feeding in the post-larval rearing phase (Table 9).

- Concerning sea-bass, recent research on the weaning carried out in our station, but which has not yet been edited, has given rise to a new feeding sequence, which improves greatly the production cost of fry. Figure 3 describes the feeding sequence applied up to a recent date.

1.3. Brood-stock

- The objective to reach, and the nutritional requirements of the brood-stock are now fully understood. Thus, a great part of the compound food is generally replaced by fresh food.

If for P. vannamei research has proven the influence of the distribution of pellets on the quantity of eggs spawned, the feed for P. japonicus brood-stock is based essentially on fresh mussels and the animals are fed ad libitum, which is around 5 % fresh weight per day of the body weight. For the sea-bass, table 10 shows that the ration given is 1.5 % dry weight per day, of the body weight. 25 % (dry weight) of fresh food seems sufficient so as to obtain satisfactory spawnings.

- Feeding has but a feeble influence on the production cost of brood-stock as is shown in Table 9 (P. japonicus). It is thus illusive to try and make an important productivity benefit from feeding at this phase of rearing. On the contrary, it is advisable to supply a very diversified fresh food of good quality.

2. FATTENING PHASE

Two types of rearings requiring an exogenous feeding entails the adoption of two different policy methods of feeding :

Semi-intensive rearing when the animals mostly obtain their food from the environment. Exogenous feeding is then only a complement of this natural food of high nutritional quality.

On the contrary, in intensive rearing, the food obtained from the environment is more often very scarce. Exogenous food must then cover all the requirements of the animals both in quality and quantity.

- The quality of the food distributed is fundamental and its misappreciation can cause real catastrophies in rearings. This quality can be judged from two points :

* Nutritional : cf other reports of the session.

* Organoleptic

The fineness of the grinding has a great influence on the transformation of the pellet.

The hardness of the pellet : When too hard it is badly accepted.

Buoyancy : Shrimp and sea-bass do not ingest food in suspension.

Powder or dust forms are not ingested.

The granulometry, or diameter of the pellet must be adapted to the size of the animal.

The diameter and the length of the pellet must not be too important for the sea-bass as it will refuse to fed $(\frac{\text{length}}{\text{diameter}} \quad 2)$

The stability of the pellet in water is very important for shrimp.

- The compound food can be conserved 3 to 4 months from the date of manufacture which will be furnished on the label and that of the following conditions are respected.

* Temperature: The speed of the chemical reactions, degradation, (Maillard reaction, peroxydation of fats) depends on the temperature. A well aered room will avoid important increases of temperature.

* The relative humidity of the air : certain vitamins are destroyed, mildew can develop if this value becomes important (70 %). The storage place must be dry.

* The food delivered in bulk must also be stored in a place which has shelter from the light.

It must be underlined that a badly stored food will not only lack vitamins (vitamin C) but will be especially toxic (peroxyde fats, mildew) and cause irrevocable lesions (kidneys, liver), to the sea-bass.

- Three ratio will permit the definition for feeding in rearing.

* Food rate = Tn = $100\,\frac{QN}{BM}$

QN = quality of food distributed per day to a define of biomass up to present (BM)

* Food conversion rate : $TCA = \frac{TN}{TC}$

where TC represents the growth rate.

This parameter which does not integrate mortality has a more scientific than economic value and is taken more so as the instantaneous value. It is especially used to verify the justification of the food rate.

* Conversion index : $Ic = \frac{QA}{BMf - BMi}$

This index is considered either at technical and economic level or at scientific level. In this case, QA represents the quantity of food brought for this rearing or the rearing period and BMf the biomass truely commercialized for example (Waste).

- The evolution of the TCA depending on the food rate (figure 4) shows clearly that this is kept at a minimum which also corresponds, at economic level, to a minimum of the production cost. This point is theoretical and depends on several parameters (average weight of the animals, their origin, small sized large sized fish, temperature,

salinity, etc...). The aquaculturist whose objective is to reach the maximum growth will try to attain this food rate by giving excess.

- This is how the fishfeed manufacturers define the average values of the feed rate in their technical documents. Table II shows our recommendations for sea-bass rearing in normal French Mediterranean conditions.

3. FOOD DISTRIBUTION METHODS

A good distribution of food tends to minimize the conversion index of the food. This distribution takes into account the behaviour of the animals : For semi-intensive rearings of shrimp, the food is distributed once per day, in the evening before sunset.

- The frequency of meals and their repartition during the day, this for the sea-bass depends on its weight in knowing that the more the distributions are divided out, the better the conversion and growth index will be. On the contrary, while taking into account the behaviour of P. japonicus, the rearings will only receive one distribution per day before sunset.

- The geographical repartition of the distribution in the rearing enclosure : Thus for shrimp, the pellets are distributed over the maximum surface area of the pond, and for sea-bass fattening reared in cages the pellets are only distributed in the center of the cage.

The methods employed: manual or automatic distribution.

3.1. Manual distribution

- Advantages
  - Little or no investment
  - Surveyence of the animal behaviour in rearing
  - With fish one can see the animals eating, this permits ad libitum feeding without waste.
- Disadvantages
  - Expensive in manual labour
  - Very demanding for the organization of the manual labour planning.
  - A quasi Utopian dividing up of the ration in numerous feeds.
  - Preferential hours for feeding which are not compatible with the normal working hours.

Technologically, it is difficult to avoid in certain cases : fresh food, pelleted for the semi-intensive rearing of shrimp.

3.2. Automatic distribution

- Advantages
  - Distribution of the pellets 7 days a week whatever the manual labour planning may be.
  - Economy from a manual labour viewpoint
  - Ad libitum dividing up of the feeds even self-feeding by the animals when a self-feeder is employed.

- Disadvantages

  - Supplementary investment
  - Upkeep of the facilities
  - Risk of over or under feeding if the rations pre-established have been calculated incorrectly.

Different devices can be seen in figures 5,6, 7 and 8.

Important remark

Automatic distribution can be accompanied by a reduced manual distribution once per day. The behaviour of the fish towards the food (indifference or heavy consumption), permits the avoidance of a notory over or under feeding.

Certainly, the present food is likely to be improved. From a nutritional viewpoint, through research, it is being perfected year after year permitting (this is obligatory) important productivity gains.

But the performances of the rearing -growth, survival, conversion index- and thus the profitability of the aquaculture Enterprise will always be compromised, by bad technology in manufacture, by bad storing and by bad distribution of the food no matter how perfected the formulation may be. Field work carried out at the IFREMER Station of PALAVAS shows that many of the "diseases" encountered by aquacultists are due to a bad management of the feeding.

Table on the consumption of prey, air and water	Tank **n° 2,3,4** Rearing period	:17/04/29-06		Special Sea-bass D. labrax		
	Rotifers	Nauplii	Métanauplii A1	Métanauplii A2	Water	Air
Distribution period	11 - 14	11 - 21	21 - 26	22 - 45	0 - 45	0 - 45
Duration in days	4	11	16	24	45	45
Peak day	12	20	21	45	45	45
Total quantity/JO	125	355	325	7184	5,5	0,5
Total quantity/JN	195	540	492	10942	8,4	0,8

TABLE 1 : LARVAL REARING OF SEA BASS : Feeding example

TABLE 2 : ALGAE - ECONOMIC APPROACH

	Unit Cost 100 1 FHT	Without manual labour %	With manual Labour %
Energy	30	60.6	23.5
Salt	7	14.1	5.5
Fresh water	0.5	1.0	0.4
Air + CO_2	7	14.1	5.5
Material	5	10.1	4.0
Manual labour	78	-	61.0
Total without manual labour	49.5	100	-
Total including manual labour	127.5	-	100

Production cost of 100 1 of algae

TABLE 3 : ROTIFERS : ECONOMIC APPROACH

Cost of 1.10^6 rotifers

NATURE	QUANTITY	U.P.Tax free	TOTAL PRICE Tax free	PRICE OF 1 $\bar{m}$ ROTIFERS Tax free	%
Food					
Yeast	75 kg	5.97 F/kg	447.75	0.0526	1.40 %
Algae	77001	1.27 F/1002	9779.00	1.15	26.30
Electricity					
Light	1036,8 kw	0.162	168.00	0.079	0.43
Air conditioning	58104,0 kw	0.162	9472.90	1.107	25.40
Brine (30 mn/week)	2.394	0.162	0.40	0.00004	0.009
Aeration					
1) Tank (x2)	20736	0.003	14	0.008	0.18
2) Brine (100 1/35 days	5040	0.003	15.12	0.001	0.02
Water					
Fresh	120 m3	4.3 F/m3	516.00	0.06	0.90
Sea water	25 m3	0.0243 F.	0.6	0.0007	0.076
Manual labour	290 H	49.00 F	14210.00	1.67	38.30 %
Salt	2400 kg	1.077 F/kg	2440.8	0.28	6.40 %
		TOTAL	37064.50 F	4.36	

Total cost for 10^6 rotifers : 4.36 F Tax free

TABLE 4 : ARTEMIA - ECONOMIC APPROACH (Cost Ao)

	Unit cost **Cheptel = 10^8 A_o FHT**	Without manual labour %	With manual labour %
Pumping	-	-	-
Heating	15	4.6	4.0
Air	5	1.5	1.3
Treatment	15	4.6	4.0
Cysts	290	89	76.4
Manual labour	55		14.5
TOTAL without manual labour	325	100	-
TOTAL including manual labour	380	-	100

PRODUCTION COST OF 10^8 Ao

TABLE 5 : ARTEMIA : ECONOMIC APPROACH (Cost A1)

NATURE	QUANTITY	U.P. Tax free	TOTAL PRICE Tax free	PRICE OF 1 $\overline{m}$ Tax free	PRICE OF 100 $\overline{m}$ Tax free	%
Number of Ao in fattening	19,01$\overline{m}$	372 F/100$\overline{m}$	70717.2	3.72	372	72.80
Warm sea Water	350 m3	14.85kw/h	1055.60	0.055	5.55	1.086
Waste water	60 m3	14,85	180	0.014	1.44	0.28
Lighting (2 x 60 x 2)	1036,8	0.162	167.9	0.008	0.88	0.772
6 months Air	10400 m3	0.003	933.12	0.049	4.90	0.95
Feeding Ao_9 bis	104.08 kg	149,70	15580.7	0.81	81.96	16.00
Manual labour	175 h	49.00	8375	0.45	45.10	8.80
TOTAL			97209.50	5.10	511	100.00 %

Production cost of 1 $\overline{m}$ of Ai : 5,11 F

TABLE 6 : ARTEMIA - ECONOMIC APPROACH (Cost A 2)

NATURE	QUANTITY	U.P. Tax Free	TOTAL PRICE Tax free	PRICE OF 1 $\overline{m}$ Tax free	PRICE OF 100 $\overline{m}$ Tax free	%
Number of Ao in fattening	12.52 $\overline{m}$	372F/100$\overline{m}$	46574.40	3.72	372	58.60
Warm sea water	570 m3	14,85kw/h	1719.1	0.137	13.73	2.16
Waste water	60 m3	14.85kw/h	181.0	0.014	1.44	0.226
Lighting	1728 kw	0.162	279.9	0.02	2.22	0.349
Air	29600 m3	0.003	388.2	0.031	3.10	0.48
Feeding Ao_9 Bis	150.22 kg	147.70	22487.90	1.79	179.6	28.2
Manual labour	160 H	49.00	7840.00	0.62	62.61	9.8
		TOTAL	79469.50	6.3	634.10	100.00

Thus production cost of 10^6A2 : **6.34 F.**

PRODUCTION COST

	PER UNIT of 2 m^3	For 100.000 45 day old		
	Cost FF	Cost FF	%	%
Manual Labour	**1850**	**2056**		**18**
Larvae	**122**	**136**	**1**	**1**
Prey treatment	**8203**	**9114**	**96**	**79**
Fluids	**266**	**296**	**3**	**2**
Total without manual labour	**8591**	**9546**	**100**	
General total	**10441**	**11602**		**100**

TABLE 7 : Estimate elements of the production cost of sea-bass larvae of 45 days.

Economic approach : Larval rearing of P. japonicus

	Cost of the rearing unit	Production cost of the population 1 000 P3		
	FHT	FHT	%	%
Animals	1 200	2.2	43	36
Heating	200	0.4	7.8	6.5
Pumping	2	-	-	-
Air	8	-	-	-
Food	1 400	2.5	49	41
Manual labour	600	1.	-	16.4
Total without manual labour	2 810	5.1	100	-
TOTAL including manual labour	3 410	6.1	-	100

TABLE 8 : PRODUCTION COST OF 1 000 P 3

The most significant profits on the production cost are expected from feeding by the replacement of Artemia nauplii and in part algae by microparticles.

	Breeders	Nauplii	P 3 : Larval rearing	P 23 : Post Larval rearing
Animals	11%	75%	36%	26,5%
Heating	27,5%	10%	6,5%	11,8%
Pumping	2,7%	0,6%	/	1,2%
Air	0,3%	/	/	0,6%
Food	5,1%	/	41	56,5%
Manual Labour	53,2%	15.5%	16,4%	3,5%
Unit Price Tax free	120,9 F/ 0	1,62/1000 N	9/1000 P3	48/1000 P23

TABLE 9 : ANALYSIS OF THE PRODUCTION COSTS

TABLE 10 : The relation between the productivity of sea-bream and sea-bass broodstocks and their daily average ration along with the proportion of fresh feeds in comparison with the total ration.

SPECIES	SEASON	TANK	PROGRAMMING of SPAWNING	DAILY AVERAGE RATION IN DRY WEIGHT (%)	PROPORTION OF FRESH FOOD/TOTAL RATION IN DRY WEIGHT (%)	PRODUCTIVITY NUMBER OF EGGS/KG OF FEMALE
SEA-BREAM	1984-84	G1	Staggered	2.9	45	362.000
	1984-85	G2	Staggered	2.7	44	1.000.000
SEA-BASS	1984-85	G4	Staggered	1.2	35	194.000
		G7		1.5	36	128.000
		G8	Advanced	1.7	31	93.000
		G9	Normal	1.3	37	206.000
		G10	Normal	1.2	39	281.000
		G3	Staggered Delayed	1.2	31	154.000
	1985-86	G4	Staggered	1.8	18	292.000
		G7	Advanced	1.6	25	117.000
		G8		1.4	27	120.000
		G 10	Normal	1.4	28	284.000

TABLE 11 - FEEDING RATE

- Around 1/3 of the ration with be given at each feed (3 feeds per day)
- Granulometry and average weight of the fish

AVERAGE WEIGHT	GRANULOMETRY
From 5 to 8 g	"Crumbs"
8 to 15 g	1,5 mm
15 to 30 g	2 mm
30 to 80 g	3,2 mm
80 to 200 g	4,5 mm
More than 200 g	6 mm

- Feeding rate depending on the average weight and the temperature

AVERAGE WEIGHT	Feeding rate/kg of pellets to be distributed for 100 kg of fish		
	< 15° C	From 15 to 19° C	From 19 to 25° C
From 5 to 8 g	Ad libitum feeding	2,8	4
8 to 15 g		2,5	3
15 to 30 g		1,7	2
30 to 80 g		1,3	1,8
80 to 200 g		1,2	1,5
More than 200 g		1	1

It is possible to replace part of the ration in pellet form by minced fish knowing that 1 kg of fresh fish = 250 g of pellets.

When manually distributed, and especially at low and high temperatures adjust the feeding ration visually.

FIGURE 1 : P. JAPONICUS - LARVAL REARING – FEEDING

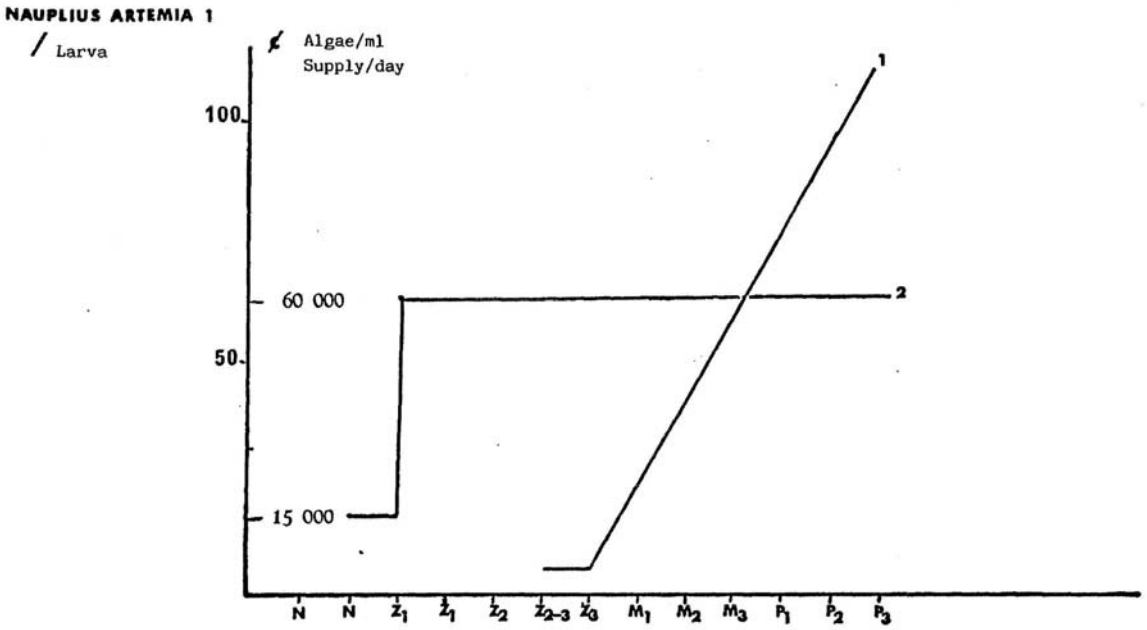


FIGURE 2 : P. JAPONICUS : Post Larval rearing

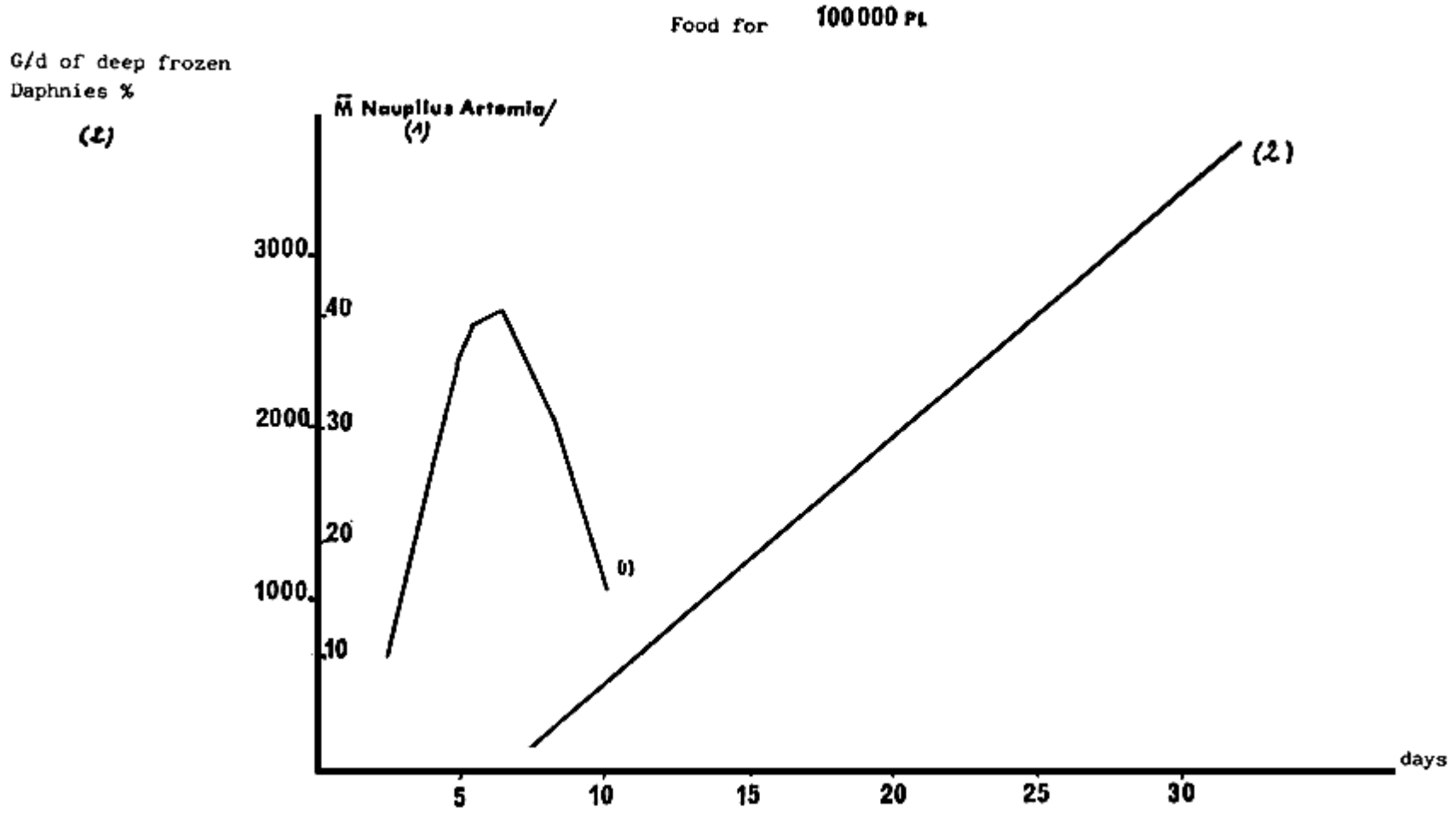
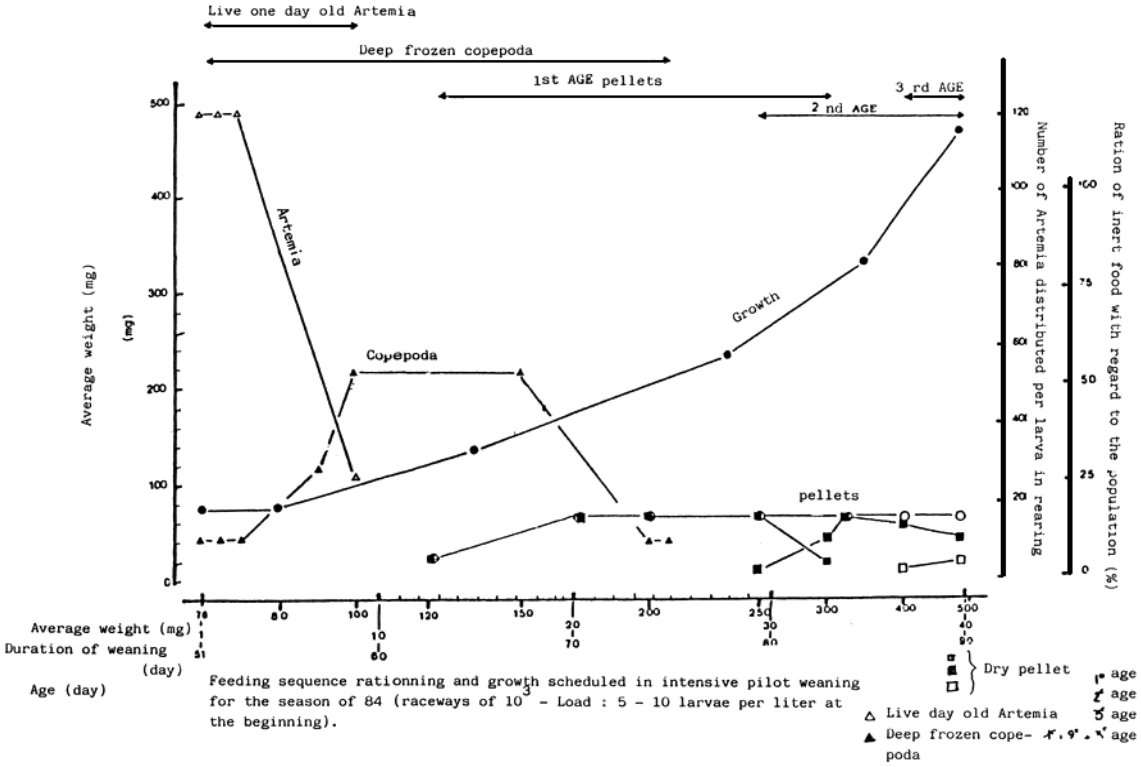


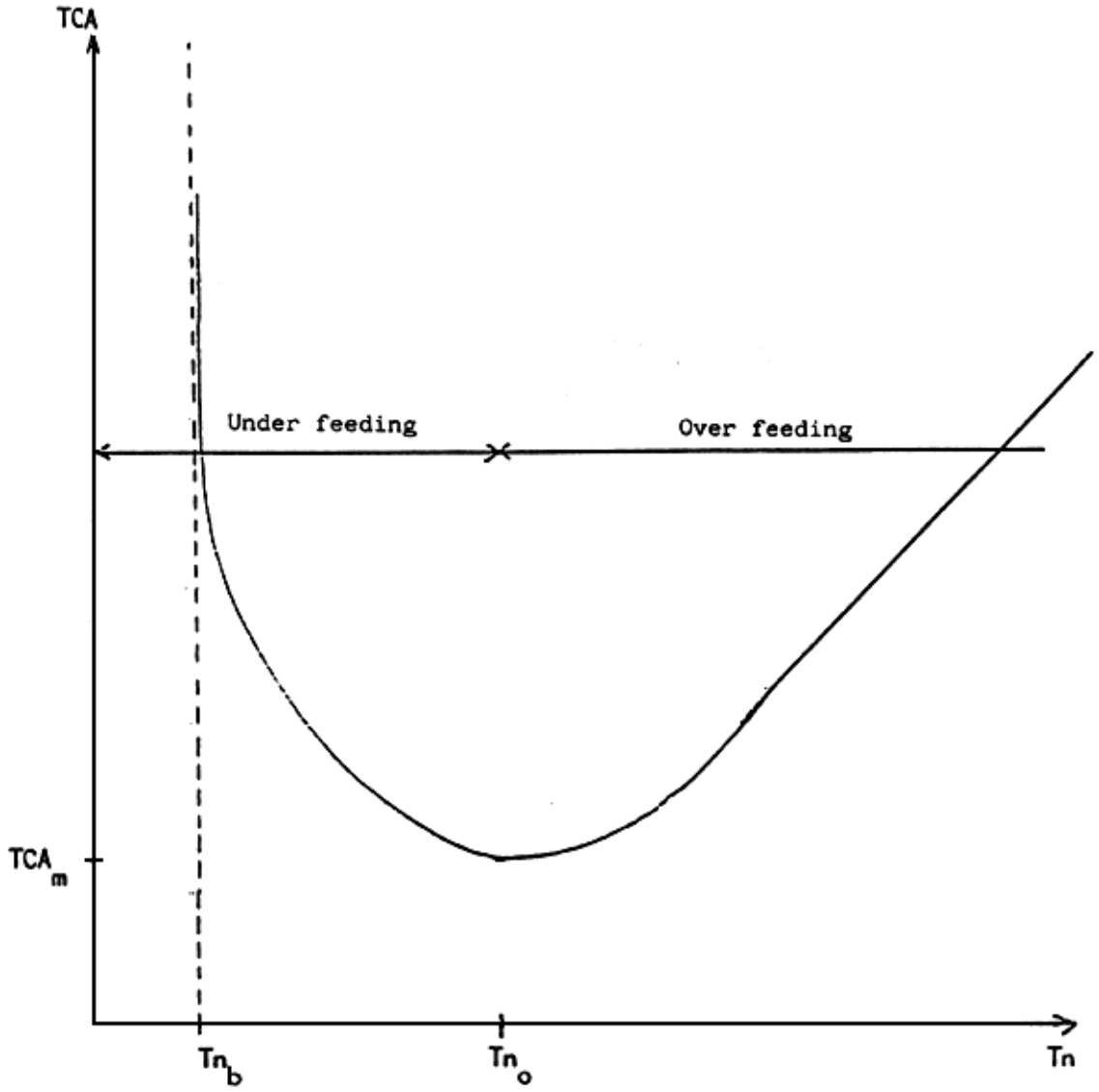
FIGURE n° 3 : WEANING TEST PROTOCOL FOR D.Labrax

Ration of inert food with regard to the population (%)



Feeding sequence rationning and growth scheduled in intensive pilot weaning for the season of 84 (raceways of 10^3 - Load : 5 - 10 larvae per liter at the beginning).

FIGURE 4 : EVOLUTION OF THE FEED CONVERSION RATE DEPENDING ON THE FOOD RATE

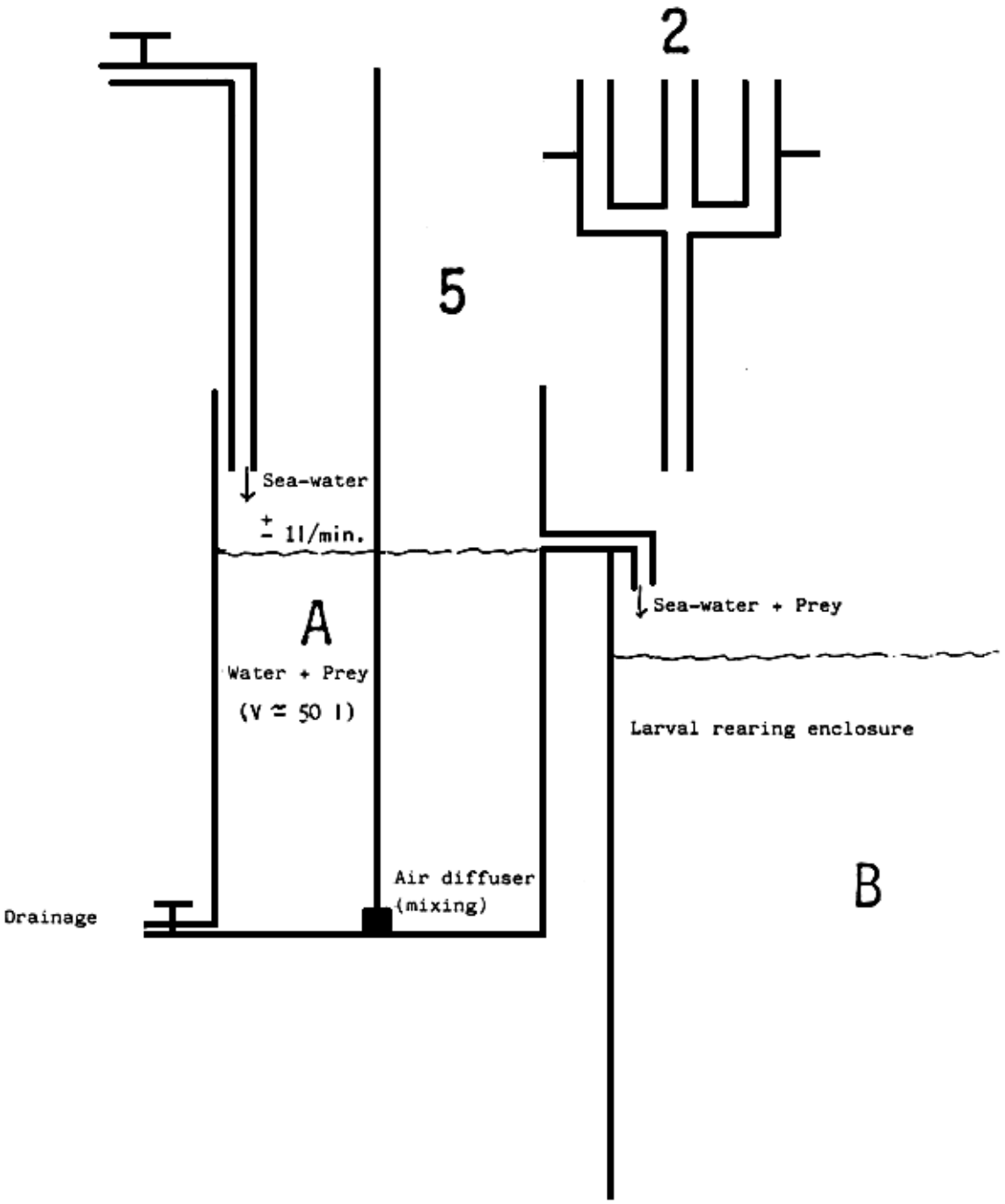


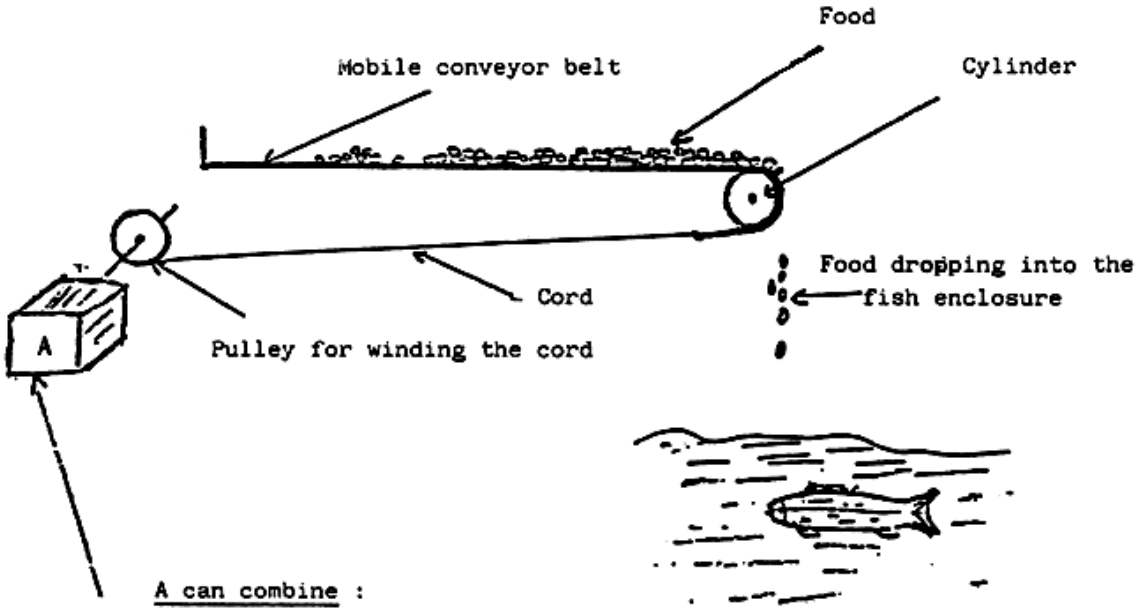
Tn_b : Feeding rate covering the basic metabolic requirements

Tn_o : Optimum feeding rate

TCA_m : Minimum feeding conversion rate

FIGURE 5 : AUTOMATIC DISTRIBUTER OF LIVE PREY





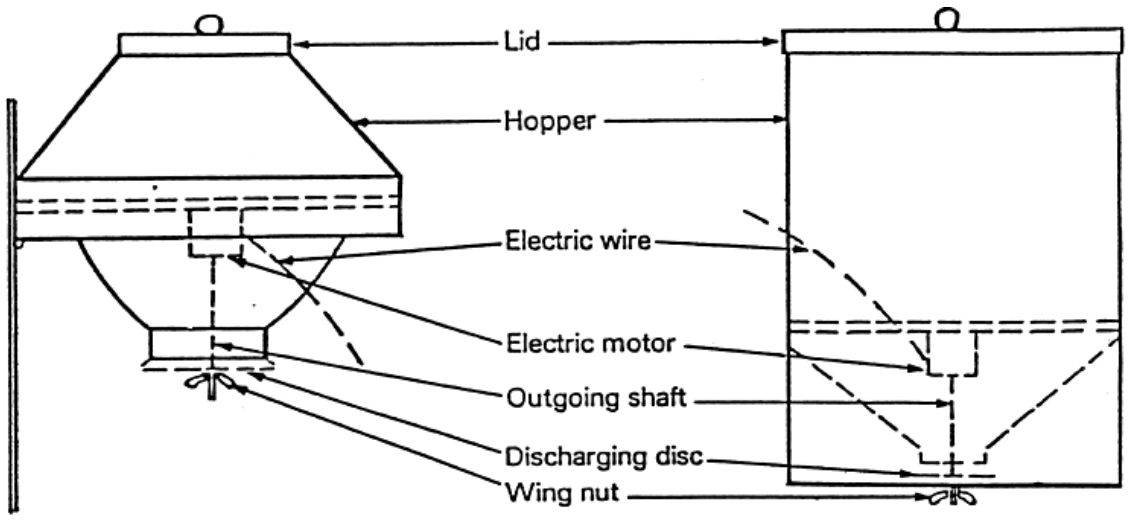
A can Combine :

- a simple spring
- a clock system
- a device programming the distribution

The distribution takes places as the belt advances

FIGURE 6 : Distribution employing conveyor belt

(Rehydrated or dry pellets)

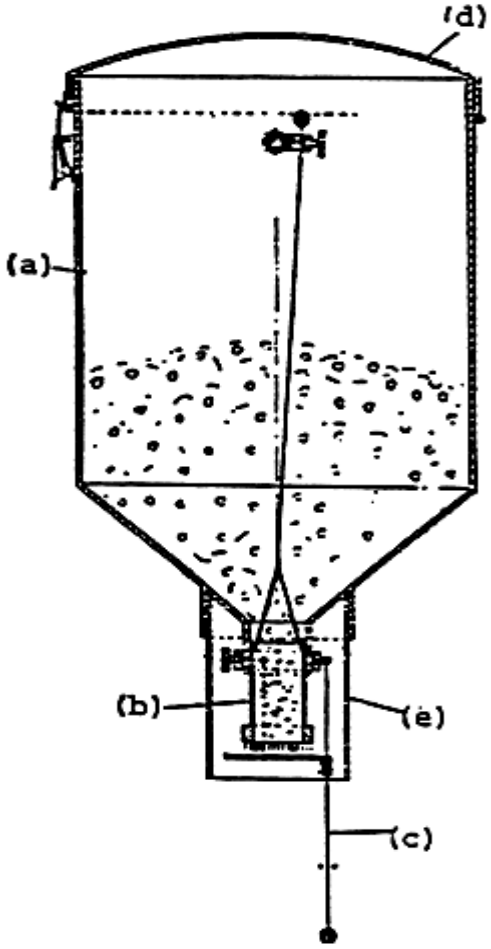


To be installated with a double delay time programmer, for example.

FIGURE 7 : VIBRATING FEEDER (Dry pellets)

FIGURE 8 : EXEMPLE OF SELF FEEDERS (PENDULUM FEEDERS)
(dry pellets)

Nos : 610, 630, 650



a = Feed hopper

b = Tube to set the feed supply.

c = Pendulum

d = Cover

e = Plexiglass tube

FUNCTIONAL MORPHOLOGY OF THE DIGESTIVE SYSTEM AND BIOENERGETICS IN FISH

J. GABAUDAN

1. INTRODUCTION

Let us consider the fish as a transformer of raw materials of various origins (often by-products of the food industry) into high quality proteins for human consumption. Firstly, the structure and function of the organs responsible for ingestion, digestion (hydrolysis) and absorbtion will be examined. A good understanding of the histology of the digestive system can also help to evaluate the nutritional status of the fish and detent possible nutritional deficiencies or imbalances:

Secondly digestibility of raw materials will be discussed as a quality criterion and quantitative data required for accurate fish formulation. This will include a description of the methods used for digestibility measurements. Techniques for the determination of gastro-intestinal evacuation time will be mentioned as well with nutritional and environmental factors affecting this parameter. Lastly some aspects of the nutritional bioenergetics of fish will be presented, including energy metabolism, energy requirements and sources of energy.

2. FUNCTIONAL MORPHOLOGY OF THE DIGESTIVE SYSTEM

2.1 General organisation of the digestive system

* Fish with a stomach

The digestive tract includes the following parts: oral cavity, pharynx, esophagus, stomach, pyloric ceca and intestine. Besides, there are two digestive glands: liver and exocrine pancreas, the latter beings often a disseminated organ. The shape of the stomach varies with the species and can be described as:

- straight
- Y shaped
- siphon shaped

Roughly, the lenght of the digestive tube depends on the feeding regime (Smith, 1980)

- 0.2 to 2.5 times the length of the fish for the carnivores,
- 0.6 to 8.0 times for omnivores,
- 0.8 to 15.0 times for herbivores:

However, other factors affect the Length of the digestive tube, such as the proportion of ingested sediments:

* Stomach-less fish

The general organisation is the same except that the stomach is missing.. It is sometimes replaced by an expansion of the intestine called the intestinal bulb but its histology does not differ from that of the anterior intestine. Therefore, stomach-less fish have no acid digestion of their food. The absence of a stomach cannot be related to the feeding regime (Cyprinidae, Scaridae, Sc-mberesox, some species of Syngnatidae).

2.2 Histoanatomy of the digestive system

Since histologic differences exist among species, only the most commonly encountered structures will be described here. Further details can be found in the reviews of Kapoor et al. (19750, Fange and Grove (1979) and Gas and Noaillac Depeyre (1981). The wall of the digestive tube is constituted by 3 layers : (1) the mucosa which

includes the epithelium and the sub epithelial connective tissue, (2) the muscularis externa formed by a circular and a longitudinal muscular layer and (3) the serosa

a) Esophagus

* Structure

- mucosa : stratified squamous epithelium, (inthe eel, the epithelium becomes simple after adaptation to seawater); numerous goblet cells present in the epithelium; sub epithelial connective tissue : generally formed by an inner dense layer and an outer loose layer. Presence of numerous leucocytes.
- muscularis externa : composed by two layers of striated muscle, an inner longitudinal-one and an outer circular-one.
- serosa : mesothelium plus connectivetissue

* Function

The esophagus conveys the food to the stomach. This is made easier by its ability to distend and its strong layers of striated musce.

b) Stomach

* Structure

The esophagus to stomach transition is abrupt for the epithelium while it is progressive for the muscularis externa.

The stomach can be divided into 3 regions: cardiac and fundic which contain gastric glands and pyloric region which has no glands.

- mucosa : high columnar, simple epithelium. The luminal surface of the epitheliocytes has short and widely spaced micro-villi and it is covered by a mucous coat. Usually there are no goblet cells but the apical cytoplasm contains mucous granules which are secreted into the gastric lumen by exocytosis.
  Exocrine glandular cells are located in the connective tissue. They are radially arranged around the gland lumen and of a single type.
- muscularis externa : it is composed of two layers of smooth muscle, an inner circular-one and an outer longitudinal-one.
- serosa : it is made of a relatevely thick connective tissue layer and a masothelium.

* Function

Gastric glands secrete hydrochloric acid and pepsinogene which becomes pepsin in the presence of a low pH. Therefore the stomach is the site of acid digestion of proteins. The pH in its lumen is equal to 2. The presence of lipases and chitinase has also been reported in the stomach of some species.

c) Intestine

* Structure

On the basis of ultrastructural and functional criteria, the intestine can be divided in 3 regions : (1) anterior intestine, (2) posterior intestine and (3) rectum.

- mucosa : Anterior intestine : it forms numerous branched folds. The epithelium is composed of a simple layer of high columnar cells, the enterocytes, among which lay goblet cells. The apical plasma membrane forms microvilli which constitute the brush border thus increasing the exchange surface between the lumen and the enterocytes. The cytoplasm contains lipid vacuoles and lipid particles.

  Posterior intestine : the folds of the mucosa are thicker and shorter. The cytoplasm of enterocytes has numerous supranuclear eosinophilic globules. Transmission electron microscopy reveals in this region of the cells numerous small channels and electron dense vacuoles. Histochemical methods demonstrated that these enterocytes are able to ingest macromolecules by endocytosis.

  Rectum : the epithelial cells usually secrete mucopoly-sacharides in abundance. This region is very short and just precedes the anal opening.

- muscularis externa and serosa : these Layers are similar to those found in the stomach. At the anal opening, the circular smooth muscle thickens to form a sphincter.

* Function

The intestine has two major functions : digestion and absorption.

Digestion : digestive enzymes active in the intestinal lumen at a pH close to neutrality are secreted either by exocrine pancreas or by the intestinal mucosa. The latter secretes :

+ proteases : aminopeptidase
  di- and tripeptidase
+ alcaline and acid nucleosidases
+ polynucleotidases
+ lecithinase, lipase and esterase
+ amylase, maltase, laminarinase etc....

Absorption : nutriments are absorbed by the enterocytes and enter into the circulatory system through blood capillaries located in the subepithelial connective tissue. Then, they are carried to the liver by the portal vein.
Histochemical studies suggest that amino acids, fatty acids and glucose are mostly absorbed by the anterior intestine while macromolecules (including proteins), water and minerals would be absorbed by the posterior intestine.

Note concerning pyloric ceca:

Pyloric ceca are finger-like projections of the anterior intestine wall located just past the pyloric sphincter. Their histology and function is comparable to that of the anterior intestine. By retaining food, they increase the intestinal evacuation time.

d) Liver

* Structure

The hepatic parenchyma is composed of polygonal cells called hepatocytes. Hepatocytes are radially arranged around a central vein in two-cell thick laminae separated by sinusoids. The cytoplasm contains glycogen and lipids. The hepatic artery and the portal vein enter into the liver. The junction of several hepatocytes form the bile canaliculi which drain the bile to the gall bladder where it is stored. The bile is then delivered to the intestinal lumen by the common bile duct.

* Function

Bile secretion is the major digestive ole of the liver. The bile emulsifies the lipids in the intestinal lumen thus facilitating enzymatic activity. Th ebile also neutralizes the intestinal content which is strongly acidic whwn it enters into the intestine. The liver has numerous other functions which are not digestive :

+ storage of energy (in the form of lipids and glycogen)
+ protein synthesis (plasma proetins, for example)
+ site of gluconeogenesis and deamination of aminoacids
+ detoxification and inactivation of toxic substances.

e) Pancreas

* Structure

Pancreas is composed by endocrine glands which secrete hormones (glucagone and insuline) and exocrine glands which secrete digestive enzymes. Exocrine pancreas is usually diffuse and consists of scattered acini in the esemteries and within the liver surrounding branches of the hepatic portal vein. A pancreatic canal opens into the intestine next to the common bile duct. The pyramidal exocrine cells secrete their products into the lumen of the acini. Their nucleus is basal and spherical, the cytoplasm is basophilic except at the apex where numerous acidophilic granules can be found. These granules are zymogen granules containing the digestive proenzymes.

* Function

The exocrine pancreas secretes the following digestive enzymes :

+ proteases : trypsine, chymotrypsine, carboxypeptidase and elastase (all are stored and secreted as proenzymes and activated in the intestianl lumen)
+ amylase
+ lipases
+ chitinases (in some species)

2.2 Digestion and absorption

The process of digestion is well described in the papars of Fange and Grove (1979). Table 1 shows the origin and the role of major digestive enzymes and thus indicates how proteins, carbohydrates and lipids of raw materials are hydrolized into aminoacids, glucose and fatty acids.

Absorption of digestion products takes place by diffusion, active transport and endocytosis.

* Proteins : free amino acids and peptides od low molecular weight are actively absorbed by enterocytes of the anterior intestine.

  In some species, proteins and polypeptodes are absorbed by endocitosis and digested within the cells. This process would allow, among other things, to partially recycle some digestive enzymes.

* Lipids : fatty acids would be absorbed by enterocytes of the anterior intestine and pyloric ceca.

* Carbohydrates : absorption of glucose occurs by active transport.

3. DIGESTIBILITY OF FEED INGREDIENTS

2.1 Definitions

The nutritional value of a feed ingredient depends on its chemical composition and on the ability of the fish to digest and absorb its nutrients.

Digestibility represents all the processes leading to absorption by the intestinal mucosa of the ingested food. Therefore it can be evaluated indirectly as the difference between ingested nutriments and nutriments excreted in the faeces.

The apparent digestibility coefficient (DCA) is calculated with the following formula :

$$DCA\,(\%) = \frac{\text{ingested nutrient - fecal nutrient}}{\text{ingested nutrient}} \times 100$$

The true digestibility coefficient (DCT) takes into account excreted endogenous material such as spent enzymes, mucus, desquamated cells and bacteria. Its formula is:

$$DCT\,(\%) = \frac{\text{ingested nutrient - (fecal nutrient - fecal endogenous material)}}{\text{ingested nutrient}}$$

Since it is technically difficult to measure endogenous excretions, apparent digestibility is usually determined.

3.2 Methods for measuring digestibility coefficient

Measuring digestibility coefficients is simple in its principle : one must collect the faeces and titratethe substance of interest in the feed and in the faeces. Technically it is not simple since the faeces are released into the water and therefore leaching of soluble compounds must be controlled.

Two methods, direct and indirect, and several techniques for faeces collection allow the evaluation of apparent digestibility.

* Direct method

  All of the ingested food and excreted faeces must be quantitatively determined. This is done in a metabolism chamber which allows the simultaneous and separate collection of faecal, gill and urinary excretions (Smith, 1971). The chamber (fig.1) consists of a cylinder separeted in two parts by a flexible dam stiched to the body of the fish. Gill excretions are collected in the anterior part, faeces (and water) in the posterior part and urine is collected through a catheter introduced in the ureter.

The collection of excretory products is therefore qualitative and quantitative. This makes possible the determination of apparent digestibility coefficients of any desired nutrient and the matabolisable energy of the tested diet.

$$\text{DCA nutrient \%} = \frac{\text{ingested nutrient - fecal nutrient}}{\text{ingested nutrient}} \times 100$$

ME = IE - (FE + ZE + UE)

Where :
- IE gross intake energy
- FE Fecal energy
- ZE Enery in gill excretions
- UE urinary energy

* Indirect method

The addition in the diet of an inert, undigestible and not absorbable marker eliminates the need for quantitative faeces collection. Indeed the variation of the ratio nutrient maker in the diet and in the faeces measures the digestibility as follows :

$$\text{DCA (\%)} = \left\{ 1 - \frac{\text{\% nutrient in faeces}}{\text{\% marker in faces}} \times \frac{\text{\% marker indiet}}{\text{\% nutrient indiet}} \right\} \times 100$$

The most frequently used marker in digestibility studies is chromic oxyde (Cr_2O_3). It is usually incompored into the diet at a rate of 1%. It can be titrated rapidely and easily by the method of Bolin et al. (1952) and it is believed not to interfere with the digestive process and gastro-intestinal transit.

Faeces collection in fish, even when not quantitative, is more problematic than for land animals. Indeed, leaching of soluble compounds occurs just after their release and can lead to significant overestimations of CDA. Therefore, particular attention must be paid to the choice of a collection technique and to its possible adaptation to a given situation.

In order to eliminate leaching losses, the fish can be taken out of the water and the faeces removed by :

- stripping : the fish being under light sedation, pressure is applied with the fingers on the abdomen in the antero posterior direction
- analsuction : a glass tube is introduced into the anus and the rectum content is removed by suction
- dissection : the fish is sacrified, the rectum is dissected out and its content sampled.

Faeces collection can also be done continously by one of the following methods :

- netting : the faeces are picked up with a net as soon as they are released
- settling : figure 2 shows the experimental setup used at IFREMER which is derived from the disign proposed by Cho et al. (1982). A settling column is connected to the base of a cylindro-conical tank. The

water flows through the column in which faecal material settles. Faeces remain in stagnant water until they are collected with a little water as possible through an opening located at the bottom of the column. The sampling, therefore does not cause any disturbance to the fish.

- continous filtration : the aparatus, shown in fig.3, was designed by Choubert et al. (1982). The water flows through screens moving linearly, thus separating the faeces and droping them into a collecting pan.

* Validity of the methods

The methods described above were compared using the same raw materials and sea bass coming from the same origin at IFREMER, Brest. The results are summarized in table II. This work and data available in the literature (Windell et al. 1978, Smith et al. 1980, Vens Capell, 1985) lead to table III which lists the advantages and inconvenients of the different collection methods.

There is probably no perfect method. It can be seen in table II that stripping, suction or dissection tend to underestimate protein CDA since they may be further absorbed in the posterior intestine. On the other hand, it is very difficult to avoid completely leaching from faeces naturally released into the water. Continous filtration seems to be quite efficient but the faeces must be cohesive for them not to stick on the screens. The incorporation of an undigestible binder into the diet contributes to the good physical properties of the faeces but can affect digestibility. In sea bass, 4% sodium alginate improve the cohesion of the fecal material without altering protein digestibility, while 6% result in a decrease of protein DC.

To conclude, a method for faeces collection should be selected keeping the following ideas in mind :

- handling of the fish must be kept to a minimum in order to reduce stress,
- the faeces collected must be representative of physiological processes,
- Leaching of soluble substances must be avoided as much as possible

3.3 Digestibility of some foodstuffs

Feed formulation by linear programming takes into account the fish nutritional requirements, the chemical composition of feedstuffs, their digestibility and price.

The DCA values given for protein (table IV), lipids (table V) and carbohydrates (table VI) in various sources illustrate the effects of a few factors on nutrient digestibility and the relevance of these data in fish feed formulation.

4. KINETICS OF GASTRO-INTESTINAL EVACUATION

4.1 Definitions

Gastric evacuation time is the time taken by a meal to evacuate completely the stomach. Gastric evacuation rate represents teh evacuation kinetics of the food, it is expressed in units of weight per unit of time. These two parameters can also be determined for the intestine or for the whole digestive tube. In the intestine, the

evacuation rate is difficult to measure since absorption must be accounted for. Therefore gastric evacuation is the usually preferred parameter. Also it is useful to quantify gastric evacuation is studies concerning feed intake, return of appetite and digestion.

4.2 Methodology

The objective is to examine the gastric content at given time intervals. Several methods have been reviewed in detail by Talbot (1980).

a) Serial slaughter

A group of fish is fed ad libitum and sub-samples (8 to 10 specimens minimum) are killed immediately after in order to measure consumption and at intervals thereafter. The digestive tube is dissected out and gastric and intestinal contents are removed and weighed. The fish are usually starved before and after the tested meal so that the results are not confused by additional food intake.

This method has several incovenients :

- it requires the slaughtering of a large number of animals,
- the data are collected on different animals at each sampling time,
- food deprivation before and after the tested meal may introduce an error since normal feeding frequency is altered.

b) Gastric flushing

In order to avoid killing the fish, the stomach content can be pumped out from live animals. Giles (1980) described a device made of two syringes strapped together. Water is alternately forced into the stomach with one syringe and remove with food with the other syringe. According to Giles (1980), 95% of the gastric content can thus be recovered.

c) X-Ray radiography

A radio-opaque compound must be incorporated to the feed. Barium sulphate is often used at alevel of 20% minimum which can require forcefeeding. Such a high incorporation rate has two incovenients : (1) it modifies substantially the composition of the diet and (2) it has been shown that force-feeding can decrease up to 50% the gastric evacuation time. Talbot and Higgins (1983) used metallic iron powder (particle size 100 to 200 µm) at a low incorporation level (5-8%) and established the relationship between iron particle number and food weight. Since iron particle are comratevely heavy, they may separate from the food and be retained in the stomach longher than the food. Grove et al. (1985) solved this possible problem by using barium suphate coated polystyrene spheroids. The position of the food in the digestive tube is followed by X-raying the live fish at successive time.

This method is elegant and simple. It is quantitative and therefore allows the measurements of gastric evacuation rates on small as well as large animals.

4.3 Utilisation of the data and factors affecting the transit

Grove et al. (1978) compared return of appetite and gastric evacuation rate in rainbow trout (fig.4). The graph describing the return of appetite is the mirror image of that describing the gastric evacuation. This demonstrates that tha strong correlation existing between gastric content and appetite. Although gastric evacuation, return of

appetite and feed consuption are correlated in time, the control mechanisms of appetite are most likely of metabolic or nervous origin (Fletcher, 1984). Knowledge of gastric evacuation can thus allow a rational approach to determining feeding frequency and ration size. This illustrated by the diagram in fig.5 which gives gastric evacuation time for rainbow trout in ralation to body weight and temperature.

The following factors modify gastric evacuation :

* Temperature : gastric evacuation time and temperature are inversely related (table VII)
* Body weight : gastric evacuation time increases with increasing body weight for a meal size expressed as a percentage of body weight (Flowerdew and Grove, 1979)
* Ration : GET is longer for when the ration is increased, but the relationship is non-linear (Jobling et al., 1977)
* Energy concentration of the diet : diet with low energy content move more rapidely through the stomach than highly energetic diets (Jobling, 1981)

5. NUTRITIONAL BIOENERGETICS

5.1 Energy flow in fish

The energy metabolism of fish differ from that of higher vertebrates in three major aspects :

- fish do not maintain a costant body temperature,
- it spends little energy to maintain its position in water,
- the major product of nitrogenous excretion is ammonia rather than urea or uric acid.

These three features allow the fish to "save energy" which results in more protein deposited per unit of energy intake than for other farm animals (Lovell, 1979).

The energy flow in fish is schematically represented in fig.6 (from Cho and Kauslik, 1985).

Digestible energy is the difference between gross energy intake (IE) and energy excreted in the faeces (FE) :

$$DE = IE-FE$$

Hence DE depends on protein, lipid and carbohydrate digestibility.

Metabolizable energy (ME) is a more defined evaluation of available energy since it takes into account nitrogenous losses due to amino acid oxidation and which are excreted through gills (ZE) and in the urine (UE) :

$$ME = DE - (ZE + UE)$$

ME is therefore related to the proportion of dietary proteins used as source and can be modified by changing, for instance, the proteins to lipid ratio.

The heat increment of feeding (HiE) represents the energy spent by digestive and absorption processes, metabolic transformations and interconversions of compounds and formation of metabolic excretory products: The determination of HiE can

be done either by measuring directly the heat released by the fish. Net energy is then calculated as follows :

$$EN = EM - HiE$$

Net energy is the most accurate estimate of the dietary energy available for maintenance (basal metabolism and resting activity) and production (growth and reproduction). Unfortunately it is technically very difficult to measure and its use is still controversial.

Cho and Kaushik (1985) suggest a method to evaluate ME, NE and maintenance energy which does not require the collect of the gill and urinary excretions and the measurement of HiE. Their procedure is as follow :

- measure digestibility coefficient of the diet
- analyse diet and whole body composition at the begining and end of the feeding trial
- calculate digestible N (DN) and digestible energy (DE) consumed by the fish
- calculate retained N (RN) and energy (RE)
- non-fecal nitrogen loss = DN – RN
  non-fecal energy loss (ZE + UE) = (DN –RN) x 25 KJ/gN
  ME = DE - (ZE + UE)
- HiE = DN x 28 KJ/gN (salmonids at 15C)
  NE = ME – HiE
- maintenance energy = NE - ER

It is important, when formulating feeds, to be able to determine dietary energy losses since they depend to a large extend on the balance between digestible protein and energy (HiE resulting from dietary protein is much greater than that from dietary lipids or carbohydartes). Generally speaking, energy losses for ZE + UE represent 4 to 8% of DE and HiE represents 9 to 14 % of DE.

5.2 Energy requirements

A fish will first use available dietary energy to meet the maintenance requirements. An energy deficient diet will therefore penalize growth. On the other hand, an excess in dietary energy will reduce food consumption since fish, as other animals, tend to adjust their feed intake on their energy requirements. If the feed is highly concentrated in energy, feed intake may decrease and intake of essential nutrients may become insufficient.

The determination of energy requirements is not simple since they depend on species, temperature, ration, rearing conditions, etc... Few data are available in the literature and they must be applied with caution. For a warmwater fish such as catfish the DE/Protein ratio should be 8 to 9 Kcal/g protein for diets containing 32 to 35 % proteins (NCR, 1981). For seabass juveniles the ratio is 7 to 8 Kcal ME per g protein.

5.3 Energy sources

Energy is "stored" in the chemical structure of feed ingredient molecules which are digested, absorbed and oxidized. Oxidation releases energy. Lipids, proteins and carbohydrates are all providing energy.

a) Lipids

Lipids are the most energy concentrated compounds. Their gross energy value is 9.1 Kcal/g. A good quality oil will usually provide 8 to 8.5 Kcal DE/g.

Lipids are therefore an appropriate source of energy to spare proteins. Dietary lipid levels are usually 9 to 10%.for sea- bream, 12% for sea-bass and 15 to 18 % for rainbow trout

b) Proteins

Fish utilize proteins very efficiently as energy source. Gross energy value is 5.65 Kcal/g and DE commonly varies from 4 to 4.5 Kcal/G

c) Carbohydrates

Carbohydrates are an inexpensive source of energy. Unfortunately they are poorly utilized by fish. It has been found that fish have limitated glucidase activity and do not control well glicaemia. Groos energy of carbohydrates is 4.5 Kcal/g but DE values varies from 1.2 to 3.6 Kcal/g.

Table I Summary of digestive processes

Organ	Enzyme	Activation	Substrate	Product
Stomach	Pepsine (HCL)	acidity pH= 2	proteins : peptide bond between TYR, TRP, PHE and between ASP and GLU	polypeptides
Liver	(secretes bile which emulsifies lipids and neutralizes intestinal content)			
Pancreas	Trypsine	enterokinase from int.mucosa	proteins : peptide bond when carbonyl group belongs to ARG or LYS.	polypeptides
	Chymotrypsine	by trypsine	proteins : peptide bond when carbonyl group belongs to TYR, TRP, PHE.	
	Carboxypeptidase	" "	polypeptides : from the end with a free carboxyl group	amino acids
	Elastase	" "	elastine	
	Amylase	" "	starch and glycogen	glucose maltose dextrines
	Chitinase	" "	chitine	di- and trimere of N-acetyl-D glucosamine
	Lipases and Esterases		triglycerides	fatty acids monoglycerides glycerol
Intestine	Aminopeptidase		polypeptides : from the end with a free amino group	amino acids
	Dipeptidase		dipeptides	amino acids
	Tripeptidase		tripeptides	amino acids
	Maltase		maltose	glucose
	Nucleotidase		nucleic acids	nucleotides
	Nucteosidase		nucleosides	Purine pyrimidine pentose

Table II Comparison of different faeces collection methods

	Stripping	Anal suction	Dissection	Netting	Settling	Continous filtration
DCA proteins %	82	87	84	91	94	92
DCA Lipids %	94	96	95	97	96	

Table III Advantages and Incovenients of seven methods for faeces collection

Method	Inconvenients	Advantages
Stripping	• Contamination of sample with urine, blood, sperm: underestimation of DCA • Stress • little sample collected • is the sample representative of naturally released faeces?	• Simple to do
Anal suction	• stress • very little sample collected • is the sample representative ?	• simple
Dissection	• requires large numbers of fish • little sample collected • is the sample rapresentative ?	• simple
Netting	• labor intensive • tedious • stress	• simple • faeces are released naturally
Settling	• some leaching may occur	• faeces released naturally • no stress • continous collect
Continous filtration	• cost of apparatus • leaching ?	• same as sattling
Metabolism chamber	• stress • fish may not adapt to confining • requires excellent water quality	• quantitative collect • ME can be determined

Table IV Digestibility of proteins

Source	Crude Protein (%)	DCA (%)	
Alfaalfa meat	17.0	61.0	
Cotton seed meal	50.5	77.7	
Anchovy meal	70.1	83.5	effect of the source (1) (rainbow trout)
Meat and bone meal	52.9	70.3	
Whole soybean 127 C,10 mn	42.9	45.4	effect of processing (1) (destruction of anti-trypsic factor (rainbow trout)
175 C, 5 mn	42.5	56.6	
232 C, 8 mn	41.1	75.1	
Commercial feed	50.0		
ration = 3.3g/Kg		71.20	effect of ration (2) (catfish)
10.0g/Kg		67.26	
16.7g/Kg		60.03	

(1) Smith, 1976. (2) Henken et al. 1985

Table V Digestibility of lipids (for rainbow trout. from Austreng et al., 1979)

Source	DCA Lipid (%)	
Soybean oil	87.9	effect of source
Cod liver oil	90.6	
Hydrogenated capelin oil		
fusion point 21 C	74.6	effect of fusion point
" " 41 C	46.4	

Table VI Digestibility of starch (for trout, Jolivet, 1986)

Source	DCA Starch (%)
Cooked corn starch	85.5
Raw corn starch	47.6

Table VII Gastric evacuation time for 30 g. Tilapias fed 3% of body weigh (from Ross and Jauncey, 1981)

Temperature (C)	Gastric evacuation time (h)
20	16.4
25	10.8
30	8.5

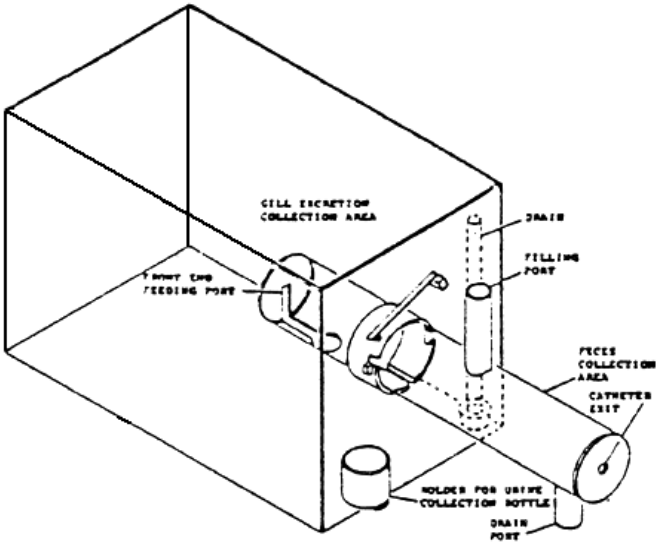
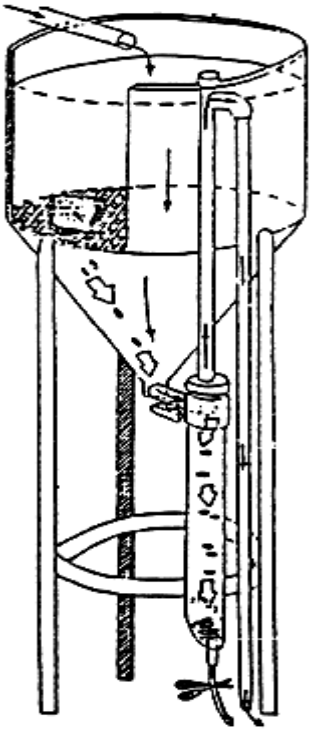


Figure 1 - Metabolism chamber (in Gabaudan, 1979)

Figure 2 - Experimental set-up for collection of faeces by settling (from Jolivet, 1986)



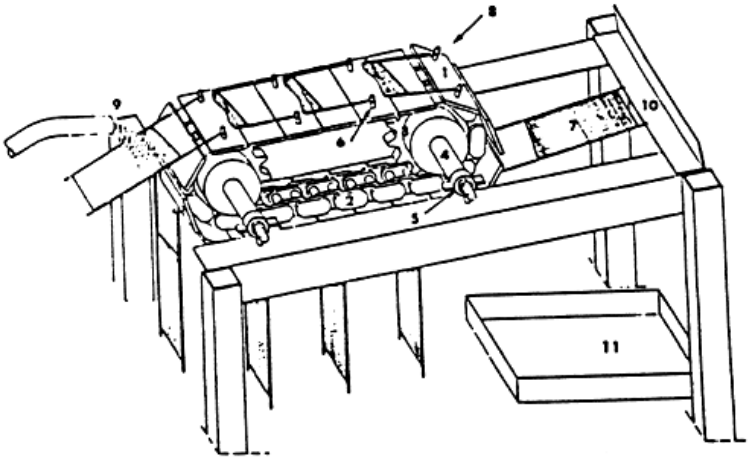


Figure 3 - Apparatus for continous faeces collection by filtration (in: Choubert et al., 1982)

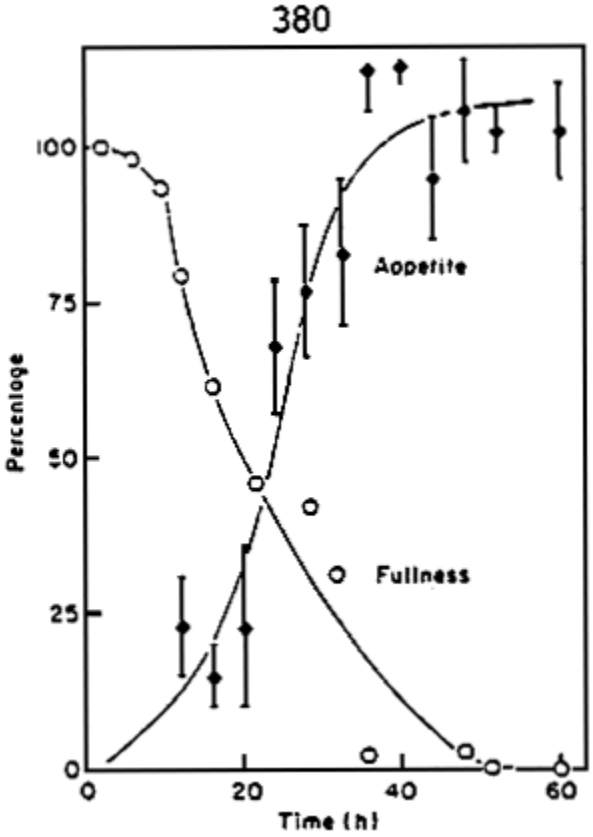


Figure 4 - Comparison between return of appetite and gastric evacuation rate for rainbow trout at 11 -12 C. (in : Grove et al., 1978)

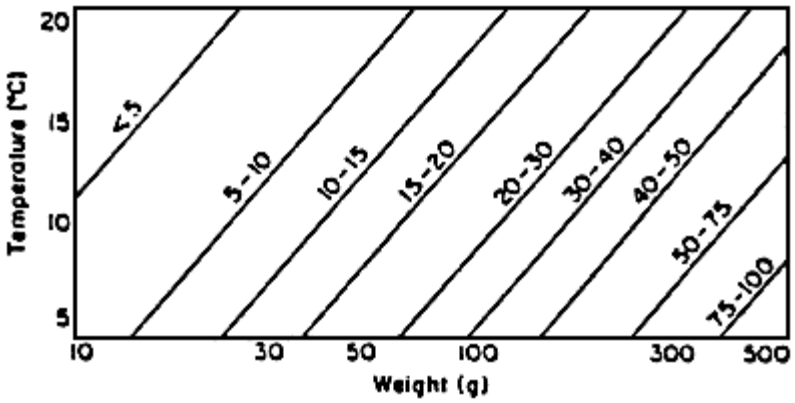


FIGURE 5 - Gastric evacuation time in hours at different temperatures and for different body weights. (in : Grove et al. ,1978)

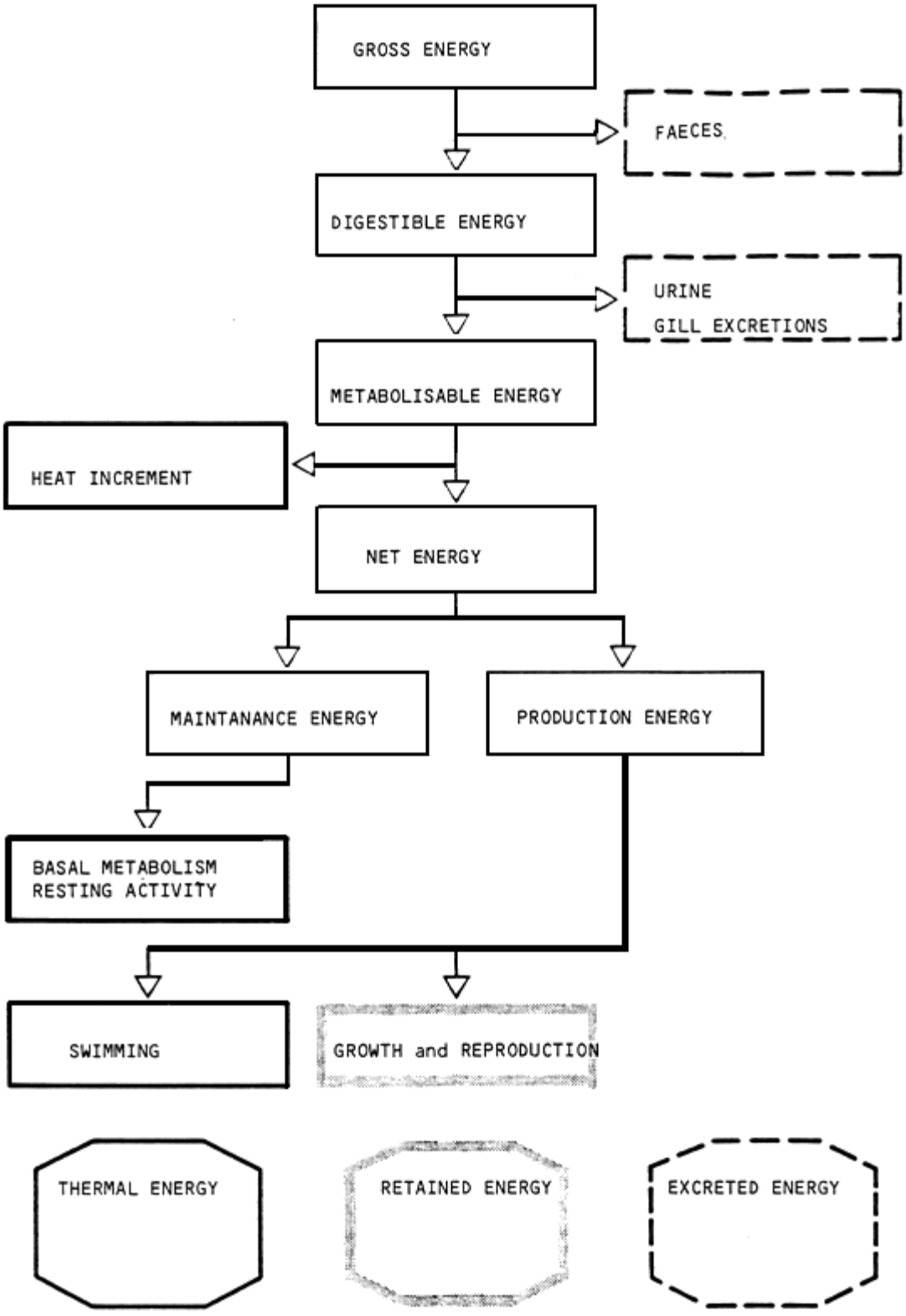


Figure 6 - Energy flow in fish

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ANNEXES

ANNEX I

PROGRAMME

19/10/86	- Welcome of participants	
20/12/86	- Opening of the session	
	- Digestion and energetic metabolism	
		J. GABAUDAN
21/10/86	- Proteins and amino-acids	C.B. COWEY
	- Carbohydrates	C.B. COWEY
	- Lipids and fatty acids	C. LEGER
22/10/86	- Vitamins and mineral salts	J.G. KOENIG
23/10/86	- Nutritional requirements of Crustacea	
		M.J. CECCALDI
	- Nutrition of molluscs	J.M. DESLOUS PAOLI
24/10/86	- Raw materials	J. GUILLAUME
	- Silage - Theory and practical work	
		I. BATISTA
25/26/10/86	- <u>Study tour</u>	
	- VIVAL - VIVEIROS de RIA S.A.R.L	
	Salgados - Boine, 8500 PORTIMAO (Mr. TEUNISSEN)	
	- SINEXPLAL Ltd	
	OLHAO (Mr. DELFIN AGOSTINHO)	
	- I.N.I.P - Centro de Investigacao Pesqueira de FARO	
	FARO (Mr. POUSAO FERREIRA)	
27/10/86	- Formulation - CRESPO FOLGADO	
	- Fish feed processing and technology	
		J.P. MELCION
28/10/86	- Feeding stimulants	A.M. MACKIE
	- Microparticles	J.P. MELCION
	- Experimentation in nutrition	R. METAILLER
29/10/86	- <u>Presentation of commercial feed</u>	
	- AQUALIM (J. SABAUT)	
	- EWOS BIOTER S.A. (A. TIANA MARISCAL)	
	- TROUW International (H. HOGENDOORN)	
	- <u>Visit</u>	
	- QUIMICAL (CRESPO FOLGADO)	
30/10/86	- Feeding in marine aquaculture	
		- C. de la POMELIE
31/10/86	- Departure of participants	

ANNEX 2

PARTICIPANTS

ABOUHALA ABDERRAHMANE	-	S. té MAROST ATALAYOUN - B.P. n.4 NADOR (MAROC)
ABDELKADER ROUABAH	-	C.E.R.P. DE BOUISMAIL WILAYA DE TIPAZA (ALGERIE)
ATTIA EL HILI HEDIA	-	I.N.S.T.O.P. 2025 SALAMBO (TUNISIE)
MEDHJOUB AMEL	-	C.N.A. de MONASTIR MONASTIR (TUNISIE)
WAGDI AHMED HAFEZ GAMAL AHMED TALAAT EL SAWAF	-	GENERAL AUTHORITY FOR AQUATIC RESOURCES 4, Tyran Street - NASR CITY (Cairo) EGYPT
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MURAT ERSOY	-	AQUACULTURE RESEARCH INSTITUTE ANTALAYA (TURKEY)
CARMELO GALEA	-	THE BIOLOGY DEPARTMENT, THE UNIVERSITY OF MALTA TAL AXOQQ - MSIDAQ (MALTA)
NICHOLAS BONANNO	-	CARTER MDINA ROAD ZEBBUG (MALTA)
SKARAMUCA BOSKO	-	BIOLOSKI ZAVOD DUBROVNIK (YUGOSLAVIA)
IVOS IGOR	-	CENMAR ZADAR (YUGOSLAVIA)
DAMIR MUSIN	-	BIOLOGICAL INSTITUTE DUBROVNIK (YUGOSLAVIA)
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ANNEX III

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