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The feeding of fish larvae : present « state of the art » and perspectives (*)

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Summary. The aim of the present paper was to outline the major achievements in larval fish rearing and, when possible, to speculate on further useful research. The effect of the parents' nutritional history is mentioned as affecting larval vitality. Several environmental factors which may influence larval behaviour in enclosures are discussed. Of particular interest are the aspects of larval fish digestive tract morphology and physiology but, up to now, information in this field is fragmentary. From information presented in this review, the processes of digestion, absorption and assimilation appear to differ considerably according to life stage-larval, juvenile or adult. Data on the biochemical composition of zooplankton, the natural food of fish larvae, is of interest because of its use in commercial rearing procedures when dry compound diets are not available. Furthermore, zooplankton can be used as a model for the formulation of an « artificial » feed. A great deal still remains to be learned about the chemical composition of zooplankton, its enzyme characteristics and its interaction with the fish digestive apparatus after the live organisms are ingested. Finally, the more or less successful results of rearing larval fish on compound diets are discussed. Diets based on single-cell protein (SCP) have proven to be the best in several trials since they support fish growth and survival as well as zooplankton does. Each section of the present review includes suggestions for further research.

Introduction.

It is not easy to estimate the nutrient requirements of larval and juvenile fish by traditional nutritional methods and procedures such as standard, semi-purified or commercially-formulated diets which do not support the growth of the larvae of several fish species. The interactions of various macro and micro-nutrients in compound diet formulations for adult fish appear to be of little help when studying the requirements of larval stages. The morphological, histological and functional aspects of larval fish development show that, during a short period of

(*) This paper is dedicated to Mrs Izabela Bontemps.

its early life-history, the larva is a very different organism from the adult. It is natural that during the development of the larva — understood in a broad sense as changes in the digestion, absorption, transport and assimilation of chemical compounds — its nutrient requirements change also. As compared to the juvenile stage, any nutrient deficiency in the larval stage is manifested in an accelerated way because of the very small initial weight and resulting rapid growth of the larva. There is a paucity of information on both the qualitative and quantitative nutrient requirements of the various life-stages of several species wich are currently being reared in large numbers in fish hatcheries. Some recent speculation (Flüchter, 1982 ; Dabrowski and Kaushik, 1982 ; Tacon, 1981) has related unsuccessful larval rearing with a lack of the essential dietary elements.

In the last 2 to 3 years, there has been an increase in the literature dealing with the different aspects of larval fish rearing. The most useful studies concern the physiological aspects of digestive tract morphology and the biochemical characteristics of zooplankton, the natural food of the larvae. The larvae of several species are highly dependent on behavioural performance and a knowledge of these aspects is obviously essential to intensive hatchery techniques. Finally, dry compound diets have been much improved in the last few years and, in some cases, have ensured appreciable success in the rearing of larval fish. However, there is still much to be learned in this field.

Since these topics appear to be important in larval fish nutrition and they have not been reviewed recently, the present article discusses new literature on these subjects with additional recommendations for future research.

I. The problem of the origin of fish larvae.

The nutritional status of the mother fish influences the chemical composition of the yolk sac material serving as endogenous food for the metamorphosing fish larvae (Stroband and Dabrowski, 1981). Shimma and Tsujigado (1981), using an activity index as a criterium of survival time during starvation in *Sebastes marmoratus,* have shown that the larvae with higher indexes had better survival rates in the feeding experiments. A relationship was found between the survival index of starving larvae and the weight of the fatty tissue in the abdominal cavity of the female ($r = 0.3637$, $n = 43$).

Although dietary fatty acids are very often a primary cause of the unsuccessful rearing of marine fish larvae (Watanabe, 1979), Yu *et al.* (1979) indicate that when mature rainbow trout grown from fish of 0.43 g initial individual weight were fed on diets containing either linolenate or linolenate + linoleate, the fertilization rate of the eggs of the groups was not different. Furthermore, the groups did not differ in the percentage of viable fry or in the growth and mortality of second generation fry up to 3 months.

Properly-matured ovulated carp eggs do not differ greatly (9.6 %) in diameter and their size is not correlated with the survival of the embryos. The size of the eggs does not affect their survival up to the stage of embryo formation ; these

data are based on the examination of 172 females (Zonova, 1973). Eldridge *et al.* (1982) demonstrated that egg size in striped bass (*Morone soxatilis*) influences only the early period of growth up to the beginning of feeding. The larvae from the smaller eggs showed later compensatory growth. The instantaneous growth coefficient resulting from feeding larvae at different food concentrations was double in those from eggs ranging between 210 and 373 µg in initial size. Compensatory growth has already been shown in salmonid fish, but this recent evidence from studies on warm-water fish argues for taking more caution in directly relating egg size to larval vitality.

II. — Behavioural constraints in the feeding performance of fish larvae.

Environmental factors like water temperature, salinity, light intensity and colour, day/night periodicity and water quality all influence feeding behaviour but will not be reviewed in this article.

The most intense growth in larval fish occurs in the posterior part of the body (Fuiman, 1983), promoting increased swimming performance in early life. Khono *et al.* (1983) have documented ontogenic changes in the swimming and feeding functions of marine fish larvae. The sequential development of the fin-fold and the fins suggests that, at a certain size, the larvae change their mode of swimming from that using a so-called cruising speed and occasional sinuous feeding posture (Hunter, 1980) to that of active caudal propulsion. This change is accompanied by a transition in feeding from swallowing the prey to biting them. Hartman (1983) studying freshwater fish larvae came to a similar conclusion independently and proposed some early-life feeding strategies, skill and swimming speed ; he found that survival was first limited by the mode of feeding, followed by mouth size and finally by the size of the available food particles. Several arguments support these three factors of feeding behaviour in larval and juvenile fish, but there are also exceptions to these arguments.

Other senses than visual perception and postural ability are involved in larval feeding behaviour. Walleye larvae do not ingest copepod nauplii and reluctantly accept rotifers when given in high density (Mathias and Li, 1982). The dinoflagellate, *Gymnodinium splendens,* is accepted as a preliminary food by the larvae of several marine fish, but their depressed growth rate after several days cannot be solely explained by comparing the energy value of the food and the energy needs of the growing fish (Hunter, 1980). Several marine fish larvae utilize and grow well on protozoans, rotifers (Divanach and Kentouri, 1982) and copepod nauplii (Houde and Schekter, 1983).

Mouth size is a sure criterium to consider when feeding larval fish on either a natural or an inert compound diet. The mouth size of cyprinid larvae varies greatly ; estimated food sizes for silver carp, grass carp and bighead carp larvae are 50-90, 90-150 and 150-270 µm, respectively (Dabrowski and Bardega, 1984). Common carp larvae accept much larger food particles (0.3-0.4 mm) right from the beginning (Dabrowski *et al.,* 1983). Coregonid larvae readily accept food particles 0.2 mm in diameter (Dabrowski *et al.,* 1984 a, b).

Chemical stimulus as a factor in the feeding of fish larvae has been less extensively studied but it might contribute to the development of a satisfactory larval diet. The larvae respond to several criteria of food-particle properties such as size, texture and flavor. Gunkel (1979) observed that coregonid larvae, after taking food granules into the mouth, spit them out. Appelbaum *et al.* (1983) also observed in Dover sole larvae that the final selection of the food particles occurred in the mouth since they could be either swallowed or rejected ; these authors found taste buds in the buccal cavity which were particularly frequent near the entrance to the oesophagus in 4-5 mm larvae, although these buds were much smaller than the external buds on the head. In carp larvae, structures permitting the perception of odours are present in the olfactory region, but only further neurophysiological investigation can tell whether they are functional at an early larval stage (Appelbaum, 1981). Indirect evidence from Dempsey (1978) suggests that feeding responses in larvae are very specific ; washed and dialysed *Balanus* nauplii extract (molecule size : < 12,000-14,000), as well as glycine and proline, induced a response in the larvae before feeding. No response to *Artemia* nauplii extract was reported at this stage but a response appeared when the larvae began to feed on them (Dempsey, 1978).

There is no study on larval fish which introduces a food attractant into the compound diet, but it might be a way of achieving an increased rate of food ingestion. In juvenile fish, Tandler *et al.* (1982) showed an increase in appetite by a basal diet supplemented with either muscle extracts of *Mytilus edulis* or a synthetic mixture of chemicals imitating the extract. Carp feeding response was highly augmented by methanol extracts of silkworm pupa (Tsushima and Ina, 1978), and further studies on the isolation of the attractant have confirmed that this was due to the mixture of glycine, alanine, aminobutyric acid and glutamic acid (Murofushi and Ina, 1981). In certain species, inosine receptors have been shown to be present in areas of the mouth where the taste buds are found (Mitchell and Mackie, 1983). Studies on inosine binding sites have only been carried out so far on one species as the other fish mainly respond to amino acids.

III. The morphology and physiology of the digestive tract.

Dabrowski (1982) indicated that for practical purposes larval fish can be divided into three groups according to alimentary tract morphology and the enzymes secreted in the gut (figs. 1-3). Salmonids appear to have a functional stomach before changing from endogenous to external food. Takahashi *et al.* (1978) observed gastric glands several days before the emergence of salmon, *i.e.* coming up from the bottom to feed. The pyloric caecum was distinguishable in this fish 21 days after hatching (10 °C) and zymogen granules were observed in the pancreas 9 days after hatching (fig. 1). During early ontogenesis, salmonids and other fish with a functional stomach usually adapt easily to a dry compound diet. In the ontogeny of the cichlid digestive tract, the small stomach is visible before yolk-sac absorption and, as the fish take their first external food, the

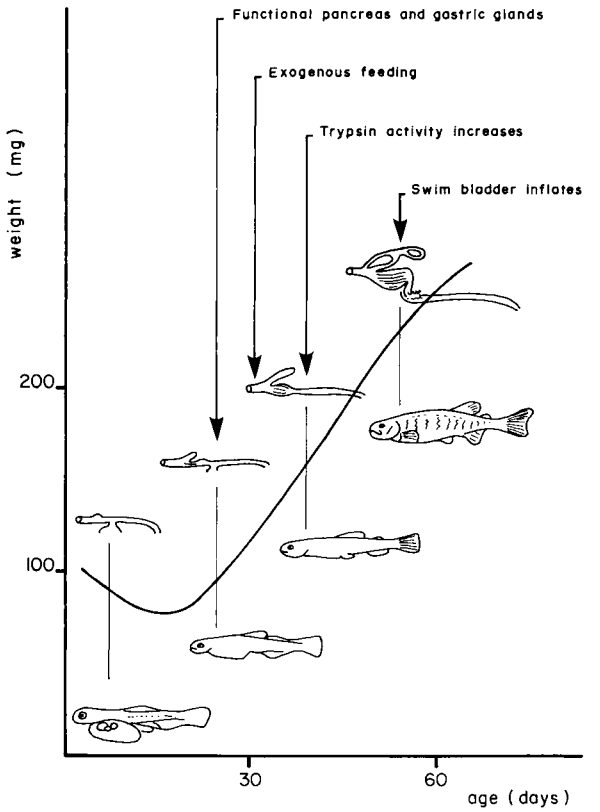

FIG. 1. — *Ontogenic development of the salmonid fish digestive tract, exemplified by rainbow trout.* Modified from Dabrowski (1982).

stomach appears as a sizeable blind pouch at the left side of the intestine (Zihler, 1982). In spite of the extremely different feeding habits of this species (algae, plants, fruits, detritus, insects or fish), the overall appearance of the digestive tract is the same.

As in the case of stomachless fish, the intestine of the second group of fish larvae can be differentiated into three segments (Stroband and Kroon, 1981). The enterocytes in these segments have the same histological, and possibly functional, characteristics as in adult fish. In *Clarias lazera,* an African catfish, the first functional cells develop in the corpus of the stomach which contains a tubular gland system secreting pepsinogen and HCl. The functional stomach is present in catfish juveniles when their body length nearly doubles after hatching. Hogendoorn (1980) and Msiska (1981) reported very unsatisfactory growth in catfish larvae fed on dry diets, and even freeze-dried zooplankton significantly decreased growth in comparison to live food. Hecht (1982) in a 10-day experiment obtained the increment of *Clarias gariepinus* larvae with negligible

mortality when they were fed exclusively on a dry diet. This difference may be species-dependent but generally fish having no functional stomach at the larval stage cause severe problems when reared solely on dry diets.

The most numerous fishes are probably those which at larval stage have no functional stomach or gastric glands but later develop digestive organs (Govoni, 1980 ; Vu, 1983). Using histoenzymological methods, it was found that proteolytic activity at alkaline pH occurred only in the middle and posterior intestine in larval sea bass, whereas gastric glands did not develop before 25 days of life (Vu, 1983).

Studies by Mähr *et al.* (1983) and Lauff and Hofer (1984) throw a new light on the development of the stomach in coregonid larvae in which the appearance and functioning of that organ are known to be delayed (Dabrowski, 1981 ; Stroband and Dabrowski, 1981) (fig. 2). In these larvae reared at 10 °C, the

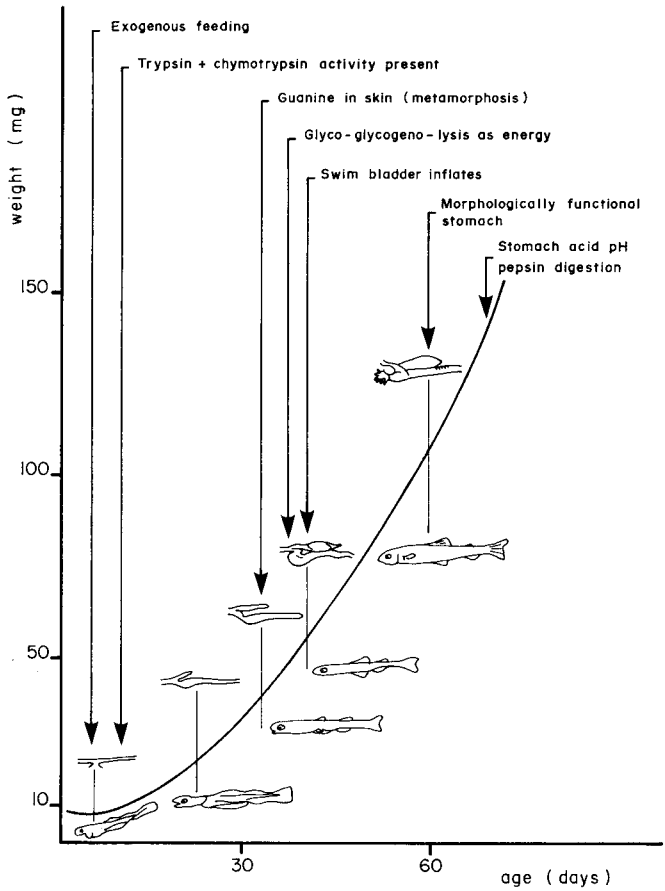

FIG. 2. — *Ontogenic development of the coregonid fish digestive tract, exemplified by Coregonus pollan.* Modified from Dabrowski (1981, 1982). Information on other coregonids from Mähr *et al.* (1983), Lauff and Hofer (1984) and Forstner *et al.* (1983) is found in the text.

gastric glands first appear in the « corpus » of the stomach between days 23 and 30. Glands were seen much later in other parts of the stomach, and at least three types of gland cells were distinguished. However, the secretory activity of gland cells in the lumen was not associated with a marked change of pH.

The pH is alkaline (6.7-7.1) in the fully-developed stomach, and acidification in the gastric lumen increases only from day 49, slowly dropping to a pH above 5.0 on day 97 (approximate weight : 60 mg) (Mähr *et al.,* 1983). Since at a pH above 5.1 pepsin activity is only about 20 %, peptic digestion cannot take place in coregenid juveniles until the age of 97 days. On the other hand, the development of gastric glands in catfish is closely associated with changes in pH, and in 15-day larvae of this species, the stomach pH drops to values of 3.3-5.2 (Stroband and Kroon, 1981). In sea bass, Vu (1983) observed proteolytic activity at pH 2.0 in the posterior intestine as early as 15 days after hatching, whereas activity at pH 9.0 was present throughout the rest of the intestine. Since stomachless fish digest acid-denatured proteins at a faster rate than native proteins (Hofer *et al.,* 1982), little advantage is taken of peptic digestion, even when the gastric glands are functional in juveniles with a stomach alkaline pH. In fact, the stomach is missing in pike larvae at the commencement of feeding (Applegate, 1982) ; the length of pike larvae has increased from 13-14 mm to 10 mm by day 11 when the formation of the stomach is visible. Szlaminka (1980) noticed proteolytic activity in the pike stomach only 18 days after hatching.

Trypsin, chymotrypsin and aminopeptidase activities also undergo ontogenic changes in coregonid larvae (Lauff and Hofer, 1984). Tryptic activity in these larvae is only slightly lower than that of rainbow trout, whereas chymotrypsin activity is much lower in the former species up to day 50 of life. A change in the pattern of trypsin isozymes also occurs during coregonid development, suggesting that the larval form of the enzyme disappears. Lauff and Hofer (1984) conclude that external enzymes obtained from zooplankton may contribute 70-80 % of the total enzyme activity in the fish digestive tract, confirming the earlier results of Dabrowski and Glogowski (1977).

These results contain valuable information which must be verified in feeding experiments using denatured pepsin or even pepsin, predigested protein sources which can be used in the formulation of dry diets for fish larvae. Protease activity was also found to depend on the fish population or the strain (Torrissen and Torrissen, 1982). Salmon fry with a significantly higher protease activity also had a higher growth rate. It may be deduced that even within a species, some strains differ in their ability to utilize compound diets.

Hjelmeland *et al.* (1984) were the first to introduce and measure proteolytic activity in fish using a sensitive quantitative method. By radioimmunoassay, these authors were able to follow the amount of trypsinogen and trypsin in developing cod larvae. After an initial rise in trypsinogen and trypsin following hatching, the time of the first exogenous feeding was symptomatic of a drastic drop in the amounts of the enzymes. This low level was maintained up to 14 days after hatching, but growth was not reported in dry weight. An increase in weight between days 14 and 18 was accompanied by an increase in trypsin-like activity and in the trypsinogen/trypsin content of larvae. The fish fed diets which did not

support their growth showed trypsogen/trypsin at detection levels. Hjelmeland *et al.* (1984) speculated that the high trypsin activity of cod larvae allows this fish to hydrolyse 25 μg of protein per hour at a weight of 40 μg of dry matter, but this point needs further study.

The third group of fish are those which remain stomachless throughout life (fig. 3). However, this was questioned recently by Labhart and Ziswiler (1979), at least as concerns some cyprinidonts. These authors contend that the epithelial region between the oesophagus and the intestine is histologically and physiologically similar to stomach epithelium. Bremer (1980) and Bergot (personal communication) were able to show that, in early life, cyprinid fish have certain cells which show acid histochemical reactivity, thus providing evidence of a secondary absence of the stomach. However, in this group of mostly cyprinid

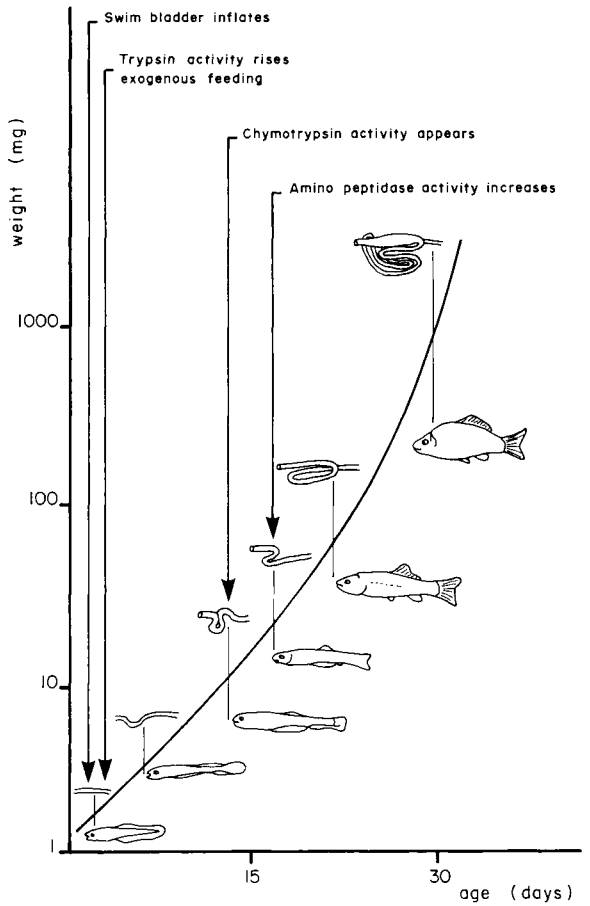

FIG. 3. — *Ontogenic development of the cyprinid fish digestive tract, exemplified by common carp.* Modified from Dabrowski (1982). Information on other cyprinids from Lauff and Hofer (1984) is found in the text.

fishes, the coiling pattern of the intestine undergoes ontogenetical changes ; noting the increase in the relative length of the intestine is the simplest way of measuring this process (see Dabrowski, 1982).

The second part of the intestine is involved in the intracellular digestion of absorbed intact protein (Stroband *et al.,* 1979). However, although this ability appears to be more evident in larvae than in juveniles, Watanabe (1982) found that intracellular digestion of horseradish peroxidase took place over a relatively long time (10 hours to 3 days) in several species. This would indicate the immunological rather than the nutritional importance of the process, bearing in mind the very fast growth rate of fish larvae.

However, Ash (personal communication, 1983) observed an increase in horseradish peroxidase in the circulating blood following ingestion in common carp juveniles. This might suggest that the process of intact protein degradation is not necessarily restricted to the enterocytes which absorbed it from the lumen, but that other organs, presumably the liver, may be involved in the utilization process.

The specific activity of chymotrypsin and trypsin in carp larvae did not differ whether the fish were fed live zooplankton or a dry diet (Ragyanszki, 1980). In the procedure applied, the zymogens were not activated during the assay, and the extension of this *in vitro* test to the dynamic process of enzyme secretion and digestion makes the conclusion uncertain. Lauff and Hofer (1984) show a rapid increase in the trypsin activity of stomachless roach larvae which reached a level nearly 7 times higher at day 30 when they weighed approximately 20 mg each. On the contrary, these authors did not detect chymotrypsin activity during early growth, and the sharp increase in the activity of this enzyme took place only when the fish weighed more than 25 mg. Aminopeptidease activity in roach larvae was several times higher than in coregonid or rainbow trout alevins, but a drastic increase in activity occurred after the same size was reached 30 to 40 days after the first feeding. Although these authors reported successful rearing of roach larvae with an artificial diet, it is characteristic that the increase in the activity of digestive enzymes and of those associated with the epithelium occurred when the larvae reached the size considered to be critical for cyprinids reared on commercial dry diets (Bryant and Matty, 1981 ; Dabrowski, 1984).

Recently, Forstner *et al.* (1983) reported a biochemical and physiological process connected with morphological changes in coregonid ontogenesis. They suggest that up to 40-50 days after hatching, *i.e.* 50-60 mg of individual weight (at 10 °C), the source of energy is fatty or amino acids metabolized via the citrate cycle. Glycogenolysis and glycolysis become the main pathway of energy metabolism when enzymes like phosphofruktokinase increase vastly and anaerobic metabolism in the muscle fibers begins to dominate. Inversely, in the ontogenesis of rainbow trout larvae, the glycolytic pathway precedes the oxidative pathway.

Eldridge *et al.* (1982) when examining the bioenergetics and growth of fish larvae fed on live *Artemia,* emphasized, for the first time to my knowledge, the importance of yolk lipid and oil globule utilization in concordance with exogenous food. The larvae fed with progressively higher concentrations of live *Artemia*

consumed energy at a faster rate, whereas the starved larvae conserved the oil (Eldridge *et al.,* 1983). It is not yet clear what this process means in terms of larval fish assimilation of the first exogenous food. These authors observed poor digestion of *Artemia* nauplii in older larvae of striped bass and nearly intact zooplankters were passing through the intestine, especially in the high concentrations of food. Non-assimilation increased when the lipid stores were exhausted, suggesting that when using *Artemia* exclusively as the first exogenous food, *Artemia* lipid should be enriched quantitatively and/or qualitatively. Kaushik and Dabrowski (1983a) found that carp larvae did not utilize nitrogen efficiently. A total daily loss of nearly 36 % of body nitrogen stores occurred at the first exogenous feeding. Buckley and Dillmann (1982) recently reported that primary amines are excreted by fish larvae with a postprandial increase corresponding to an increase in ammonia excretion. Cetta and Capuzzo (1982) indicated that primary amines and other unidentified nitrogenous substances constitute more than 50 % of the total nitrogen loss in winter flounder larvae. There are still many things to be learned as concerns the metabolic pathways of transfer from endogenous to exogenous feeding in fish larvae.

Johns *et al.* (1981) have shown that a range of temperatures or cyclic temperatures do not affect the efficiency or length of yolk sac absorption in marine fish larvae ; but the situation is different in other species of larval fish which show the best performance at lower temperatures (Dabrowski and Luczynski, 1984).

Ehrlich and Muszynski (1982) have shown recently that food availability and temperature selection must be considered together when interpreting larval bioenergetics. Pretein mobilization was very active at the lowest possible temperatures for the larval grunion, a marine fish, whereas oil metabolization decreased drastically. At the highest temperatures tolerated by the larvae of this species, both fat and protein utilization decreased, the latter more strongly. Since the temperature selected was 5 to 7 °C higher for fed than unfed larvae, the latter, even after one day of fasting, showed specific metabolism in the different conditions ; the colder water reduced the metabolic rate and conserved energy when the food was limited, allowing maximization of daily bioenergetic efficiency. Another aspect, however, should be taken into account, namely, the regulatory role of hormones during diurnal activity. Meier and Fivizzani (1980) indicated that, when injected during the day, prolactin, the most potent lipogenic hormone in teleosts, could more than double body fat stores within one week. The response to hormonal rhythmicity was so firmly anchored in the daily photoperiod that injections of prolactin at other times were either ineffective or reduced the fat stores. Both temperature and daylength influence the prolactin rhythm in fish. Since it has been found that the metabolic rate of carp larvae depends highly on cyclically fluctuating temperatures and that survival is better than in constant temperatures (Jezierska *et al.,* 1979), performance can be improved by putting the fish in conditions imitating the natural ones.

Jezierska *et al.* (1979) proposed that not only activity but other factors must be responsible for the metabolic adjustement of carp to fluctuating temperatures. Although these authors did not vary the light during their

experiment, Kaushik *et al.* (1982) found very pronounced rhythmicity in larval carp due to day-night changes in the illumination. So, the effects of temperature and of light intensity may potentialize the behavioural, metabolic and hormonal responses of young fish. This is certainly an interesting direction to explore.

IV. The natural food of fish larvae.

A feasible alternative to the less successful artificial diet for larval fish is inert, preserved natural food or natural food cultured in intensive systems. There is already much work in the literature dealing with the quality of various species of invertebrates used as fish food ; their quality depends on the food offered to them and on their origin. The preparation of a fully successful diet for several species of zooplankters is not an easier task than to prepare an artificial diet for fish larvae.

The starting point in considering zooplanktonic organisms as larval food should be their biochemical characteristics. However, the problems is more complex since the proximal mineral, protein and fatty acid compositions of zooplankton depend on origin, season and food and most of all, on species.

Phytoplankton, when offered alone, has no nutritional value. Although phytoplankton is frequently encountered in the larval digestive tract during the first feeding (Lasker, 1975), larval distribution and survival can occur only in a chlorophyll maximal layer when the phytoplankton is supplemented with microzooplankton. Dinoflagellates support the growth of larval anchovies in the longer period (Scura and Jerde, 1977).

Moksness (1982) found that the dominant organism that capelin (*Mallotus villosus*) larvae feed on during the first two months of growth are the larvae of *Spionidae.* Veligers of *Littorina* appear in the gut, demonstrating a close relation to the density of these organisms in the enclosures.

Watanabe (1979) and Watanabe *et al.* (1983) have reviewed most of the research done on the chemical composition of zooplankton and its nutritional value for fish larvae. Unfortunately, when comparing the principal factors of these living feeds, their dietary values were referred to in relation to the requirements of juvenile or adult fish, neglecting possible differences in ontogenesis.

In the main larval food, protein content varied between 45 and 65 %, but crude lipid and crude ash contents varied more. Lipid content in *Artemia* nauplii was 17.5 to 25.8 % and in *Brachionus plicatilis* it even reached 31 % of dry matter (Watanabe *et al.,* 1983). Iron content was very variable depending on *Artemia* origin, but the authors were of the opinion that this was not the crucial factor in the larval fish diet.

Arginine, the major free amino acid in zooplankton, was found in amounts up to 1.43 %. Bearing in mind the specific metabolic importance of this amino acid, Dabrowski and Rusiecki (1983) have discussed its possible role. Arginine concentration, expressed as a molar value, is 10 mM and, together with free amino acids in zooplankton, makes a solution of 30-40 mM. This value compared

to the molar concentration of free amino acids in the digestive tract of adult fish (Dabrowski, 1983a) leads to the conclusion that only the mechanical destruction of zooplanckton, without any proteolytic activity, creates a high concentration of available amino acids in the lumen of the larval fish gut. This is no doubt important information, but there was no attempt to formulate an artificial diet for larval fish which would include free amino acids in a proportion similar to the natural living food.

The nutritional quality of living feeds is attributed mostly to their fatty acid composition which is affected by the culture environment. Rotifers cultured with baker's yeast contain very small amounts of ω3 highly unsaturated fatty acids (ω3 HUFA), such as 20 : 5 ω3, and high amounts of monoenoic fatty acids such as 16 : 1 and 18 : 1. In comparison to those cultured on baker's yeast, rotifers which contain 1.4-1.9 % of 20 : 5 ω3 in total lipid or those cultured on yeast and *Chlorella* or on *Chlorella* only increase the content of this fatty acid up to 8.1-11.8 and 22.8 and 31.8 %, respectively. Freshwater *Chlorella* is rich in linoleic and linolenic fatty acids, whereas marine *Chlorella* is rich in ω3 HUFA (Watanabe *et al.,* 1983). The lipid fatty acid composition in other organisms like *Artemia* and, to a lesser extent, the marine copepods, *Tigriopus* and *Acartia,* and the freshwater copepods, *Moina* and *Daphnia,* corresponds in a similar way to the culture medium.

Salmon, carp and eel require a certain proportion of linoleic and linolenic fatty acids to achieve maximal growth, whereas marine fish respond much better to the ω3 HUFA content in the diet. Watanabe *et al.* (1983) found that both the growth and survival of marine fish larvae can be considerably improved when they are fed live food with a high ω3 HUFA content. Compared to a group fed a low level of ω3 HUFA, survival increased from about 20 to 50-80 % and the length of the fish doubled when they were offered ω3 HUFA-rich zooplankton. Consequently, Watanabe *et al.* (1983) developed two methods to increase the ω3 HUFA level in zooplankton and in this way to improve its nutritional quality. They fed the zooplankton either yeast or *Chlorella* containing a high level of the desired fatty acids or added emulsified ω3 HUFA to the medium ; both methods raised the level of essential fatty acids within 12 h. Oka *et al.* (1982) presented results on the fatty acid composition of larval ayu (*Plecoglossus altivelis*) after 20 days of feeding with *Moina* of different origins. *Moina* fed on baker's yeast, *Chlorella* or special ω-yeast affected considerably the ω3 HUFA content in fish (9.6, 16.3 and 22.5 % of lipids, respectively).

The fatty acid composition of the krill, *Euphasia superba,* suggests that it could be an interesting option as a source of 20 : 5 ω3 (30-31 % of the total) (uncorrected detector response) (Clarke, 1980).

Bromley and Howell (1983) indicate that pre-feeding *Artemia* metanauplii with algae for 2 days significantly increased the nutritional value of *Artemia* nauplii and that, consequently, fish fed on the metanauplii accepted and grew better on the artificial diet. Another way of directly improving *Artemia* value was proposed by Sakamoto *et al.* (1982). For *Artemia* nauplii, these authors formulated an artificial diet by encapsulation which increased the level of ω3 HUFA and thus the nutritional value of *Artemia* for the fish larvae.

Robin (1982), working with the larvae of *Dicentrarchus labrax* has shown that *Artemia* nauplii pre-fed on a compound diet gave more satisfactory growth results than nauplii fed only on yeast. Fish weight was 209.9 and 73.1 mg and survival 24 and 54 % with the former and latter *Artemia* sources, respectively.

Although *Chlorella* has proved to be the best food for cultured rotifers, it is expensive to raise, requiring equipment, space and man-power. Teshima *et al.* (1981) developed a micro-encapsulated diet for rotifers based on casein (48.26 %), glucose, sucrose, α-starch which, without storage or preservation, supported good rotifer growth for 9 days. A similar procedure was attempted by Gatesoupe and Luquet (1981) and Gatesoupe and Robin (1981). These authors formulated practical diets for rotifer culture, substituting freeze-dried algae, and obtained relatively good success in rearing sea bass larvae fed on the rotifer. Other products like yeast and the commercial algae, *Spirulina* and *Chlorella,* were found to be suitable food sources for the rotifer, *Brachionus plicatilis.*

Yamasaki and Canto (1980) reported a very interesting larval food, namely, an euryhaline harpacticoid copepod, *Tisbintra elongata.* This species is easily cultured on rice bran and fermented fish solubles and, when offered to milkfish (*Chanos chanos*) larvae, it gives good growth and survival not significantly different from those of fish fed on *Artemia* nauplii.

Vanhaecke and Sorgeloos (1983) have shown that the nutritional value of *Artemia* nauplii from nine different sources did not differ when tested with common carp larvae and that survival (over 90 %) was a criterium of food quality. Growth differences in carp were attributed to the size, *i.e.* the weight, of nauplii and highly correlated with it. In *Menidia menidia,* nauplii size was a limiting factor and a significant increase in fish mortality was observed as nauplii length increased from 430 to 510 μm (Beck and Bengtson, 1982).

Klein-MacPhee *et al.* (1982) found very pronounced differences in winter flounder when *Artemia* strains of different geographical origin were fed. *Artemia* nauplii from San Pablo Bay gave significantly lower survival (15 %) than the other ones. Nauplii of this origin had only 1.68 of 20 : 5 ω3 and 33.9 % of linoleic acids, whereas the other strains had 8.01-15.35 % of 20 : 5 ω3. These authors also pointed out that the pesticide content could be only a contributing factor to fish mortality as nauplii heavily contaminated with DDT sometimes gave excellent larval survival.

Farkas (1979) observed that temperature has a pronounced effect on the HUFA content in copepods. When copepods pre-adapted to warm summer temperatures were gradually placed under low temperatures (4-5 °C) for 48 h, their fatty acid composition changed drastically with the docosahexaenoic acid (22 : ω3) level, increasing from 10 to 24 %. It is useful to know that the nutritional value of natural plankton can be improved simply by decreasing the environmental temperature before the food is offered to larval fish. However, cladocerans did not have a similar response.

Lee *et al.* (1981) did not encounter many differences in the larvae of black sea bream (*Mylio macrocephalus*) reared up to day 10 on either *Chlorella*-fed rotifers or those cultured on baking yeast. The differences in performance became

apparent at day 18 of rearing when the group fed the yeast rotifers displayed poorer growth and survival.

Knowledge of the role of invertebrate proteases may also contribute to a better understanding of the digestion process in larval fish (Dabrowski and Glogowski, 1977 ; Lauff and Hofer, 1984). There is strong evidence that both acid proteinases and trypsin-like proteinases are present in crustaceans. The hydrated embryos of *Artemia salina* have an acid protease and two accompanying inhibitors controlling the activity of the protease (Nagainis and Warner, 1979). It is possible that these inhibitors remain in newly hatched *Artemia* nauplii. The hydrolysing capacity and specificity of crustacean serine proteases and the trypsin-like protease of vertebrates show some differences (Kimoto *et al.,* 1983). Invertebrate proteases are very resistant to self-digestion ; at pH 8 and 30 °C, crayfish trypsin retains 90 to 100 % of its initial activity after 2 to 3 days. Chymotrypsin activity is absent in crayfish, and the proteolytic enzymes are immediately and irreversibly inactivated at pH 3.0 (Zwilling and Neurath, 1981). This limits the possible role of enzymes when the functional stomach of fish reaches that acid pH (Lauff and Hofer, 1984). Crustaceans also have low molecular weight proteases which are isolated from both the hepatopancreas and the digestive fluid without prior activation like trypsin and carboxipeptidase. These alkaline-stable proteases with optimum alkaline pH (Armstrong and De Villez, 1978) can be easily washed out of frozen material and recorded in the medium (Grabner *et al.,* 1981). Neither low-temperature freezing (Ahmed *et al.,* 1976) nor freeze-drying (Zwilling and Neurath, 1981) destroy invertebrate proteolytic activity, although a cell-free extract of copepods at − 20 °C showed substantial activity loss. The role of zooplankton proteases as activators of fish zymogen is undeniable (Jancarik, 1964) as is the direct contribution of proteolytic activity to the autolytic processes of food organisms. The process of protein digestion in the very short gut of larval fish is an enigma. Considering the short evacuation rate of the straight larval tube, it is difficult to explain the fast growth of the larvae. Carp juveniles evacuate 50 % of ingested zooplankton within 45 min after feeding (Kaushik and Dabrowski, 1983a) ; when comparing this value of 10-mg fish to the initial larval weight of 1 mg, the zooplankton seems to have required only 30 min to pass through the intestine.

Indeed, Fossum (1983) indicated that evacuation time might not be a good criterium of the process of zooplankton digestion in the larval fish intestine. This author concludes that, although the passage time in larval herring is 12.5 to 22.5 h at 6 °C, the digestion time is much shorter, *i.e.* it takes 1.5 h for copepod nauplii to become transparent. The use of this criterium in estimating the digestive process in the larval fish gut is debatable, but the evacuation rate itself is certainly not sufficient for measuring food digestion. The activation time of catfish trypsinogen and chymotrypsinogen by porcine enteropeptidase at 37 °C is 1 and 2 h, respectively (Yoshinaka *et al.,* 1981).

Autolysis of cyprinid proteases during gut passage is very low (3-5 % of the total activity) (Hofer, 1982), and it can be assumed that the activation time of larval zymogens is much shorter and/or that intestinal enzymes and zooplankton proteases are more efficient than mammalian enzymes when activated. Self-

activation of carp zymogens in optimal conditions is a long-term process (Cohen *et al.,* 1981a). It has been shown recently that the catalytic action of fish trypsin and chymotrypsin on specific substrates is enhanced 10 to 100 times in comparison to mammalian enzymes (Cohen *et al.,* 1981b) or several times as concerns the proteolytic efficiency of both enzymes (Krogdahl and Holm, 1983). To conclude, it is difficult to draw definite conclusions as to the role of zooplankton proteolytic enzymes, but the autolysis of plankton crustacea and the activation of fish zymogens definitely contribute to food utilization in larval fish.

Another enzyme pool of physiological importance in the digestive process is that of alkaline phosphatase. Wynne and Gophen (1981) indicated that the activity of this enzyme in zooplankton is related to the quality of the ingested food, whereas acid phosphatase activity is unrelated. The activity of this enzyme has been compared in zooplankton and fish. Available data in the literature refer to the intestines of the common carp and catfish ; the activity of alkaline phosphatase was 50 nM of 4-nitrophenol released per mg of protein per min (25 °C) (Fraisse *et al.,* 1981). Cvancara and Huang (1978) studying a number of fish, reported that the alkaline phosphatase activity of liver and kidney was 10-20 and 100-200 nM/mg protein/min (37 °C), respectively. However, alkaline phosphatase activity in *Mesocyclops leucarti,* recalculated to a comparable unit, was higher by several orders of magnitude than that found in fish tissues (4.10^4 to 5.10^5 nM/mg protein min^{-1}) (Wynne and Gophen, 1981). Surprisingly, alkaline phosphatase was not detected in *Bosmina longirostris.* It remains to be determined if the enormous alkaline phosphatase activity of zooplankton ingested by larval fish could have some physiological significance in the digestion and assimilation processes.

I will now discuss zooplankton processed by freeze-drying. Kentouri (1981) divided several marine fish into groups according to the acceptance of frozen plankton and the growth results of the larvae. The larvae of *Mugil auratus, Sparus auratus* and *Dicentrarchus labrax* showed satisfactory growth and survival when fed frozen zooplankton. The larvae of *Gobius* sp. refused to hunt immobile prey, while the larvae of *Trigla corax* rejected the prey after examination. Although they accepted and ingested thawed zooplankton, sole and turbot larvae had a high mortality and poor growth rate ; the authors reported that these fishes were unable to assimilate the ingested food. In a study by Kentouri (1981), the frozen food was either distributed constantly at one fixed point or sporadically by a floating glass block. Medgyesy and Wieser (1982) successfully reared larval *Coregonus* by using an apparatus which decreased to a minimum the leaching time of substances in thawed zooplankton before it was consumed by the fish. Unsuitable distribution and the leaching of free amino acids and enzymes (Grabner *et al.,* 1981) from the frozen zooplankton might be why Raisanen and Behmer (1982) were not successful in rearing the Canadian whitefish, *C. clupeaformis.* However, the results of Medgyesy and Wieser (1982) cast a doubt on the Flüchter (1982) hypothesis of the essentiality of shock-frozen zooplankton preserving a high nutritional value as compared to slow-frozen zooplankton.

It has been shown recently that not only prefeeding *Artemia* on algae (Bromley and Howell, 1983) but also treating the rotifer, *Brachionus*

plicatilis, with an antibacterial drug had a highly significant effect on the survival of turbot larvae (Gatesoupe, 1982). It was concluded that pathological infection possibly occurred in the course of rearing and that drug ingestion in rotifers can be very effective. However, the antibacterial drug might have affected the nutritional value of the food. Reduced microbial activity could significantly reduce the hydrogenation of unsaturated fatty acids (linoleic and linolenic) which are especially essential to fish. In pigs with reduced microflora in the digestive tract, Eggum *et al.* (1982) observed 44.0 and 7.7 % of linoleic and linolenic acids, respectively, in the feces, whereas in control animals these rates were 6.2 and 3.8 %, respectively. The importance of essential fatty acids in zooplankton has already been emphasized.

Freeze-dried zooplankton was used by Beck and Bengtson (1979) in the larvae of the silverside, *Menidia menidia,* but with little success as concerned growth and survival. On the other hand, Sulaiman (1974) when studying several feeds for common carp larvae, reported excellent growth (30.8 and 72.5 mg individual weight after 3 and 4 weeks of rearing, respectively) when using lyophilized *Daphnia.* The fish fed live *Daphnia* weighed 48.5 and 65-123.8 mg respectively, at the same time but survival in both groups was relatively low (23-36 %). Surprisingly, there is very little data on rearing fish larvae using freeze-dried zooplankton and the results of Dabrowski *et al.* (1978) contradict those of Sulaiman (1974). The former authors included up to 60 % of freeze-dried zooplankton in their diet formulations and the results in common carp and grass carp (Dabrowski *et al.,* 1979) were negative.

The method of preparing zooplankton by freeze-drying needs further study.

V. The formulation of compound diets.

A formulated dry diet for larval fish must certainly correspond to certain physical properties, *i.e.* the size and texture of the food particles, to be acceptable. The presence of food after a certain time period in the fish gut can be expressed as the percentage of animals feeding and is an index of acceptance (Halver *et al.,* 1981). The index of gut fullness is measured as a proportion of the total to the maximum number of feed particles in the gut. It is believed, in the case of the larvae of some species like pike or coregonid, that high acceptance coefficients (AC) already indicate a successful start. These AC appear to be good measurements of behaviourally oriented larval fish ; however, they cannot be used on cyprinid larvae which accept food particles well and which later have no nutritional value. Acceptance is strongly influenced by the aroma of the food, and pronounced differences are found between larval fish in this respect (Appelbaum, 1980) ; carp larvae would not accept sweet-tasting food, whereas tilapia always showed a positive reaction to food with a sweet, sour, bitter or salty taste.

Attractants when added to larval diets may prove equally effective, as shown in experiments with juveniles of 110 mg individual weight (Cadena Roa *et al.,* 1982). The addition of a mixture of betaine, glycine, alanine, arginine, glutamic acid and inosine increased sole survival and individual weight after 40 days from

50.3 to 78.0 % and from 633 to 837 mg, respectively, when the amounts of attractants added were increased from 1.5 to 6.9 %.

The chemical composition of the compound diet should imitate as closely as possible the already-reviewed chemical composition of natural zooplankton. This gives a wide range of tolerance in terms of percentages of protein, lipid and minerals, but less is known about the vitamin content of zooplankton as a reference for the vitamin mixture of a dry diet. The nutritional value of zooplankton and of dietary ingredients tested on juvenile (Watanabe *et al.,* 1983) and adult (Pigott *et al.,* 1982 ; Gabauda *et al.,* 1980) fish provides little information or is even misleading. Evaluated in terms of the protein efficiency ratio (PER) or net protein utilization (NPU) of 1 to 2-g common carp, rotifers and *Artemia* seemed to be inferior to casein, although casein has no nutritional value for larval carp which grow well on both types of zooplankton. Digestibility tests of pepsin-digested fish protein hydrolysate gave very satisfactory results in rainbow trout, the coefficients being well over 90 % ; however, gut bleeding, probably due to the highly hygroscopic nature of this product, was observed. Without more test results, it is over-optimistic to say that this material could be used as the main ingredient of the larval fish diet. The amino acid profile of the ingredients considered for larval diets does not provide much information either. As mentioned earlier (Dabrowski *et al.,* 1978), several commercial proteins are richer in essential amino acids (particularly sulfur amino acids) than zooplankton proteins, but they have no nutritional value in larval diets.

An alternative way of preparing compound diets for fish larvae is microencapsulation. This method was suggested for the feeding of aquatic organisms sometime ago but, as Jones and Gabbott noted in 1976, no experimental results had been reported up to that time. Most of the work published up to now has dealt with invertebrates (Kanazawa *et al.,* 1982). The preparation of microcapsules from aqueous microdroplets was described by Chang *et al.* (1966). A nylon-protein wall is prepared in which the basic amino acids are cross-linked to the nylon wall, but blocking the side-chain NH_2 groups prevents tryptic digestion ; this limitation can be avoided by preparing starch derivatives with a free NH_2 group susceptible to amylase digestion in the larval gut. Capsules containing 25 % nylon can be digested by chymotrypsin but, as mentioned above, the activity of this enzyme is very low in larval fish.

There are several advantages to the microencapsulated diets (reviewed by Jones and Gabbott, 1976), particularly when they are used for experimental purposes and contain ingredients of high nutritional value for the larvae. This method should prove fruitful in the future.

Kanazawa *et al.* (1982) prepared a microparticulate diet, creating a zein membrane by using NaOH or ethanol ; the growth and survival of the prawns was almost comparable to those of animals grown on live feeds.

Microencapsulation as a way of preparing larval diets, needs to be studied further ; even the first experiment using it was not successful (Bryant, personal communication). Sasaki (1981) used freeze-dried homogenate of adult *Artemia* to prepare microcapsules bound with either carboxymethyl-cellulase (CMC) or nylon-protein. The growth of *Fundulus heteroclitus* larvae was negligible in these two

diets in comparison to fish fed on *Artemia* nauplii. The author suggested that leaching the material (40 % of dry matter) during microencapsulation was the cause of these unsuccessful results.

Halver *et al.* (1981) mentioned a special plastein starch-bound diet and indicated its good acceptability by both marine and freshwater fish larvae, but no growth data were available.

Murai *et al.* (1981) proposed coating synthetic amino acids with casein to increase their retention time in the gut and to improve their utilization. There is some indication that this process improves amino acid assimilation in carp juveniles, but it was not certain ; in Murai's study, diet 6 should have contained the same amount of casein as used for coating in diet 7, rather than a higher level of gelatin. These points were clarified in a later work by the same authors (Murai *et al.*, 1982) who showed that, as the casein was simply added to the diet instead of using it as a binder of synthetic amino acids, its effect on fish growth was greater than when it was used as a coating. Dabrowski (1983b) used coating and polymerized lysine in zein base diets, and neither process improved crystalline amino acid utilization much. No studies of this kind using semi-purified diets and amino acids have been done on fish larvae.

Another method of improving the nutritional quality of food proteins is through the covalent binding of free amino acids (Voutsinas and Nakai, 1979) ; due to carbodiimide binding, the tryptophan and methionine contents in protein were increased 11 and 6 times, respectively. Bound amino acids are readily released, as demontrated *in vitro* and *in vivo* by Puigserver *et al.* (1978). Although these methods are interesting and they avoid the objectionable odors and reactions with other components, which are inevitable when free amino acids are added to the diet, the enzymatic enrichment of dietary proteins appears more promising (Arai *et al.*, 1978). One of the advantages of this latter method is that reaction is streospecific and only L-racemic ester is used as a substrate. Bound amino acid can be increased 5.1-fold, as in the case of lysine (Ikura *et al.*, 1981) or lead to 32.5 % of methionine or 22.5 % of tryptophan, if the protein is enriched (Ashley *et al.*, 1983). The latter authors studying rats showed that amino acids in plastein form gave the same results as in a free form. Plastein proteins containing peptides and bound synthetic amino acids represent a new direction of exploration in nutritional studies on fish. Protein of designed amino acid composition provides a unique opportunity to study fish amino acid requirements using nitrogenous compounds of known molecular weight.

The initial manufacturing phase of a partial hydrolysate was described by Beddows and Ardeshir (1979). Conditions of temperature, pH and enzyme activity can be established to obtain a product containing the desired concentration of nitrogenous compounds. Furthermore, it can be enriched with specific amino acids by plastein reaction.

Buddington and Doroshov (1983) have reported the successful rearing of sturgeon larvae on a semi-moist (22 % water) diet only. The fish willingly accepted « Biodiet » in comparison to other dry food and mortality was negligible. After 38-40 days of trial, the sturgeon juveniles grown on semi-moist « Biodiet » were significantly smaller than the control group fed on live tubificids. These results

closely corroborate those of first-feeding salmon fry given « Biodiet » (Lemm, 1983).

Molvik *et al.* (1984) recorded the initial growth of cod larvae fed on a diet based on cod roe, but metamorphosed fish were not obtained. The roe, boiled, fortified with vitamins, antioxidants, vegetable oil and freeze-dried, contained a high level (23.2 %) of docasahexaenoic acid, the fatty acid required by fish larvae. Cod roe were used by Hodal (1983) in a successful attempt to grow eels in a recirculated system. This food was gradually replaced by a dry trout diet as the fish reached 1 g of individual weight.

Rothbard (1982) has described a method of microencapsulating whole egg for a larval diet (after Chow, 1980). No data on carp larvae growth or survival are given, but Woynarovich (personal communication, 1983) considers this method only as a preliminary measure before stocking the fish in ponds. Lyophilized hen egg yolk was found to be useless as an exclusive diet for carp larvae (Sulaiman, 1974), and freeze-dried carp eggs, although giving a relatively high survival of carp larvae up to week 4 of rearing (30-57 %), resulted in negligible growth (Sulaiman, 1974). When freeze-dried and offered to carp larvae, other parts of the carp body (liver, muscle), or even the whole fish, had no value (von Lukowicz, 1977 ; Dabrowski *et al.,* 1978). Flüchter (1982) found that an acetone extract of *Artemia*, when added to a standard-formula diet, supported the growth and metamorphosis of *Coregonus* sp. larvae. This requires further verification ; Huse *et al.* (1982) were not able to confirm the use of the zooplankton extract as a successful rearing diet when fed to cod larvae on dry diet alone.

The products which have been somewhat successful so far in rearing larval fish solely on dry compound diets are single-cell proteins (SCP) (Dabrowski *et al.,* 1983). A certain kind of yeast used as the major ingredient of the diet ensures the growth and appreciable survival of several species of cyprinid (Dabrowski, 1984) and coregonid (Dabrowski *et al.,* 1984b) larvae. Preliminary studies have indicated that the optimal yeast content is about 60 %, but the accompanying ingredient is important. Freeze-dried pork liver gave more successful results than the freeze-dried spleen or fish meal ; a similar formulation (57 % of SCP and 28 % of freeze-dried pork liver) was used in an experiment with coregonid larvae. Bacterial protein was shown to be of lower value than two sources of yeast, methanol-grown yeast (Protibel) and IFP (Institut Français de Pétrole) yeast (Dabrowski *et al.,* 1984b).

Some species of cyprinid larvae like those of common carp seem to be « difficult » and others, like those of silver carp, seem to be « easy » (Kainz and Gollmann, 1981 ; Dabrowski, 1984). An alternative to the exclusive feeding of a dry diet is to determine the « adaptation weight » of the larvae (Bryant and Matty, 1981) and then offer them dry food when they reach that size. With the diet formulation given above, the adaptation weight is about 5-6 mg for the four cyprinids studied, which means 4 to 6 days of initial zooplankton feeding. Gatesoupe (1983) weaned sole larvae by initially feeding them on live *Artemia* for 10-15 days and then changing to a semi-moist compound diet containing 31 % casein, 27.7 % fish meal, 4.8 % clam flesh and other additives.

Freeze-dried krill cannot be used as a dietary ingredient at initial feeding, but after adaptation weight is reached, a diet containing krill has been proved to be equally good in some cyprinids (Dabrowski, 1984). Braaten *et al.* (1983) indicated that when cod fry were fed a diet composed of 45.5 % herring meal and 27.3 % krill meal, high growth and survival rates were obtained.

Several problems in the formulation of dry diets need more study because appreciable growth and survival of larval fish have been obtained with these diets. Dabrowski *et al.* (1983) indicated wide differences in the mineral composition (P and Ca) between fish fed zooplankton and those fed a fry diet, although the total mineral content of those diets did not suggest these variations. It remains to be elucidated why some SCP products have high nutritional value for larval fish and others do not.

Conclusions and recommendations

Fish larvae are susceptible to dietary deficiency in a more spectacular way than juvenile or adult fish, but a small size of the eggs or larvae does not imply lower vitality ; in some fish species, the inverse has been convincingly documented. The nutritional history of the parents and its effect on progeny growth and survival should be explored in detail. The evidence gathered so far suggests that nutrient allocation for reproduction might result from some dietary deficiency.

Several environmental conditions affect the feed acceptance of both the live and the compound diets by larval fish and, even crucial factors like water temperature and day-night cycle have not been sufficiently studied in a fish species of major aquacultural interest. Generally, it appears desirable to increase water temperature above that natural to the species (Blaxter *et al.*, 1983) when rearing in intensive systems. There is already enough data to suggest the positive effect of variable cyclic water temperature and illumination on behaviour, hormonal balance and metabolism.

Morphological and functional differences between fish species and during ontogenesis may, to some extent, explain the nutritional constraints in the acceptance and assimilation of a dry compound diet. Despite some excellent works on digestive enzymes in larval and juvenile fish in 1983 and 1984, this area of research still requires further study.

In a number of cases, the nutritional value of the zooplankton is due to its fatty acid (ω3, HUFA) content ; this problem can be dealt with in several ways and possibly also by regulating the water temperature. Much less is known about the other biochemical consituents of zooplankton, *i. e.* protein, peptides, amino acids, vitamins and enzymes. Knowledge of these components is essential to understanding the processes of digestion, absorption and assimilation in larval fish.

Finally, the formulation of a dry compound diet should take more account of zooplankton composition. Several basic problems, as freeze-drying the

zooplankton, need further research to evaluate its effectiveness. New methods designed to increase the nutritional value of dietary proteins have been discussed ; these include the preparation of zooplankton substitutes, taking into account the molecular weight and amino acid composition of the proteins. A compound diet based on SCP proteins and freeze-dried tissues has given good growth and survival when offered at weaning. This line of research should be continued since fish performance can be improved by modifying diet formulations and rearing conditions.

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Résumé. *Alimentation des larves de poisson : savoir-faire et perspectives.*

La présente revue a pour objet l'exposé des principaux acquis en matière d'alimentation des larves de poisson et de proposer des hypothèses de travail pour le développement des recherches. L'historique alimentaire des reproducteurs est en premier lieu évoqué pour ses effets sur la vitalité des larves ; de même les facteurs environnementaux peuvent affecter le comportement des larves dans les enceintes d'élevage. Bien que fragmentaires, les connaissances relatives à la morphologie et à la physiologie du système digestif s'avèrent d'un grand intérêt. Ainsi les processus de digestion et d'absorption apparaissent affectés de façon considérable par le stade physiologique : larve, juvénile, adulte. La connaissance de la composition biochimique du zooplancton — aliment naturel des larves — de son équipement enzymatique et la participation de celui-ci aux processus de la digestion méritent une attention soutenue en particulier pour les pratiques d'alimentation lorsque des formulations composées ne peuvent pas être utilisées. Celles-ci n'ont procuré jusqu'à l'heure que peu de résultats satisfaisants, toutefois il apparaît que l'incorporation de protéines unicellulaires améliore considérablement la survie et la croissance de larves et permet de procurer des performances proches de celles obtenues avec une alimentation à base de zooplancton. Chaque section de cette revue comporte des suggestions quant au développement souhaitable d'axes de recherche.

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