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Studies on the suitability of commercial dry diets for rearing of larval *Coregonus lavaretus* from Lake Constance

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Abstract

The suitability of two commercial dry diets for rearing *Coregonus lavaretus* larvae was tested in a 58-day experiment. A diet of live zooplankton was used for comparison. The trials were conducted at water temperature of 12°C in 800 l tanks, with a stocking density of 10000-25000 larvae per tank. Diets were evaluated by means of growth and liver histological analysis. Best growth was obtained with zooplankton, but one of the two dry diets (TetraWerke Az 20) also resulted in satisfactory growth. Examination of liver structure using light and electron microscopy revealed no nutritional pathology for larvae fed with dry diet Az 20. Histological diagnosis presented evidence for a close association between hepatic protein synthesis and larval growth. From the data of the present study it can be concluded that a large-scale rearing of larvae of *Coregonus lavaretus* exclusively on dry food, is feasible.

Keywords : *Coregonus lavaretus*, larval nutrition, dry diets, growth, liver histology.

Croissances comparées de larves de Coregonus lavaretus *du lac de Constance, nourries avec des aliments secs, préparés industriellement.*

Résumé

L'efficacité de deux régimes alimentaires à base d'aliments composés artificiellement pour l'élevage de larves de *Coregonus lavaretus* a été étudiée lors d'une expérimentation de 58 jours. Un régime à base de zooplancton a été utilisé pour comparaison. L'étude a été faite avec de l'eau à 12°C, dans des bassins de 800 l et avec 10000-25000 larves par bassin. Les effets de la nourriture ont été étudiés sur la croissance et par l'examen histologique du foie. La meilleure croissance a été obtenue avec du zooplancton. Mais aussi une croissance satisfaisante était obtenue avec des nourritures synthétiques (TetraWerke Az 20). L'analyse du foie chez les larves nourries avec Az 20 ne montre pas de signes de déficits nutritionnels. La diagnose histologique indique l'existence d'un rapport entre la synthèse protéinique du foie et la croissance larvaire. Les résultats obtenus montrent qu'un élevage de larves de *Coregonus lavaretus* en grande quantité est possible uniquement avec de la nourriture synthétique.

Mots-clés : *Coregonus lavaretus*, nutrition larvaire, aliments secs, croissance, histologie du foie.

INTRODUCTION

In order to maintain *Coregonus* populations in Lake Constance, the lake is regularly stocked with larvae obtained from eggs incubated in hatcheries (Löffler and Deufel, 1980). Less than 10% of these larvae are prefed indoors before release into the lake. For rearing of the larvae in the hatcheries, zooplankton collected from Lake Constance is used, although this procedure has a number of limitations, e. g. problems in obtaining sufficient quantities of plankton at any time or problems with the nutritional quality of the plankton (Eckmann *et al.*, 1986). Therefore, for intensive rearing of larval coregonids, suitable dry diets would be advantageous. In several recent laboratory studies it has been demonstrated that successful rearing of whitefish larvae exclusively on dry diets is possible (Dabrowski *et al.*, 1984; Rösch and Appelbaum, 1985; Bergot *et al.*, 1986; Luczynski *et al.*, 1986; Rösch and Dabrowski, 1986).

Two aspects remain to be clarified before artificial diets can replace zooplankton as starter feeds for routine use in hatcheries (Rösch and Dabrowski, 1986): (1) Most dry diets which gave successful results in rearing trials with larval coregonids have been tested at water temperatures of 15°C or above. For practical purposes, their performance at the temperatures available in hatcheries (10-12°C) has to be tested. (2) Until now, rearing trials have been conducted under laboratory conditions only. The performance of fish on dry diets in larger rearing units remains to be established.

The present study intends to contribute to the aforementioned questions. For this purpose, feeding trials were carried out using two commercial dry diets. Results have been evaluated by means of growth and survival. Furthermore, in accordance with the suggestion of Bergot *et al.* (1986) to use additional criteria in the development of dry diets for larval coregonids, we have employed liver histological diagnosis as a new tool in discrimination between the quality of test diets.

MATERIAL AND METHODS

Experimental procedures

The larvae used were newly hatched larvae of pelagic spawning (=Blaufelchen) *Coregonus lavaretus* from Lake Constance. Hatching occurred at about 8°C. Feeding started 4 days after hatching (equals day 0 of the experiment). The trials lasted from 25 April 1986-19 June 1986.

The feeding trials were carried out in circular 800 l plastic tanks, receiving pre-aerated ground water at a continuous flow rate of 7 $l.min^{-1}$. Each tank was gently aerated. Water temperature was held at 12 ± 0.5°C. Initial stocking density was about 25000 larvae per tank for the trials using dry diets and 10000 per tank for the live food trial. The tanks were cleaned daily and fish losses quantified. An exact measurement of mortality proved to be impossible due to the high initial density of larvae.

Two dry diets (TetraWerke starter food types Az 20 and Az 3) were tested in comparison with live zooplankton. The proximate composition of the dry diets is shown in *table* 1. Live zooplankton was collected every second day from Lake Constance-Obersee using a plankton net with a mesh size of 200 µm. Plankton was supplied to the larvae in excess twice per day. Those plankters which were not fed to the fish immediately after catching were stored in an aerated 50 l plastic tank for a maximum of 1.5 days. Plankton which sedimented during the storing period was not fed to the fish. Due to the periodicity in Lake Constance plankton availability was limited from day 34 to day 58. Therefore satiation feeding could not be maintained during this period. The formulated diets were presented as microgranules of <200 µm diameter during the first 3 weeks of rearing and as 200-500 µm granules during the following weeks. Artificial food was provided continuously between 8 a.m. and 6 p.m. using automatic feeders (Scharfling system). The initial daily ration of 10 g per tank was increased to 15 g per day from day 19 and to 20 g per day from day 27 onward. The daily ration was adjusted in order to reach a maximum daily intake. Once a week in the dry food groups Chloramin T was added to the water at a concentration of 1 $g.m^{-3}$ as a prophylactic measure against gill disease (Deufel, 1974).

Growth analysis

Samples for growth measurements were taken at days 0, 13, 21, 28, 34, 42 and 58 of the experiment. From each treatment a minimum of 20 larvae were sampled. The total length (to the nearest 0.5 mm) of each fish was measured, whereas the wet weight (to the nearest 0.1 g) was recorded for the entire sample. For measuring the dry weight of the larvae, the sample was dried at 80°C to constant weight before the dry weight was determined.

The effect of diet type on the final average total length was tested using one-way analysis of variance. The Student's t-test was used to determine differences between the means.

Table 1. — Proximate analysis of the dry diets.

Content (% of dry matter)	Az 3	Az 20
Moisture (% as fed)	6.6	7.6
Crude protein (N×6.25)	66.2	69.5
Crude fat (ether extract)	15.2	13.8
Crude ash	9.6	10.1
Gross energy (kJ/kg)	23030	22800

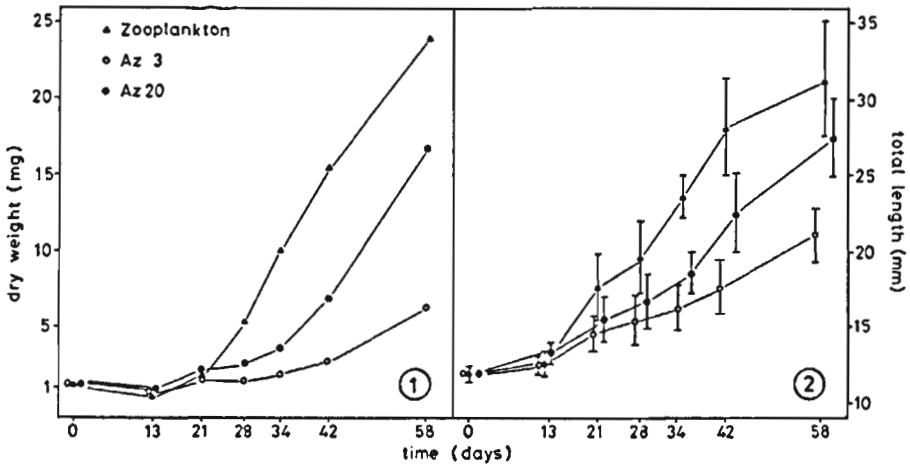

Figure 1. — Dry weight (mg) increase of larval *Coregonus lavaretus* fed on the dry diets Az 3 and Az 20 or on zooplankton collected from Lake Constance.

Figure 2. — Length (mm) increase of larval *Coregonus lavaretus* fed on the dry diets Az 3 and Az 20 or on zooplankton collected from Lake Constance. Means ±s.d. are indicated.

Liver analysis

Histological examination of the liver was conducted on samples from days 13, 21, 28, 34 and 58 of the experiment. From each treatment 5-8 specimens were fixed for light microscopy and 4-6 specimens for electron microscopy. The larvae were sampled approximately 30 minutes after initial feeding in the morning.

For light microscopy, the digestive tracts of the larvae were fixed for 24 hours at room temperature in Carnoy's fluid. After dehydration, they were embedded in paraffine wax. 7 µm thick serial transverse and sagittal sections were stained with methylgreen-thionine (Roque *et al.*, 1965) for RNA and DNA (control: acid hydrolysis) and with Best's Carmin for glycogen (Pearse, 1968) (control: diastase digestion).

For transmission electron microscopy, the livers of the larvae were dissected and fixed for 24 hours at 4°C in 2.5% glutardialdehyde in 0.1 M cacodylate buffer, pH 7.6. The tissues were then rinsed several times in buffer, postfixed for 2 hours with osmium ferrocyanide and stained overnight *en bloc* in 1% uranyl acetate. After dehydration, the tissues were embedded in araldite. Semithin (0.5 µm) and ultrathin sections were prepared with glass knives. The semithin sections were stained with methylen blue-azur II. The ultrathin sections were mounted on copper grids, stained with lead citrate and examined under a ZEISS EM 9-S2 electron microscope.

In order to obtain diet- and not hunger-related symptoms, only larvae with filled intestines were used for histological diagnosis.

Qualitative histological examination was done with coded sections in order to avoid any subjective influence by the interpreter. Quantitative measurements were made on hepatocyte nuclear area. For this purpose, the areas of hepatocyte nuclei were measured on light micrographs (final magnification of 1 100×) using a computer-based planimeter equipped with VIDS III software (AI Tektron). Statistical evaluation of the morphometrical data was made using the non-parametric U-test of Wilcoxon, Mann and Whitney (Sachs, 1984) The level of significance adopted was $p<0.05$.

RESULTS

Food acceptance

Live zooplankton was readily accepted by the larvae from the beginning of exogeneous feeding onwards, whereas only 45 to 65% of the larvae accepted the dry particles within the first 10 days of feeding. Subsequently approximately 80% of the larvae accepted the dry food.

Mortality

Because of the high number of larvae in the tanks, only estimates of mortality can be provided. Cumulative mortality was about 10% in the zooplankton trial and about 50% in the dry food-groups. Most of the losses in the dry food groups occurred during the first 2-3 weeks of the experiment and were due to the loss of non-feeding larvae.

Growth

The increase in length and dry weight of the larvae is given in *figures* 1 and 2, specific growth rates are

Table 2. – Specific growth rate (% dry weight/day) of *Coregonus lavaretus* larvae fed for a period of 58 days (25.4-19.6.1986) on a diet of zooplankton or the dry foods Az 3 and Az 20.

Period (days)	Az 3	Az 20	Zooplankton
0-13	−3.06	−2.19	−4.07
13-21	9.39	12.86	15.33
21-28	−0.98	3.09	15.33
28-34	4.27	4.57	11.16
34-58	5.21	6.82	3.75
0-34	1.19	3.11	6.43
0-58	2.84	4.63	5.31

presented in *table* 2. Growth, in terms of length and weight, was similar for all treatments during the first 3 weeks of rearing but became different during the fourth week, when zooplankton-fed larvae showed a pronounced length and weight increase. According to ANOVA, length differences among the three test groups were significantly different from day 28 onwards, although after day 34 growth in the live food-group was no longer optimal due to a limited availability of zooplankton.

The specific growth rate based on dry weight (*table* 2) reveals a fairly good growth for larvae fed on Az 20, when compared with zooplankton-fed larvae. The growth of larvae fed on Az 3 was poor.

Dabrowski *et al.* (1983) demonstrated that length and/or weight frequency distributions may indicate malnutrition of larvae. Therefore, this parameter is included in the present investigation. Frequency distributions of total length in the three test groups at day 34 are shown in *figure* 3. The range of the size distribution does not differ much between the three treatments. However, for Az 3 the diagramm is left-skewed whereas it is right-skewed in the zooplankton group.

Liver histology

During the first 28 days of the experiment, zooplankton-fed larvae showed well compartmented hepatocytes, with medium sized glycogen fields and with perinuclear and peripheral stacks of rough endoplasmic reticulum (rER) (*fig.* 5*a*). Using the methyl-green-thionine stain, the rER stacks could be visualized at the light microscopical level as intensively RNA-positive staining rods located near the nucleus or the cellular periphery. Pathological alterations of parenchymal, cellular or organelle structures were absent. Parenchymal homogeneity, *i.e.* the degree of uniformity in the response of the individual hepatocytes within the liver parenchyma, was high.

With day 34, liver glycogen contents of zooplankton-fed larvae, as estimated from light and electron micrographs, decreased. In addition, slight structural disturbances, particularly in the organization of the rER, became evident in a smaller percentage of the hepatocytes, thus giving rise to a limited increase of parenchymal heterogeneity.

Intracellular organization of the hepatocytes of Az 20-fed larvae (*fig.* 5*b*) was comparable to that of zooplankton-fed larvae. Parenchymal heterogeneity, however, was always higher than in the zooplankton group: between 10-20% of the hepatocytes showed changes in electron density, intracellular compartmentation, glycogen content and particularly disturbances of the stack-like arrangement of the rER cisternae. Moreover, the mitochondria of fish fed with

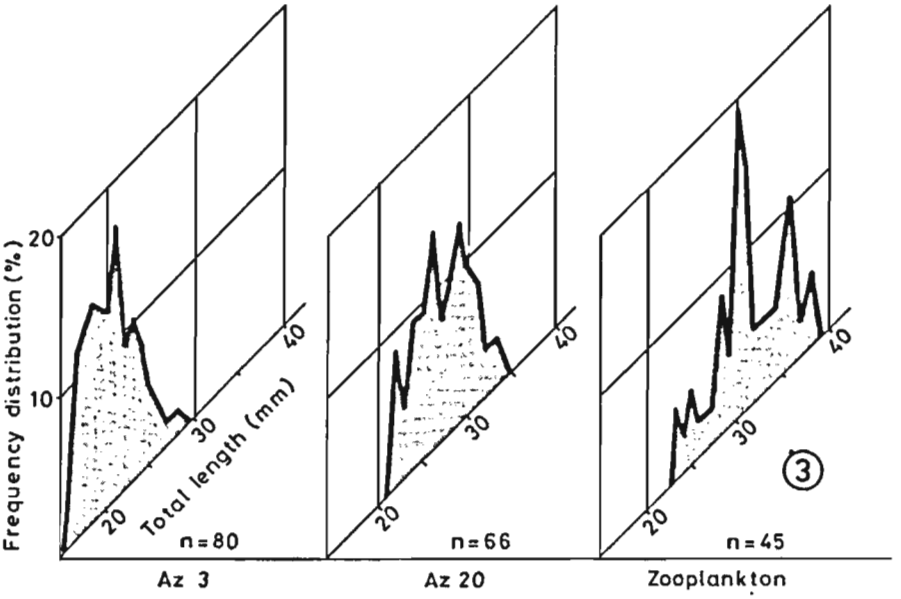

Figure 3. – Frequency distribution (%) of total length of larvae of *Coregonus lavaretus* fed on the dry diets Az 3 and Az 20 or live zooplankton after 34 days of feeding [mean total length ± s.d. (mm) = 16.1 ± 1.6 for Az 3, 18.4 ± 1.7 for Az 20 and 23.7 ± 1.7 for zooplankton].

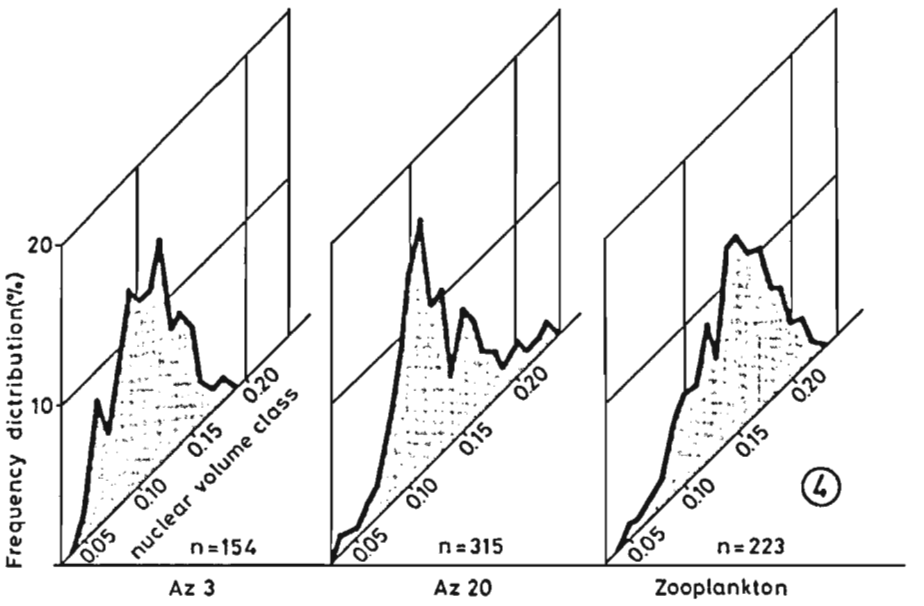

Figure 4. — Frequency distribution (%) of nuclear volume classes of larval *Coregonus lavaretus* fed on the dry diets Az 3 and Az 20 or live zooplankton after 34 days of feeding (mean nuclear volume ±s.d. (arbitrary units) = 0.11±0.03 for Az 3, 0.12±0.03 for Az 20 and 0.15±0.03 for zooplankton).

Az 20 were slightly enlarged when compared to zooplankton-fed larvae. In the remaining hepatocytes, stack-like organized cisternae of the rER were present and the glycogen content and the intracellular compartmentation were comparable to the features obtained with zooplankton-fed larvae. An impairment of liver structure towards the end of the experimental period, as noted for the zooplankton group, was not evident in fish reared on Az 20.

The structure of the hepatocytes of Az 3-fed larvae differed from the other two groups. The rER, although the number of cisternae appeared to be not lower than in the two other groups, was organized poorly (*fig.* 5*c*). In most cells of the parenchyma it failed to show a stack-like organization. The hepatic glycogen contents were low. Towards the end of the experimental period, liver structure impaired in Az 3-reared larvae and the liver developed degenerative features such as pronounced parenchymal heterogeneity, a nearly complete loss of glycogen, spherical transformation of the mitochondria and fragmentation and disorganization of the rER (*fig.* 5*d*).

Escaffre and Bergot (1986) showed that the size of hepatocyte nuclei is a good indicator of the nutritional status of a fish. We therefore measured the areas of hepatocellular nuclei in 34-day-old coregonid larvae by means of computer-aided morphometry. The frequency distribution of nuclear volumes is shown in *figure* 4. Zooplankton-fed larvae possessed significantly larger ($p<0.05$) nuclei than the hepatocytes of larvae fed with dry food. For all three test diets, the frequency distribution of nuclear volumes approximately parallels the frequency distribution of body length as shown in *figure* 3.

DISCUSSION

Under the conditions used in the present investigation (semi-technical rearing under *hatchery* conditions and using *commercial* dry diets) the larvae reared on the dry diet Az 20 were 34% and 69% of the dry body weight of larvae reared on zooplankton, after 34 or 58 days of rearing, respectively. Comparison of the final weight at day 58 must be taken with caution since from day 34 to day 58 the availability of zooplankton was restricted. Consequently the final weight does not represent the maximum growth attainable if zooplankton were given in excess.

During the first feeding period (days 0-34), the body weight of Az 20-fed larvae is within the range found in other experiments with coregonid larvae from Lake Constance using *experimental* dry diets and conducted in *laboratory* aquaria (Rösch and Appelbaum, 1985; Hofer and Bürkle, 1986; Rösch and Dabrowski, 1986). Therefore, the growth obtained with the dry diet Az 20 can be considered to be satisfactory.

Liver structural analysis, which is a sensitive tool for the assessment of nutritional effects in fish (e. g. Storch *et al.*, 1984; Segner and Juario, 1986; Mosconi-Bac, 1987; Verreth *et al.*, 1987) supports the conclusion on the quality of Az 20 as derived from the growth results: Az 20 evoked no pathological liver alterations even over the prolonged feeding period of 58 days, thus indicating that the larvae fed with Az 20 did not suffer nutritional deficiencies.

Nevertheless, although Az 20 appears to be an adequat diet for larval *Coregonus lavaretus*, growth obtained with this food is still lower than growth

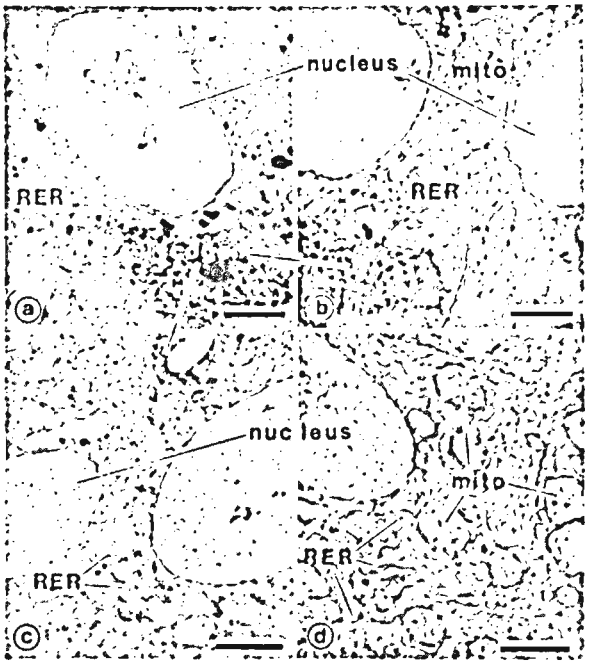

Figure 5. — Electron micrographs of the liver structure of larval *Coregonus lavaretus* fed on the dry diets Az 3 and Az 20 or live zooplankton. The bar indicates 1 µm.

Figure 5 *a*. — Hepatocyte of a 21-day-old larva fed with live zooplankton. There are well-developed stacks of rough endoplasmic reticulum (rER) and medium-sized glycogen fields. The mitochondria are electron-lucent. Such a cell-structure indicates the absence of nutritional deficiencies.

Figure 5 *b*. — Hepatocyte of a 21-day-old larva fed with dry diet Az 20. Its appearance is essentially similar to that described for zooplankton-fed larvae, only the mitochondria tend to be slighlty enlarged.

Figure 5 *c*. — Hepatocyte of a 21-day-old larva fed with dry diet Az 3. The rER is not organized in stacks but coursing in an irregular arrangement through the cytoplasm. A lower amount of glycogen is stored. The mitochondrial morphology resembels that of Az 20-fed larvae. Such a hepatocellular structure clearly indicates a less functional state of liver metabolism than the hepatocytes shown in *figures* 5 *a* and 5 *b*.

Figure 5 *d*. — Hepatocyte of a 58-day-old larva fed with dry diet Az 3. Hepatocellular structure is further detiorated, expressing the prolonged action of malnutrition of the organism.

obtained with live food-feeding. Four possible reasons for this difference should be discussed: firstly, the incompletely developed digestive system of larval fish may encounter problems in processing compound dry diet; secondly, the biochemical composition of the formulated diets may be not sufficient for the nutritional requirements of the larvae; thirdly, live food may induce a more growth-oriented metabolism of the larvae than dry food and finally, lower growth of larvae fed on dry food may be related to a reduced food intake when compared to the food intake in larvae fed with live food.

Low utilization of a diet in the digestive tract of fish larvae can be caused by insufficient enzyme secretion of the larvae (Lauff and Hofer, 1984) or by low digestibility of the diet. Although endogenous digestive enzyme production of fish larvae is low at first feeding, food consumption is also low at first feeding and the enzyme: substrate ratio may be similar in larval and in older fish, although the absolute enzyme activities may differ greatly. That the absolute amount of digestive enzyme activities of larval fish is not the crucial point for the success of a diet, is supported by the findings of Baragi and Lovell (1986), in a study on larval rearing of striped bass, *Morone saxatilis*, and of Segner *et al.* (in press), in a study on larval coregonids. In both investigations, no obvious correlation between digestive enzyme activities and growth performance of the larvae was observed. However, it remains to be clarified whether the digestibility of dry diets is comparable to that of live diets.

Discussing the biochemical diet composition as a factor contributing to growth differences, the biochemical composition of the dry diet Az 3 obviously was insufficient. This conclusion is based on the presence of pathological features in the livers of larvae reared on Az 3. Consequently, the low growth rate of larvae fed with this diet can be explained by an insufficient diet composition. On the contrary, as already mentioned above, in the livers of larvae fed with Az 20 no indications for nutritional deficiencies were found; the lower growth obtained with this diet when compared to larvae fed with live food has to be explained by the influence of other factors.

One of these other factors may be a different metabolic orientation under the live and the dry food regimen. It is well established that growth of larval fish is a function of the intensity of protein metabolism (e. g. Fauconneau *et al.*, 1986) and of the amount of RNA per cell (e. g. Buckley, 1984). The RNA is directly involved in protein synthesis. In structural terms, the intensity of cellular protein synthesis is correlated with the organization and extension of the RNA-containing ER (=rER) and it is a basic fact in histology that cells with high protein synthesis rate are characterized by extended, parallel layers of rER cisternae. In the present study, a good correlation existed between the morphological organization of the site of hepatocellular protein synthesis, the rER, and the growth rate of the larvae. Feeding the larvae with zooplankton generated numerous rER cisternae within the cytoplasm of the hepatocytes, organized in a stack-like manner. Accordingly, this diet evoked the best growth rate among the three test diets. Az 3, on the other hand, although leading to a

similar or only slightly lower number of rER membranes than Az 20 and zooplankton, showed no stack-like but an irregular arrangement of the rER cisternae. This is indicative for a disturbed functional state of the rER. Consequently, feeding coregonid larvae with Az 3 gave the lowest growth rate. Az 20 evoked hepatocellular features basically similar to zooplankton-fed larvae, but there was an enhanced number of hepatocytes with a disturbed arrangement of the rER cisternae thus leading to an overall reduced number of fully functional cells within the livers of larvae fed with Az 20. In agreement with this, the growth rate of larvae reared on Az 20 was reduced when compared to the zooplankton group, but was higher than in the Az 3 group.

The measurements of nuclear volumes, as presented in *figure* 4 further support the idea that the fast growth of zooplankton-fed larvae was related to an enhanced hepatocellular metabolism. From investigations on rat hepatocytes it is known that hepatonuclear size is positively correlated with the intensity of cellular metabolism (Hildebrand, 1980). Likewise, Escaffre and Bergot (1986) showed for rainbow trout that the diameter of hepatocyte nuclei depends on the nutritional status of the fish. In *Coregonus lavaretus* larvae from this study, feeding on zooplankton resulted in the largest nuclei, whereas the hepatocyte nuclei of larvae reared on the two dry diets were significantly smaller.

Thus, performance of the rER and nuclear volume present evidence that growth of larval coregonids is correlated with the intensity of liver metabolism, particularly the liver protein metabolism. The enhanced growth of larvae fed on live food seems to be, at least in part, caused by the greater capacity of this type of food to stimulate liver metabolic activities. Az 20, although apparently fulfilling the essential nutritional requirements of *Coregonus lavaretus* larvae, seems to be not able to stimulate growth metabolism of the larvae to the same extent as live zooplankton. Az 3, finally, neither meets the nutritional requirements of the larvae nor triggers an intensive hepatic metabolism.

It is interesting to note that the frequency distribution of nuclear volumes closely parallels the frequency distribution of total length of the larvae. This example illustrates another time that where events are visible at the organismic level they have their ultimate reason in events at the cellular level (Vogt, 1987; Segner and Braunbeck, 1988).

Finally we have to discuss the role of food consumption in the determination of larval growth. Studying the degree of intestinal filling in 13-day-old coregonid larvae during the daily feeding period, we observed that larvae fed with live food always showed freshly ingested plankters in the anterior third of the intestine, whereas in larvae fed with dry food, the anterior third of the intestine was already empty before the next food was ingested (Segner, unpublished observations). Moreover, many of the larvae reared with dry diets accepted food only occasionally but not at each time when food was offered. These observations suggest a less continuous and less intensive feeding of dry food-fed larvae when compared to the zooplankton group. A recent investigation of Troschel (1988) reports a maximum daily intake of zooplankton which is far beyond the daily ration of larvae fed on dry food as reported by Bergot *et al.* (1986). Additionally, larvae fed with living zooplankton start to feed earlier than those fed on dry diets (Rösch and Dabrowski, 1986; present investigation). To our opinion, it is an important task of future studies to investigate to what extent differences in the growth of fish larvae fed on live or dry diets are due to quantitative differences in food intake under these feeding regimens.

In conclusion, the results of this study give evidence that a large scale rearing of coregonid larvae using dry diets from hatching onwards is possible. This is confirmed by the report of Rösch (1988) on a dry food-based mass rearing of *Coregonus lavaretus* larvae in hatcheries of Lake Constance. Further research in coregonid larval rearing should deal with the improvement of dry diet composition in order to achieve a better stimulation of cellular metabolism, and, secondly, with an improvement of feeding techniques for increasing larval growth by enhanced food intake.

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