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Development of yolk complex, liver and anterior intestine in pike-perch larvae, *Stizostedion lucioperca* (Percidae), according to the first diet during rearing

Laurence Mani-Ponset [1], Jean-Pierre Diaz [1], Olivier Schlumberger [2] and Robert Connes [1]

[1] *Laboratoire de Biologie animale, Université de Montpellier II, 34095 Montpellier cedex 5, France.*

[2] *Division Aquaculture et Pêche, CEMAGREF, 361, rue J. F. Breton, BP 5095, 34033 Montpellier cedex 1, France.*

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Abstract

Pike-perch *Stizostedion lucioperca* larvae fasted or given two diets (artificial feed, zooplankton) were studied by transmission electron microscopy and using specific cytological dyes. The objective of this work was to assess the changes in the yolk sac, liver and anterior intestine following the mouth opening stage (95 °C.d, day 5 - 5.8 mm) from endo- to exotrophy periods. The yolk sac included a syncytial envelope closely bound to the proteinaceous vitellus and the oil globule. Its development was similar to that observed in trout (*Oncorhynchus mykiss* and *Salmo fario trutta*) and sea-bream (*Sparus aurata*), but without vitellus fragmentation into platelets. Vitellus resorption was never achieved in dying larvae. From the end of the endotrophic phase onwards, the amount of hepatic glycogen decreased. Carbohydrate disappeared at the beginning of the exotrophic phase then increased after 220 °C.d (day 10 - 6.0 mm), mainly in zooplankton-fed larvae. Lipids from enterocytes, sinusoids and general blood stream showed a similar development. They were abundant at 95 °C.d, progressively disappeared up to 260 °C.d (day 12 - 6.3 mm), and increased again from 350 °C.d (day 16 - 6.7 mm) onwards if larvae were fed zooplankton. Dietary lipids did not seem to immediately replace yolk lipids despite absorption in the gut. Various hypotheses are proposed to explain the physiological abnormalities of this critical larval stage. Although zooplankton appeared to have better nutritional qualities than the artificial feed, these two foods proved to be quite unsatisfactory as they induced liver cell changes typical of food deficiency, generally observed in starving larvae.

Keywords: Fish, Percidae, pike-perch, *Stizostedion lucioperca*, developmental stages, nutrition, ultrastructure.

Évolution du complexe vitellin, du foie et de l'intestin antérieur des larves du sandre Stizostedion lucioperca *(Percidae) en fonction de la première alimentation en élevage.*

Résumé

Des larves du sandre *Stizostedion lucioperca*, soumises au jeûne ou à deux régimes alimentaires différents (aliment artificiel, zooplancton) ont été étudiées au microscope électronique à transmission et par des réactions cytochimiques appropriées. Le but était d'apprécier, à partir de l'ouverture de la bouche (95 °C.j, J5 - 5,8 mm), l'évolution du complexe vitellin, du foie et de l'intestin antérieur durant le passage de l'endotrophie à l'exotrophie. Le complexe vitellin, constitué d'une masse de vitellus et d'un globule lipidique entourés d'un périblaste syncytial, évolue comme celui des truites (*Oncorhynchus mykiss* et *Salmo fario trutta*) et de la daurade (*Sparus aurata*) mais sans fragmentation en plaquettes du vitellus. Sa résorption n'est pas complète au moment de la mort de beaucoup de larves. Le glycogène, présent dans le foie à la fin de la période endotrophe, s'épuise au début de l'exotrophie et réapparaît à partir de 220 °C.j (J10 - 6,0 mm) surtout chez les larves élevées sur zooplancton. Les lipides ont une évolution

analogue, aussi bien dans les entérocytes que dans les sinusoïdes et la circulation générale. Très abondants à 95 °C.j, ils disparaissent progressivement jusqu'à 260 °C.j (J12 - 6,3 mm) pour réapparaître à partir de 350 °C.j (J16 - 6,7 mm) chez les sujets nourris de zooplancton. Les lipides d'origine alimentaire ne semblent pas prendre immédiatement le relais de ceux du complexe vitellin, en dépit d'un déroulement normal de l'absorption intestinale. Plusieurs hypothèses sont émises pour tenter d'expliquer les anomalies physiologiques de cette phase critique. Bien que le zooplancton collecté paraisse avoir de meilleures qualités nutritives que l'aliment artificiel, ces deux types de nourriture se sont montrés peu satisfaisants, entraînant au niveau du foie des modifications caractéristiques de carences, généralement observées chez les larves à jeun.

Mots-clés : Poisson, Percidae, sandre, *Stizostedion lucioperca*, développement larvaire, nutrition, ultrastructure.

INTRODUCTION

The percid teleost, *Stizostedion lucioperca*, is a fish of high economic value, which justifies efforts made to control its rearing. As in most fish, the first diet is usually incompatible with the nutrient requirements of young larvae and high mortality may result. To determine a means to avoid such failure and facilitate the passage from endotrophy to exotrophy, we studied changes in the yolk complex, the liver and the anterior intestine of larvae fasted or given different diets during this period. Few teleosts have been investigated from this angle; we give here our preliminary results. In the past, a number of studies concerning the yolk complex, such as that of Kunz (1964) have been carried out. However, if we exclude research on yolk biochemistry or restricted data on yolk utilization sequence (Avila and Juario, 1987), studies on ultrastructural development of the yolk complex concern only three species: *Oncorhynchus mykiss* (Vernier and Sire, 1977 *a* and *b*), *Salmo fario trutta* (Walzer and Schönenberger, 1979 *a* and *b*) and *Sparus aurata* (Guyot *et al.*, 1993). The early involvement of liver in the storage and mobilization of carbohydrates and lipids has only been studied in *Oncorhynchus mykiss* (Vernier, 1975, Vernier and Sire, 1976; 1977) and in *Dicentrarchus labrax* (Diaz and Connes, 1991). The first signs of intestinal absorption have been described in several teleost species reviewed by Sire *et al.* (1981), Babin and Vernier (1989) and Sire and Vernier (1992).

MATERIAL AND METHODS

The pike-perch larvae used in this work were provided by GEMAGREF (Centre Expérimental du Machinisme Agricole, du Génie Rural des Eaux et des Forêts) in Montpellier (station de Lavalette). They were kept in well water in a closed-circuit unit with an emerged biological filter in 50 l cylindrical-conical tanks at an initial density of 50 larvae per litre. Larvae were kept in darkness until mouth opening and, afterwards, maintained in natural light and photoperiod. Since the breeding period is very short for pike-perch, we could only work on two egg-layings within the same year. Larvae from the first laying, kept at 16 °C, from hatching to day 14 and at 22 °C from day 14 to day 22 (end of test), were divided into two groups: the first received no food, the second was fed "Aqualarve", manufactured by Aqualim (France): microparticles of size consistent with the mouth development (100 to 200 μm until day 15, 200 to 300 μm afterwards) and composed of 55 % crude protein, 12 % crude fat and 13 % ash. Larvae from the second laying, kept at 22 °C from hatching to day 20, were also separated in two groups: the first was fed "Aqualarve", the second was given live zooplankton collected from fertilized tanks and sieved before distribution so that the prey size was compatible with the larval gape. The prey size was from 125 to 250 μm up to day 15 (Rotifera, Cladocera, Copepod nauplii) and from 200 to 300 μm up to day 15 (Cladocera, adult Copepods). Artificial feed was distributed with a rotating Eheim 3580 feeder. The ration was 8 to 10 g per day divided into 8 feeds for 2500 larvae. Since temperature conditions were not the same in both tests, we characterized the stages of development by degree days (°C.d) rather than day.

Every two-day sampling, five larvae per group were anaesthetized in cold water and subjected to double fixation in 2.5 % glutaraldehyde (1 h) then in 1 % osmium tetroxyde (1 h) at 350 mOsm and pH 7.2. They were embedded in Epon 812 after dehydration in a graded series of alcohol, and ultrathin sections were prepared with an LKB ultramicrotome. For the morphological study, they were contrasted by double-staining with uranyl acetate (30 min) and bismuth oxynitrate (30 min). Lipids and glycogen were respectively enhanced by OTO (Seligman *et al.*, 1966) and Thiery (1967) methods on ultrathin sections layered on a gold grid. Semithin larvae sections were contrasted with argentodiamin hydroxide (Singh, 1964) to reveal glycogen and with Black Sudan to characterize lipids (Sire and Vernier, 1980).

RESULTS

As for most fishes, there are three phases in the postembryonic development of pike-perch: (1) an endotrophic phase when the pre-larva lives on its yolk reserves; (2) an endo-exotrophic phase characterized

by depletion of the yolk reserves and beginning of feeding, and (3) an exotrophic phase when feeding is the only nutrient source. The endo-exotrophic phase, which we focused on in this study, did not exceed 400 °C.d. At the end of the experiment, the survival rate was 10 % for all groups, except starved larvae which had all died by 310 °C.d. The highest death rate occurred between 250 and 300 °C.d. Better growth was obtained with the zooplankton diet (34 %) than with artificial feeding (22 %). The starved larvae shrank by 3 % over the study period. Whatever the diet, larvae began eating from mouth opening stage. Five to six days later, over 50 % of them had food in their gut and thus until the end of the experiment. The observed samples were taken from those larvae.

Since larvae reared at 22 °C had a faster development than larvae reared at 16 °C, we decided to express the results in °C.d to make their comparison easier.

Results reported hereafter are summarized in *table* 1.

General condition of larvae at mouth opening

At mouth opening (95 C.d for precocious specimens), pike-perch larvae still had a substantial vitelline vesicle under the anterior area of the gut (*fig.* 1). This vesicle had two separate parts of almost equal volume: the oil globule in front and the vitellus at the back. These two parts were surrounded by a yolk syncytial layer, or periblast, of variable thickness (*fig.* 1), the thickest area being the dorsal part closer to the gut and liver. The periblast had high levels of cisternal and vesicular endoplasmic reticulum, mitochondria and, in the middle area, Golgi vesicles (*fig.* 2). On the external side, numerous microvilli protuded into the perivitelline circulatory space, lined by an endothelium and directly linked with vessels irrigating the liver. Microvilli penetrated the vitellus on the inside part.

The liver was a voluminous mass next to the left side of the yolk complex. It was already functionally organized with differentiated and polarized hepatocytes including, for some larvae, small glycogen areas.

The gut was still straight and had four distinct parts: an œsophagus with large mucocytes, a "gastric" area where cells had few microvilli and numerous clear apical vesicles, an anterior folded intestine with well organized brush border enterocytes and a posterior intestine without folds, separated from the former part by a valvula.

The pancreas was also functional, as shown by the state of exocrine cells grouped in acini and enclosing zymogen bodies, sometimes in the exocytosis phase.

Yolk complex development

After mouth opening, the vitellin reserves were resorbed more quickly for the vitellus than for the oil globule, occurring simultaneously with the volume reduction of periblast and nuclei gathering. In spite of the penetration of periblast microvilli, the vitellus never gave rise to platelets. Many larvae died before their reserves were exhausted; some of them were still enclosed within the liver.

From 95 ° to 130 °C.d, lipids abounded in the syncytial cytoplasm, and their size (0.05 nm) and localization corresponded to those of lipoproteins. They were more numerous near the oil globule and could be found in most of the endoplasmic reticulum and in the Golgi vesicles of the medium and external areas of the periblast (*fig.* 2 and 3). They filled the perivitelline circulatory space (*fig.* 4) and the whole larva circulatory system.

From 130 °C.d, the periblast presented many changes (*fig.* 6). Its nuclei became irregular, dense bodies accumulated in the mitochondria, the endoplasmic reticulum was disorganized, the Golgi vesicles became distorted and vesicles heterogeneous in content appear (*fig.* 5). Lipids became less and less abundant in the syncytium, and lost their particulate aspect and disappeared progressively from the circulatory spaces, with a slight delay for zooplankton fed larvae.

Treatments given to larvae had little effect on periblast function and on the duration of reserve resorption. However, this period was shorter for the second test, probably due to a higher rearing temperature from the beginning.

Liver development

At mouth opening stage, hepatocytes enclosed a few scattered glycogen particles that were quickly used up; at 160 °C.d most larvae contained none. In the following days these particles are gathered in patches. The carbohydrate content differed between larvae, and completely disappeared shorlty before death.

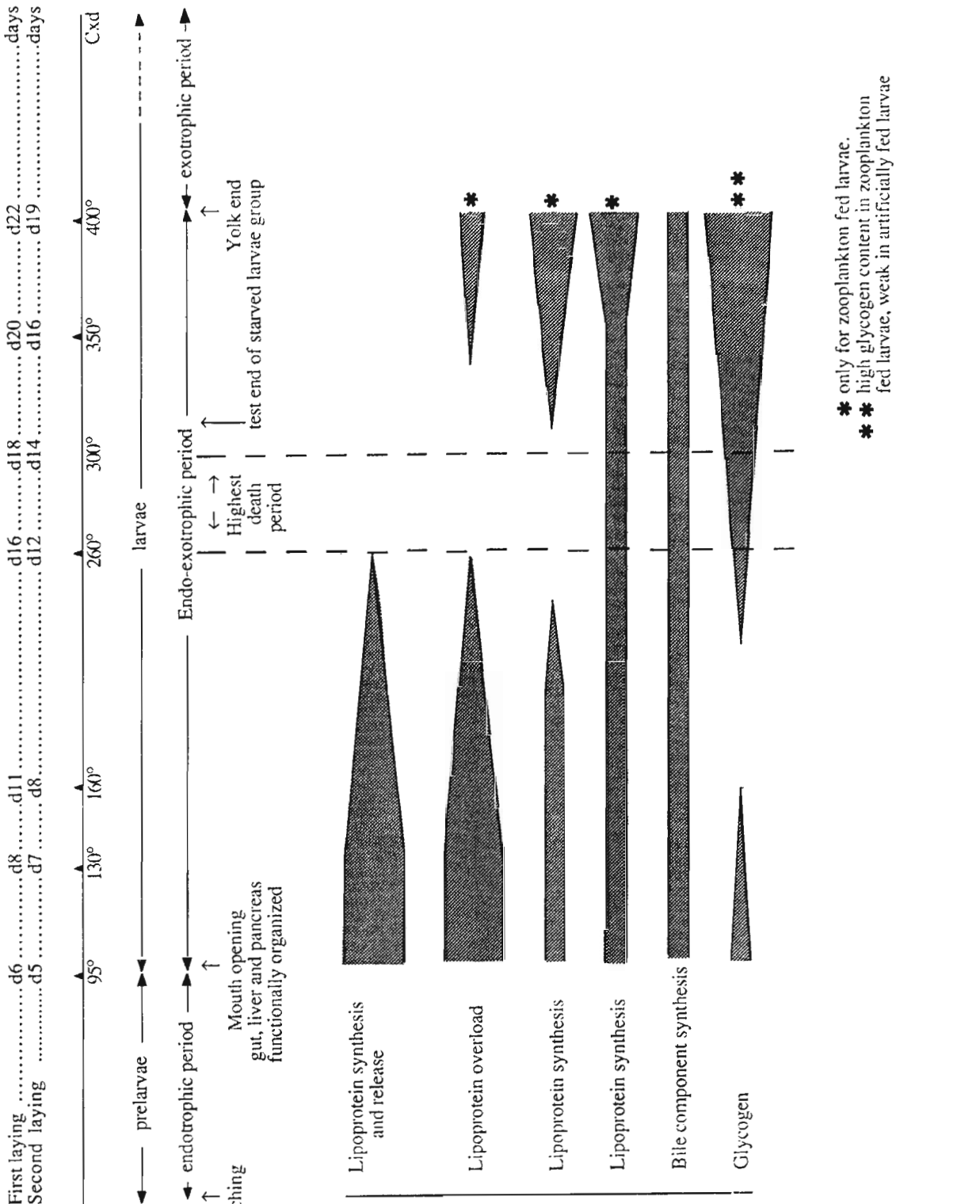
The zooplankton-fed larvae accumulated much more glycogen large areas of which led to "compartmentalization" of the hepatocytes (*fig.* 9). In contrast, glycogen stocks were markedly lower in artificial-diet fed or fasted larvae.

At the mouth opening stage, the hepatocyte lipoprotein synthesis was weak (few particules are obvious in some parts of endoplasmic reticulum and dictyosomes) (*fig.* 8). Hepatocytes stayed like this until fasting and artificially fed larvae died. On the other hand, from 350 °C.d, this synthesis was increased in zooplankton fed larvae (*fig.* 10). During this period, particles reacting positively to lipid stains were enclosed in large vesicles near the bile canaliculus, sometimes inside them, in larvae from all groups.

In both fasting and artificially fed larvae, from 160 °C.d, homogeneous and heterogeneous lipid accumulations, also found in older larvae (*fig.* 11), appeared near the sinusoids. These lipidic droplets are not obvious in zooplankton fed larvae.

At the mouth opening stage, the lipoprotein particles were numerous in the sinusoids of all larvae (*fig.* 8)

Table 1. – Sequence of the yolk complex and digestive system activities during the different phases of the pike-perch post-embryonic development.



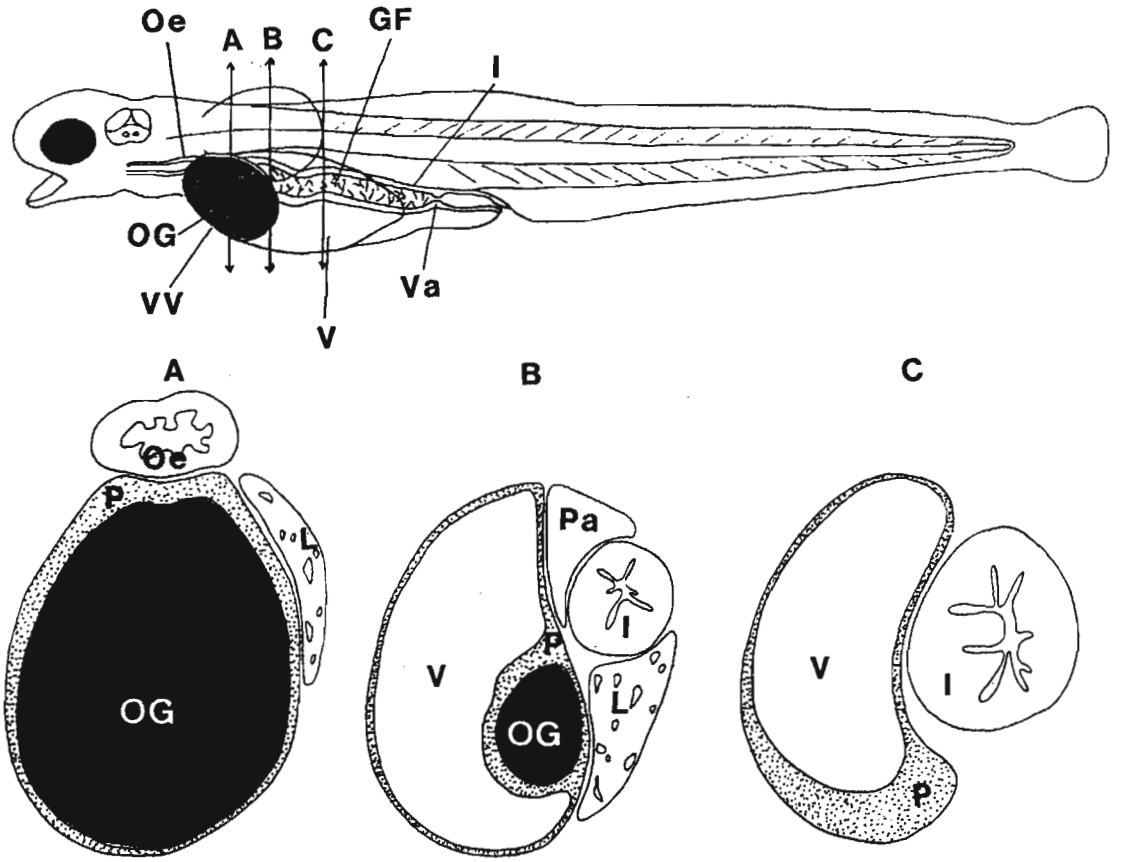


Figure 1. – Diagram of a 6 day-old (95 °C.d) pike-perch larva. Cross-sections at three different levels showing the organization of yolk reserves and the digestive tract. GF: gut mucosa folds; I: intestine; L: liver; Oe: œsophagus; OG: oil globule; P: periblast; Pa: pancreas; V: vitellus; Va: valvula; VV: vitelline vesicle.

especially in those close to the oil globule. They gradually disappeared between 200 °C.d and 260 °C.d. At 450 °C.d, only trace amounts were noted in the fasting and artificially fed larvae sinusoids (*fig.* 11). In contrast, in zooplankton fed larvae, the number of lipoprotein particules increased from 350 °C.d (*fig.* 10), but without reaching the level observed at mouth opening.

Whatever the treatment, from 160 °C.d, livers of all larvae present signs of alterationy: hepatocyte mitochondria were swollen, often distorted, or associated with dense bodies and myelinic-like structures; and sinusoids, Disse spaces and bile canaliculus were also swollen (*fig.* 7).

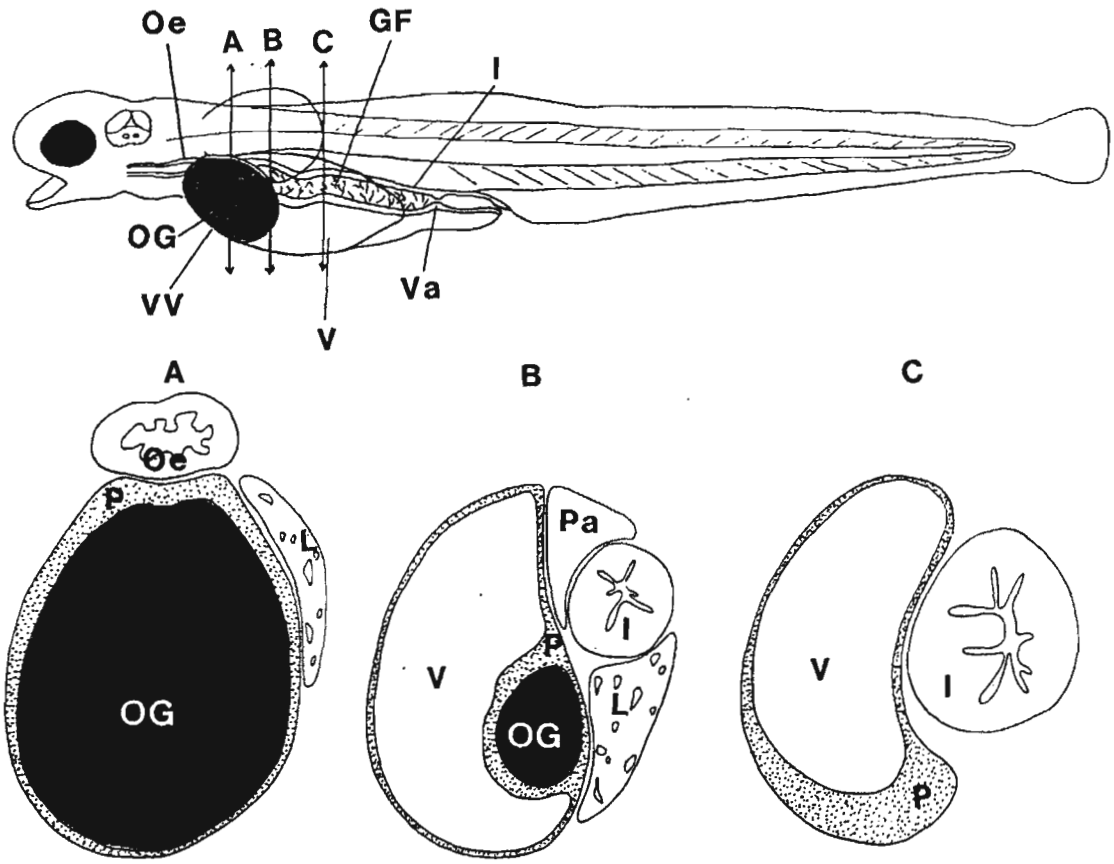
Development of intestinal absorption of lipids

Until mouth opening, lipoproteins, massively present in the general blood stream, accumulated in the sub-epithelial layer and in the enlarged interenterocytic spaces of the intestinal wall, but were absent in enterocytes (*fig.* 12).

Around 130 °C.d, soon after the first food intake, lipoproteic particles were seen in different parts of the endoplasmic reticulum, vesicles of the terminal web and vesicles of supra- and infranuclear areas. These particles were released into the interenterocytic spaces and reached the circulatory system. From 190 to 230 °C.d, lipid quantities decreased and sometimes disappeared from the enterocytes and there was no longer any transfer towards the circulatory system. From 310 °C.d, the situation was reversed: abundant amounts of lipoproteic particles were concentrated in vacuoles arising from active dictyosomes (*fig.* 15) and they were clearly transferred towards the intercellular spaces and basal lamina (*fig.* 13). Note that enterocytes in fasting larvae of 130 °C.d also contained some lipoproteins in their endoplasmic reticulum and Golgi system (*fig.* 14).

DISCUSSION

With a strictly endotrophic prelarval period of 95 °C.d, and an endo-exotrophic larval period not exceeding 400 °C.d, the postembryonic development of pike-perch *Stizostedion lucioperca* was chronologically similar to that of sea bass *Dicentrarchus labrax* (Diaz and Connes, 1991) and sea bream *Sparus aurata*



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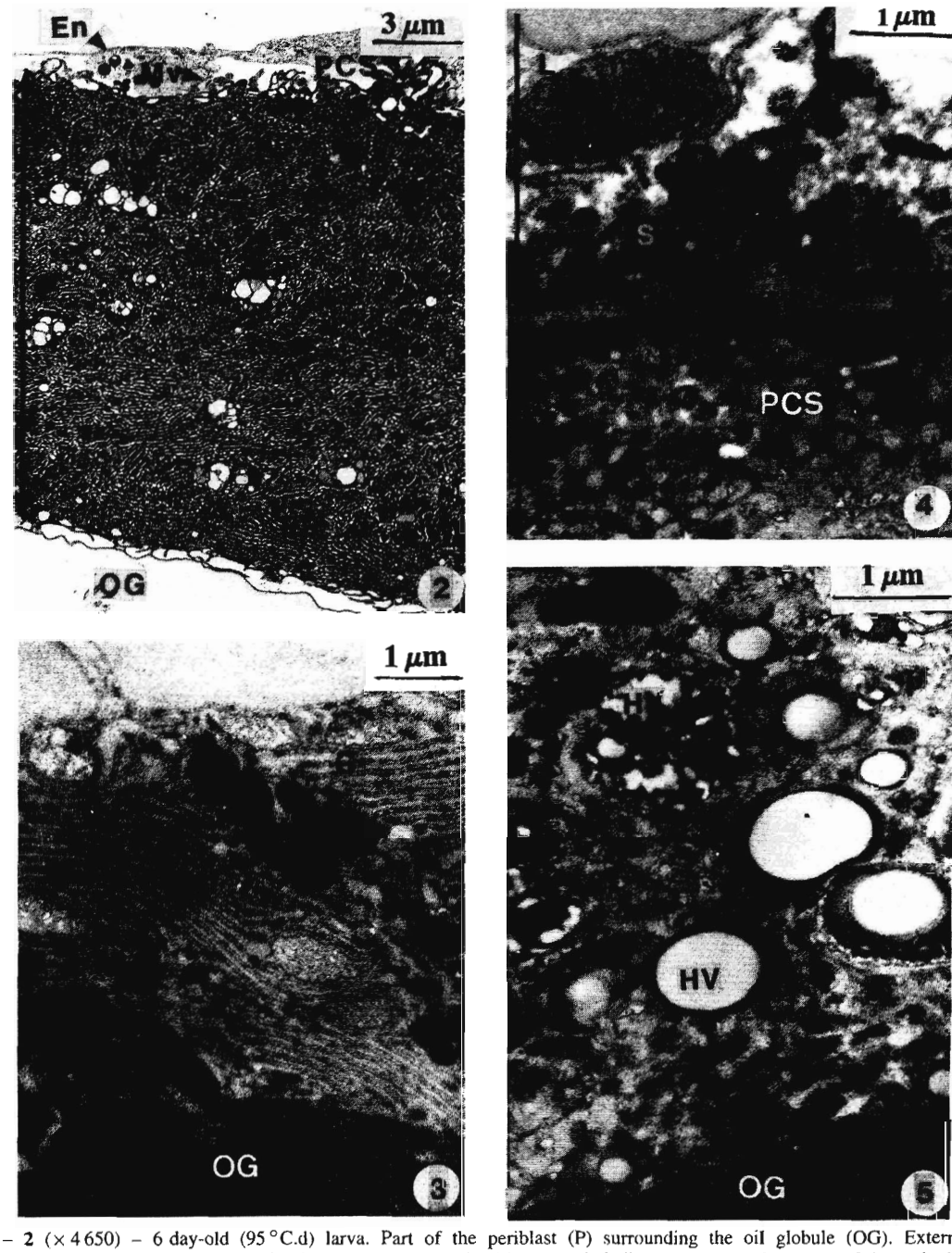
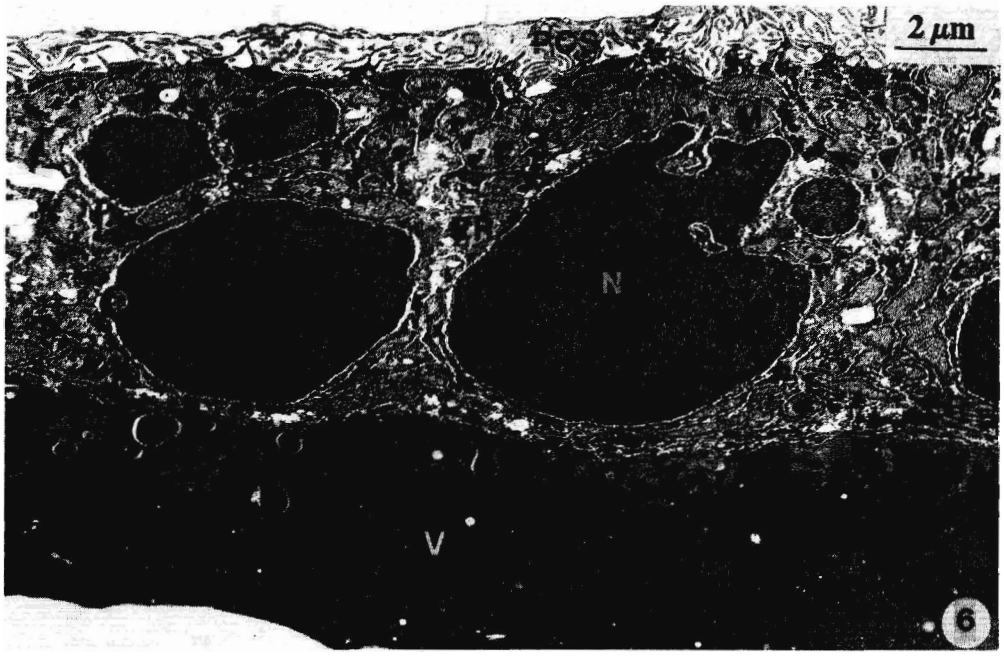
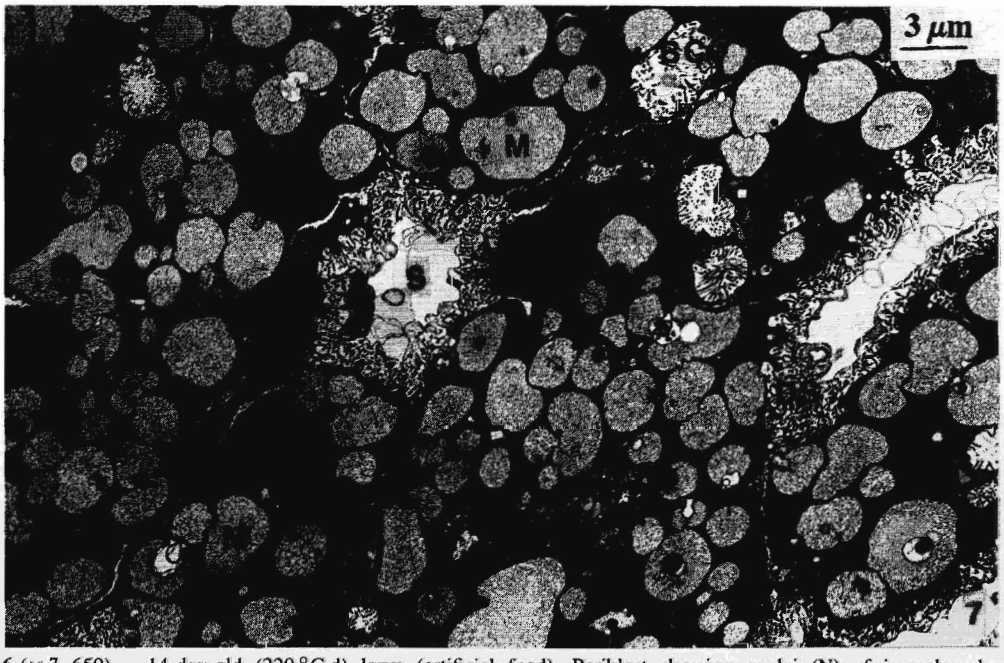


Figures 2-5. – **2** (× 4 650) – 6 day-old (95 °C.d) larva. Part of the periblast (P) surrounding the oil globule (OG). Externally, numerous microvilli (Mv) protude into the perivitelline circulatory space (PCS) lined by an endothelium (En). Note the extent of the endoplasmic reticulum (ER) and the Golgi apparatus (G). **3** (× 14 750) – 8 day-old (130 °C.d) fasted larva. Area of the periblast near the oil gobule (OG). Golgi apparatus (G) and endoplasmic reticulum (ER) contain numerous lipoproteic particles (OTO method). **4** (× 17 900) – 6 day-old (95 °C.d) larva. Contact area between the liver (L) and the periblast (P). The perivitelline circulatory space (PCS) and the adjacent sinusoid (S) are filled with lipoproteic particles (OTO method). En : endothelial cell. **5** (× 20 100) – 16 day-old (270 °C.d) larva (artificial food). Formation of lipidic heterogenous vesicles (HV) in the periblast area (P) near the oil globule (OG).

(Guyot *et al.*, 1993), but not trout *Oncorhynchus mykiss* (Vernier, 1969).

As in sea bream (Guyot *et al.*, 1993) or sea bass (unpublished observations), the yolk complex in pike-perch included a syncytial layer, or periblast, closely bound to an essentially proteic vitellus and to an oil globule. It was slightly different from the yolk complex of two trout species, *Oncorhynchus mykiss* and *Salmo fario trutta*, that have several oil globules and a stratified periblast (Vernier and Sire, 1977 *a* and *b*; Walzer and Schönenberger, 1979 *a* and *b*). Unlike the

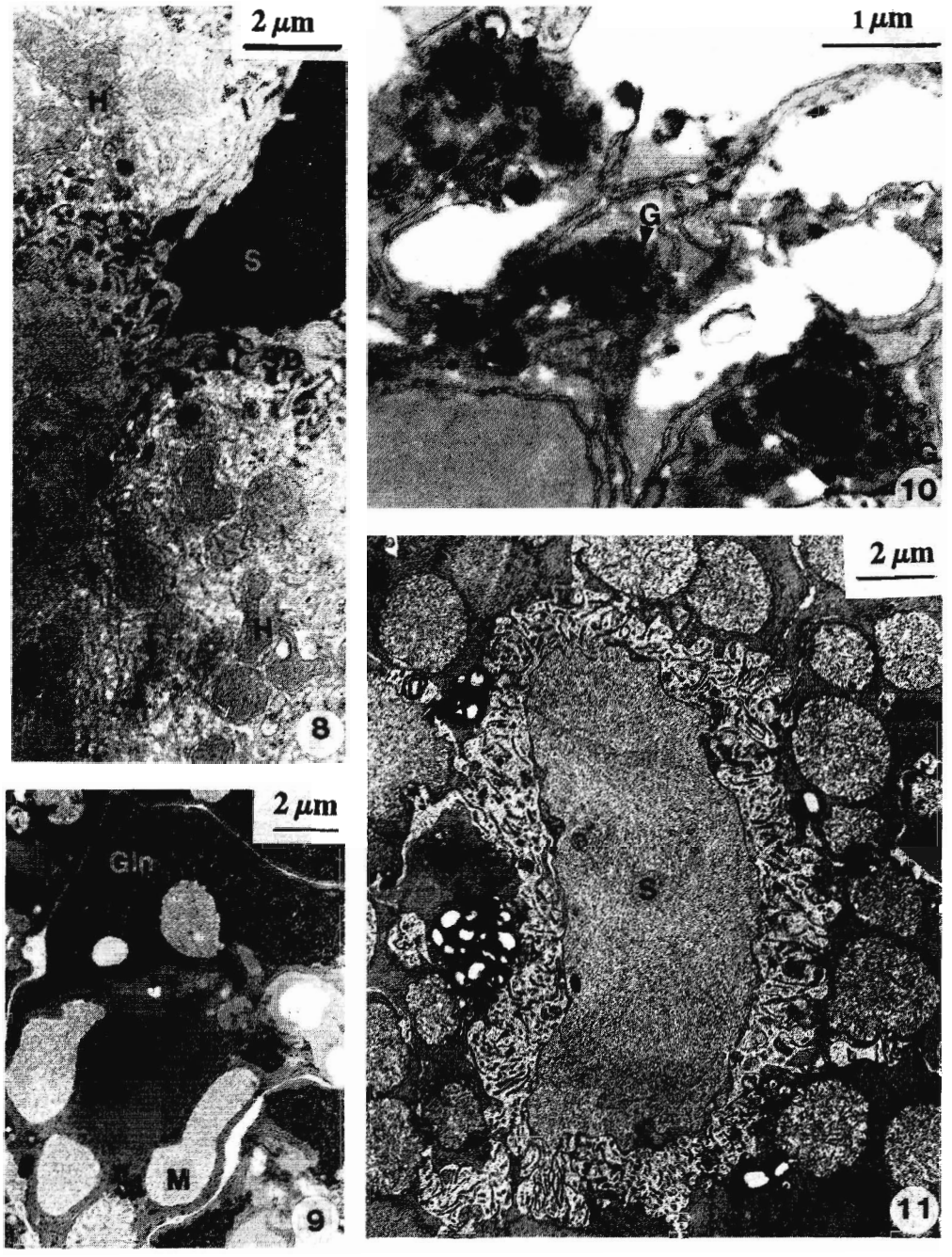




Figures 6-7. – **6** (× 7 650) – 14 day-old (220 °C.d) larva (artificial food). Periblast showing nuclei (N) of irregular shape, disorganized endoplasmic reticulum (ER) and mitochondria (M) containing dense bodies (→); PCS: perivitelline circulatory space; V: vitellus. **7** (× 3 800) – 12 day-old (190 °C.d) larva (artificial food). Hepatic parenchyme showing sinusoids (S) with an expanded Disse space (DS) and swollen bile canaliculus (BC) and mitochondria (M).

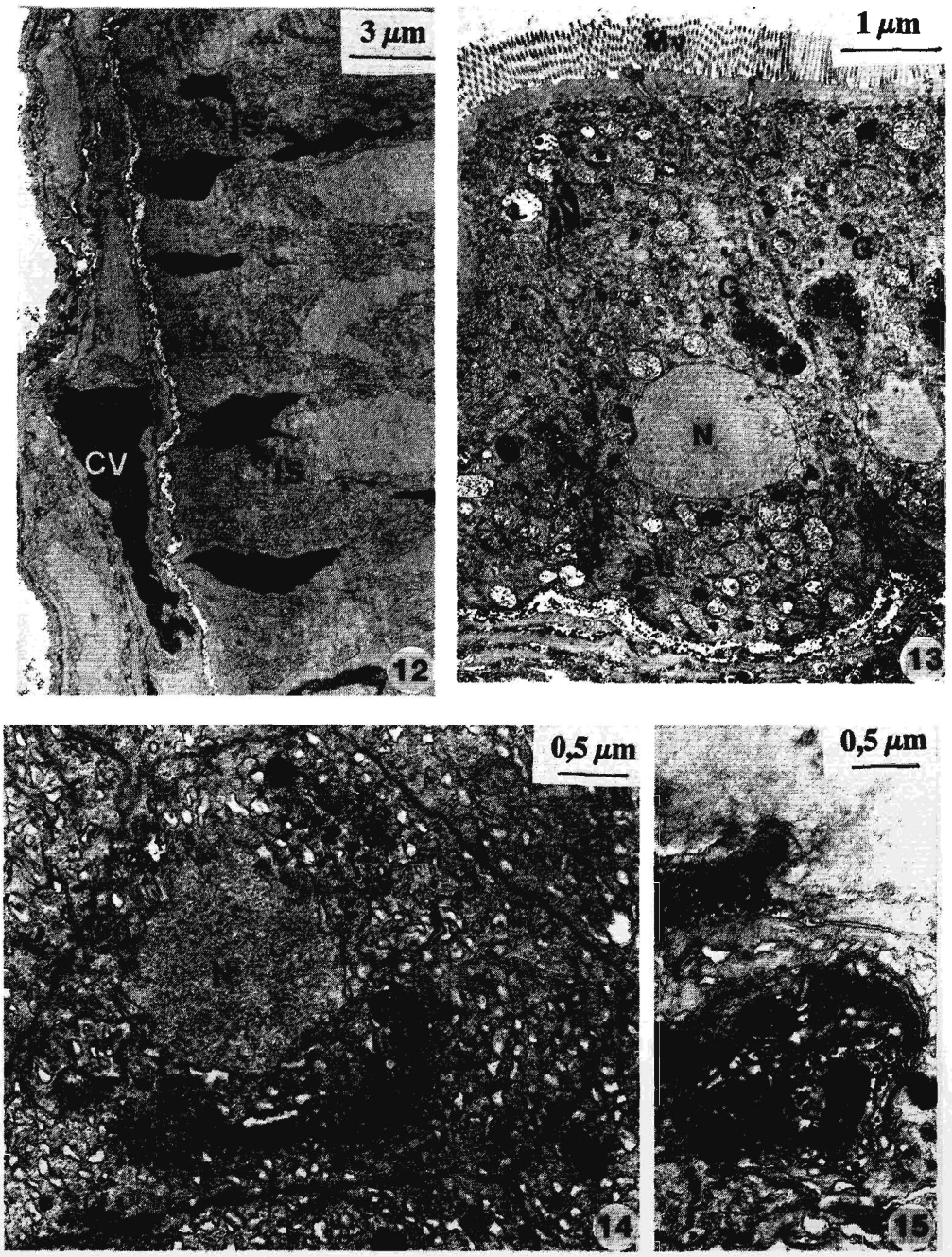
yolk complex development in sea bream, the vitellus does not become fragmented into yolk platelets and the periblast does not disintegrate before larval death during the endo-exotrophic period. At death, larvae still have some vitellus and an oil globule, which raises some questions. Are these remains of substances that cannot be assimilated by the periblast and which only disappear in surviving larvae? Or, is the first diet unsuitable for complete resorption of vitellus reserves and for new metabolic processes to ensure growth and survival? No answers are available at the present time.

The beginning of the endo-exotrophic period was marked by a substantial release of lipids from the globule to circulatory spaces through the periblast



Figures 8-11. – **8** (× 8 700) – 6 day-old (95 °C.d) larva. High lipoprotein content of a sinusoid (S) and Disse space (DS), and low concentration (→) in the hepatic cells (H). **9** (× 5 900) – 16 day-old (350 °C.d) larva (zooplankton). Glycogen (Gln) accumulation enhanced by T. C. H. silver proteinate. **10** (× 21 400) – 16 day-old (350 °C.d) larva (zooplankton). Dictyosomes of hepatocytes (G) giving rise to numerous vesicles concentrating lipoproteic particles, enhanced by the OTO method (empty spaces correspond to glycogen areas), in a hepatic cell. **11** (× 7 050) – 20 day-old (450 °C.d) larva (artificial food). Large heterogenous lipidic vesicles (HV) in the vicinity of a sinusoid (S). Note the low lipoprotein content of the sinusoid and the Disse space. Compare with *figure* 8 (OTO method).

in which lipoproteic particles were synthesized. This process was similar to those described for *Oncorhynchus mykiss* (Vernier and Sire, 1977 *a*) and *Sparus aurata* (Guyot *et al.*, 1993). Its intensity was reflected by a lipoprotein overload in the circulatory system and their storage in the sub-epithelial layer of the intestine and in the interenterocytic spaces. As suggested by Sire and Vernier (1979), we think that the lipid reserves could be used when larvae increase their motility and therefore their energetic needs. In fact, lipoproteins progressively disappeared from the circulatory spaces after 130 °C.d, while the periblast



Figures 12-15. – **12** (× 5 200) – 6 day-old (95 °C.d) larva. Part of the intestine wall showing high lipoprotein accumulation in a vessel (CV) of the sub-epithelial layer, the basal lamina (BL) and the expanded interenterocytic spaces (IS) (OTO method). **13** (17 550) – 16 day-old (350 °C.d) larva (zooplantkon). Enterocytes showing numerous lipoproteic particles in the Golgi apparatus (G) and, in some areas (→), their transfer to the intercellular space and basal lamina (BL). Mv : microvillii ; N : nucleus (OTO method). **14** (× 24 250) – 8 day-old (130 °C.d) larva (fasting). Cross-section through an enterocyte containing lipoproteic particles in Golgi vesicles (G). N : nucleus. (OTO method). **15** (× 24 100) – 18 day-old (310 °C.d) larva (artificial food). Golgi apparatus concentrating lipoproteic particles enhanced by the OTO method.

showed disturbed synthetic activity and produced heterogeneous inclusions.

Lipid absorption started after 130 °C.d since enterocytes then contained lipoproteins released in the interenterocytic spaces. It decreased from 190 to 230 °C.d, as confirmed by the low lipoprotein content in enterocytes, along with a full or partial reduction of its transfer to the circulatory system. In contrast, it increased from 310 °C.d, as lipoproteic particles were abundant in the intestinal epithelium and their transfer was intensive. Dietary lipids did not seem immediately to replace those of the yolk complex.

The capacity for absorbing lipids as soon as exogenous feeding begins has also been observed in other fish species (Iwai, 1968; Tanaka, 1972; Stroband and Dabrowski, 1979; Rombout *et al.*, 1984; Govoni *et al.* 1986; Watanabe and Sawada, 1985).

Generally, during the larval period and from initiation of exogenous feeding, it takes a more or less long time before the rise of digestive enzymatic activities takes place (Alliot *et al.*, 1977; Cousin *et al.*, 1987; Munilla-Moran *et al.*, 1989; Dabrowski and Culver, 1991). However, the presence of lipids that can be absorbed in the digestive tract of *Stizostedion lucioperca* implies a capacity for digesting food, which calls for suitable enzyme machinery from initiation of exogenous feeding. At this time, the liver synthesized bile components, as suggested by the presence of vesicles around the bile canaliculus, and the pancreas was functional. An increase in these functions is convenient at this stage to facilitate exogenous fat digestion.

How can we explain the decreased lipid load in the organism during the shift from endotrophy to exotrophy? The feed (artificial or zooplankton) given to larvae had a sufficiently high lipid content to reject the hypothesis of a quantitative deficiency; however, the qualitative deficiency should be checked. This could explain restored hepatic activity after 350 °C.d in larvae fed zooplankton, which seemed more suitable than the artificial diet for ensuring larval survival. The problem actually may be a deficiency in intestinal absorption, more or less related to failure of the regulatory mechanisms, or a defect in fatty acids absorption related to lipid digestion (low pancreatic lipase synthesis or bile salts synthesis). In any case, the level of lipoprotein release from enterocytes to the plasma cannot explain the lack of lipids in the circulatory system. Indeed, the enterocytes did not give any signs of saturation, like lipid droplet accumulation, as is the case for *Dicentrarchus labrax* larvae (Deplano *et al.*, 1991). A temporary decrease in lipid needs could be considered during the critical diet changing period? However, in *D. labrax*, we observed an identical disappearance of lipoproteins in the general circulatory system during the same period (unpubl. results) followed by a massive comeback when transition to strict exotrophy occurred. Resumption of intestinal absorption and hepatic activity at 310 °C.d in pike-perch larvae suggests a similar development in the two species. If this was confirmed, the high rate of death during the transition from endotrophy to exotrophy in fish larvae would not be the result of a dysfunction of lipid metabolim but of accompanying processes in these changes.

Are the performances of artificial-diet reared larvae identical to those fed zooplankton? How do fed specimens react compared to fasted specimens? No definitive conclusions can be drawn from this preliminary study due to the limited number of experiments carried out. The most evident fact is that none of the diets was satisfactory. All larvae presented hepatic alterations characteristic of food deficiency, generally correlated with starvation. These alterations are also found in sea bass and sea bream during difficult diet periods. With a slightly higher rate of growth, zooplankton-fed larvae had some detectable histological qualities which were missing in artificially fed subjects. Much more glycogen was stored in their hepatocytes after 160 °C.d, the period of lipoprotein synthesis by the periblast was extended, and there was a higher increase of lipoproteic synthesis by the hepatocytes after 310 °C.d. The fasted larvae kept some glycogen in their hepatocytes for a longer period and synthesized lipoproteins in their enterocytes. It is known that fasted mammals can synthesize very low density lipoproteins in the intestine from plasmatic fatty acids (Gangl and Okner, 1974; Gangl and Renner, 1978). Fasting fish might be able to use plasmatic fatty acids as well as bile lipids and lipids constituting cells rejected into the lumen (Okner and Isselbacher, 1974; Sire and Vernier, 1979; Deplano *et al.*, 1991). However, the hypothesis of a fasting interruption due to the presence of some microorganisms in the breeding water or to some type of cannibalism cannot be overlooked, although there were no signs of food in their gut.

Since none of the diets was satisfactory, zooplankton-fed larvae cannot serve as an adequate reference for nutrional state of dry food tested larvae. Thus, it seems necessary to select prey following a better knowledge of the planktonic population found in natural surroundings where the pike-perch reproduction takes place.

On the other hand, dry food should be supplemented with exogenous digestive enzymes to increase its digestibility as obtained for *Cyprinus carpio* (Dabrowski and Glogowski, 1977) and *Sparus aurata* (Kolkovski *et al.*, 1993).

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REFERENCES

Alliot E., A. Pastoureaud, J. Trellu, 1977. Évolution des activités enzymatiques dans le tractus digestif au cours de la vie larvaire du bar, *Dicentrarchus labrax*. Variations des protéinogrammes et des zymogrammes. CNEXO, Acte Colloq., **4**, 85-91.

Avila E. M., J. V. Juario, 1987. Yolk and oil globule utilization and developmental morphology of the digestive tract epithelium in larval rabbitfish, *Siganus guttatus* (Bloch). *Aquaculture*, **65**, 319-331.

Babin P. J., J. M. Vernier, 1989. Plasma lipoproteins in fish. *J. Lipid Res.*, **30**, 467-490.

Cousin J. C. B., F. Baudin-Laurencin, J. Gabaudan, 1987. Ontogeny of enzymatic activities in fed and fasting turbot, *Scophthalmus maximus* (L.) *J. Fish Biol.*, **30**, 15-33.

Dabrowski K., D. Culver, 1991. The physiology of larval fish. Digestive tract and formulation of starter diets. *Aquac. Mag.*, **17**, 49-61.

Dabrowski K., J. Glogowski, 1977. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. *Hydrobiologia*, **54**, 129-134.

Deplano M., J. P. Diaz, R. Connes, M. Kentouri-Divanach, F. Cavalier, 1991. Appearance of lipid-absorption capacities in larvae of the sea bass *Dicentrarchus labrax* during transition to the exotrophic phase. *Mar. Biol.*, **108**, 361-371.

Diaz J. P., R. Connes, 1991. Development of the liver of the sea bass, *Dicentrarchus labrax* L. (teleost, fish) II. Hepatocyte differenciation. *Biol. Struct. Morphol.*, **3**, 57-65.

Gangl A., R. K. Ockner, 1974. Intestinal metabolism of plasma free fatty acids (FFA). *Clin. Res.*, **22**, 358A.

Gangl A., M. D. Renner, 1978. Metabolism of plasma free fatty acids by mucosa of man. *Gastroenterology*, **74**, 847-850.

Govoni J. J., G. W. Boehlert, Y. Watanabe, 1986. The physiology of digestion in fish larvae. *Environ. Biol. Fish.*, **16**, 59-77.

Guyot E., R. Connes, J. P. Diaz, 1994. Résorption des réserves vitellines et passage de l'endotrophie à l'exotrophie chez la larve de Daurade (*Sparus aurata*) nourrie et à jeun. Production, environment and quality. Bordeaux Aquaculture '92. G. Barnabé, P. Kestemont eds. Europ. Aquac. Soc. Spec. Publ., Ghent, Belgium, **18**, 213-226.

Iwai T., 1968. Fine structure and absorption patterns of intestinal epithelial cells in rainbow trout alevins. *Z. Zellforsch.*, **91**, 366-379.

Kolkovski S., A. Tandler, G. W. Kissil, A. Gertler, 1993. The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata* Linnaeus, Sparidae) larvae. *Fish Physiol. Biochem.*, **12**, 203-209.

Kunz Y., 1964. Morphologische Studien über die embryonale und postembryonale Entwicklung bei Teleostiern mit besonderer Berücksichtigung des Dottersystems und der Leber. *Rev. suisse Zool.*, **71**, 445-525.

Munilla-Moran R., A. Barbour, J. R. Stark, 1989. NADPH generation in turbot larvae: a practical approach. *World Aquaculture*, **20**, 78-79.

Ockner R. K., K. J. Isselbacher, 1974. Recent concepts of intestinal fat absorption. *Rev. Physiol. Biochim. Pharmacol.*, **71**, 107-146.

Rombout J. H. W. M., H. W. J. Stroband, J. J. Taverne-Thiele, 1984. Proliferation and differenciation of intestinal cells during development of *Barbus conchonius* (Teleostei, Cyprinidae). *Cell. Tissue Res.*, **236**, 207-216.

Seligman A. M., H. L. Wasserkrug, J. S. Hanker, 1966. A new staining method (OTO) for enhancing contrast of lipid-containing membranes and droplets in osmium tetroxide-fixed tissue with osmiophilic thiocarbohydrazide (TCH). *J. Cell Biol.*, **30**, 424-432.

Singh I., 1964. A modification of the Masson Hamperl method for staining of argentaffin cells. *Anat. Anz.*, **115**, 81-82.

Sire M. F., C. Lutton, J. M. Vernier, 1981. New views on intestinal absorption of lipids in teleostean fishes: an ultrastructural and biochemical study in the rainbow trout. *J. Lipid Res.*, **22**, 81-94.

Sire M. F., J. M. Vernier, 1979. Formation de VLDL intestinales endogènes. Étude ultrastructurale sur un nouveau modèle, l embryon et l adulte, à jeun, de truite. *Biol. Cell.*, **35**, 271-280.

Sire M. F., J. M. Vernier, 1980. Lipid staining on semithin sections with Sudan black B or Nile blue Sulphate. Application to intestinal fat absorption. *Acta Histochem. Cytochem.*, **13**, 193-201.

Sire M. F., J. M. Vernier, 1992. Intestinal absorption of protein in teleost fish. *Comp. Biochem. Physiol.*, **103**A, 771-781.

Stroband H. W. J., K. R. Dabrowski, 1979. Morphological and physiological aspects of the digestive system and feeding in fresh-water fish larvae. *In:* Nutrition des poissons. Fontaine M., ed., Centre National de la Recherche Scientifique, Colloq. CNERNA. Actes, Paris, 355-376.

Tanaka M., 1972. Studies on the structure and function of the digestive system in teleost larvae. IV. Changes in the epithelium related to fat absorption in the anteromedian part of the intestine after feeding *Jpn J. Ichthyol.*, **19**, 15-25.

Thiery J. P., 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.*, **6**, 987-1018.

Vernier J. M., 1969. Table chronologique du développement embryonnaire de la truite arc-en-ciel, *Salmo gairdneri.* Rich. 1836. *Ann. Embryol. Morphol.*, **2**, 495-520.

Vernier J. M., 1975. Étude ultrastructurale des lipoprotéines hépatiques de très basse densité au cours du développement de la truite arc-en-ciel, *Salmo gairdneri* (R. J.) *Microsc. Biol. Cell.*, **23**, 39-50.

Vernier J. M., M. F. Sire, 1976. Evolution of the glycogen content and of glucose-6-phosphatase activity in the liver of *Salmo gairdneri* during development. *Tissue Cell.*, **8**, 531-546.

Vernier J. M., M. F. Sire, 1977*a*. Lipoprotéines de très basse densité et glycogène dans le syncytium vitellin, l'épithélium intestinal et le foie, aux stades précoces du développement embryonnaire chez la truite arc-en-ciel. *Biol. Cell.*, **29**, 45-54.

Vernier J. M., M. F. Sire, 1977*b*. Plaquettes vitellines et activité hydrolasique acide au cours du développement embryonnaire de la truite arc-en-ciel. Étude ultrastructurale et biochimique. *Biol. Cell.*, **29**, 99-112.

Walzer C., N. Schönenberger, 1979*a*. Ultrastructure and cytochemistry study of the yolk syncytial layer in the

alevin of trout (*Salmo fario trutta* L.) after hatching. I. The vitellolysis zone. *Cell. Tissue Res.*, **196**, 59-73.

Walzer C., N. Schönenberger, 1979*b*. Ultrastructure and cytochemistry study of the yolk syncytial layer in the alevin of trout (*Salmo fario trutta* L.) after hatching. II. The cytoplasmic zone. *Cell. Tissue Res.*, **196**, 75-93.

Watanabe Y., N. Sawada, 1985. Larval development of digestive organs and intestinal absorptive functions in the freshwater goby *Chaenogobius annularis*. *Bull. Tohoku reg. Fish. Res. Lab.*, **47**, 1-10.