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Lipid class and fatty acid composition of planktivorous larval pike *Esox lucius* living in a natural pond

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Abstract

This study was undertaken in a pond used for natural spawning of pike. Zooplankton and pike larvae were sampled using a horizontal haul net (120 μm or 1 mm mesh size) through the aquatic vegetation. Among the different class sizes of larvae, one cohort was isolated (11.1 mm $\leqslant L \leqslant$ 46 mm), in which larval pike first fed on small Cyclopoids then switched to Cladocerans until they fed on insect larvae. Length (1.2 mm/day) and weight growth were found to be fast. Between day 8 and day 13 of exogenous feeding there was a marked decrease of reserve lipids of larvae. This decrease coincided with a lower growth and seemed to be ascribable to changes from Cyclopoid (60.4 % of ingested prey) to 2 species of Cladoceran (42.4 % and 36.4 % of ingested prey), in the feeding sequence of larvae. Pike probably found difficulties in capturing Cyclopoid and digesting Cladocerans as their alimentary canal was not completely developed. After day 13, Cladoceran digestibility was good as the S-bend gut was formed, when triacylglycerol content of larvae increased until the experiment ceased. As indicators, of good growth, we detected increasing amounts of phospholipids throughout the whole study. The fatty acid composition of larval triacylglycerols appeared to be very similar to the composition of zooplankton lipids. Dietary fatty acids could have been incorporated whithout modification into triacylglycerols and into phospholipids possibly with limited elongation/desaturation. The results suggest that pike larvae require both (n-3) FA and (n-6) FA.

Keywords: Pike larvae, zooplankton, feeding behaviour, triacylglycerols, phospholipids, fatty acids.

*Composition en acides gras et évolution des classes lipidiques de larves zooplanctonophages de brochet (*Esox lucius*), en milieu naturel.*

Résumé

Les larves de brochet étudiées ont été prélevées en milieu naturel (étang de Faux, Creuse, France). La nourriture zooplanctonique et les larves de brochet ont été échantillonnées ou capturées à l'aide de filets (vide de maille : 120 μm ou 1 mm). Une cohorte de larves a pu être isolée (11,1 mm $\leqslant L \leqslant$ 46 mm), elle présentait une forte croissance (1,2 mm/jour) et un régime alimentaire évoluant successivement des Cyclopoïdes aux Cladocères, puis aux larves d'insectes. Entre le 8^e et le 13^e jour d'alimentation active, le passage d'un régime alimentaire à base de Cyclopoïdes (60,4 % des proies ingérées) à un régime dominé par 2 Cladocères (42,4 % et 36,4 % des proies ingérées) s'est traduit par une croissance plus faible et une baisse significative des lipides neutres larvaires. Après le 13^e jour d'alimentation exogène, les dépôts de lipides (triglycérides) réaugmentent grâce à une nourriture mieux digérée, la formation du tube digestif étant achevée. Traduisant une croissance continue des larves, les phospholipides ont augmenté sans cesse tout au long de l'expérimentation. L'analyse par chromatographie en phase gazeuse des acides gras larvaires et zooplanctoniques a montré une nette influence des profils d'acides gras du zooplancton sur les triglycérides de brochet. Suivant le type de proie consommé, des acides gras d'origine alimentaire comme le 18 : 2 (n-6) et le 20 : 5 (n-3) ont pu être partiellement bioconvertis et incorporés sous forme de

20:4 (n-6) et de 22:6 (n-3) dans les phospholipides. Ceci pourrait traduire des besoins en acides gras polyinsaturés des séries (n-3) et (n-6) de la part des brochets au stade larvaire.

Mots-clés : Larves de brochet, zooplancton, alimentation, triglycérides, phospholipides, acides gras.

INTRODUCTION

The northern pike *(Esox lucius)* is an important game and commercial fish. The culture of this fish is often limited to the production of fingerlings for restocking of natural waters in order to enhance sport fisheries (Huet, 1976; Horvath, 1983; van Lukowicz, 1983). Unfortunately, although restocking is necessary in new environments, it appears to be quite inefficient in waters with natural pike populations (Le Louarn, 1983). The survival of newly introduced fingerlings or yolk-sac larvae is determined by the largest sized native pike (Grimm, 1983), the abundance of aquatic plants and food availability (*see* review of Raat, 1988).

Thus, recruitment of young pike partially depends on an adequacy between nutritional requirements and prey quantity or quality. This is particularly relevant when pike larvae start exogenous feeding on zooplankton prey, in vegetated littoral areas (Frost, 1954). The same is true for extensive or intensive rearing of pike larvae, where successful production is subject to the quality and abundance of the zooplankton food available.

Conditions for efficient growth in larval stages of many fish are determined by the biochemical composition of the diet and prey (Barnabé, 1991). Watanabe (1982) has pointed out the role of lipids in fish nutrition. The level of (n-3) and (n-6) polyunsaturated fatty acids (PUFA) in the food is considered as an important factor of development of fish larvae (Pionetti *et al.*, 1986; Fraser *et al.*, 1989). The dietary requirements for PUFA vary from one species to another; but marine fish larvae generally need long chain PUFA such as 20:5 (n-3) or 22:6 (n-3) (Tocher *et al.*, 1992), whereas freshwater fish generally require shorter chain PUFA such as 18:3 (n-3) and 18:2 (n-6) (Farkas *et al.*, 1977; Léger and Frémont, 1981).

There is a general lack of knowledge on the fatty acid (FA) requirements and composition of freshwater larvae of predator fish like perch, pike-perch and pike.

Furthermore there are no data on the transfer of fatty acids from plankton to fish larvae under natural conditions in freshwater, whereas many authors have demonstrated the importance of fatty acid dynamic in marine food chains, e.g. for larval seabass and herring (Pionetti *et al.*, 1986; Fraser *et al.*, 1987). Only Muje *et al.*, (1989) have compared the distribution of fatty acids in zooplankton prey and 0^+ vendace (*Coregonus albula*) muscles.

In the present report we examined the influence of zooplankton fatty acids on the composition of pike larvae triacylglycerols (TAG) during the planktivorous phase. The growth, the changes in stomach contents and the evolution of the main lipid class are described. The transfer and bioconversions of PUFA through different levels comprising zooplankton and TAG and phospholipids (PL) of larvae are also discussed.

MATERIALS AND METHODS

Study site

The study was undertaken in a pond used for natural spawning of pike (0.6 ha surface, 2.20 maximum depth, mean pH = 6.2). The pond was surrounded by aquatic macrophytes characteristic of peat-bog flora e.g. *Menyanthes trifoliata, Carex* sp., *Myriophyllum* sp. Dominant phytoplankton included species of Cryptomonadina and Diatoms.

Sampling

Zooplankton was collected using a horizontal haul net of 120 μm mesh size towed through the littoral vegetation. One subsample was frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for lipid analysis. The other subsample was preserved in 4% formalin for species determination and length measurements.

At the same time and in the same locations we sampled larval pike. Collections were made from the hatching of larvae (9 and 10 April) until these larvae ignored zooplankton prey (12 to 16 May). We sampled at 3-5 day intervals using 0.5 m diameter nets with mesh size of 1 mm. At each collection, 10 larvae were preserved in 10% formalin while the others (from 3 to 6) were frozen immediately after fishing and stored at $-80\,^{\circ}C$ for lipid extraction. Yolk-sac embryos were omitted from analysis.

Growth of pike larvae was estimated from length measurements (mm) and wet weight determinations (mg) of formalin preserved samples.

Dietary studies

The digestive tract was removed from larvae and examined. Cladocerans were identified to species and Copepods were indentified as Cyclopoids or down to species. Insect larvae or heads of digested insects were also counted at the end of the experiment. All prey items were measured according to Lejolivet (1988). Stomach content analysis were performed using the method described by Castel (1985); two factors were employed.

Numerical percentage (Cn):

$$Cn = \frac{P}{Np} \times 100$$

P = number of individuals in a prey category

Np = total number of all ingested prey

Frequency of occurrence (Fp):

$$Fp = \frac{n}{N_1} \times 100$$

n = number of stomach containing a prey category (p)

N_1 = number of filled stomachs examined

The frequency of occurrence was used to define the dietary importance of prey.

$Fp > 50\%$: The most important prey, the number of which defines the larvae diet.

$10\% < Fp < 50\%$: Additional prey.

$Fp < 10\%$: Uncommon prey which have no influence on the diet.

Lipid analysis

Prior to analysis we separated zooplankton species in accordance with the food spectrum in the stomachs of larvae.

Lipids were extracted from 2 to 6 freeze-dried larvae or zooplankton samples by the method of Folch *et al.* (1957). Separation of pike triacylglycerol (TAG) from pike phospholipids (PL) was carried out by preparative thin layer chromatograhy (TLC) on silica gel 60 plates, using hexane : diethyl ether: formic acid (80 : 20 : 2, by volume) as the developing solvent (Christie, 1989).

The lipid extracts were saponified in methanolic NaOH (2N) for 10 min at 60 °C; conversion into fatty acid methyl esters (FAME) was performed by using methanolic H_2SO_4. FAME were analysed by gas chromatography on a capillary column (20 m × 0.34 mm i.d.) coated with a Carbowax CW 20 M phase. The methyl esters were identified by comparison with known standards (SIGMA, Chemical Co. St Louis). The presence of some unusual fatty acids such as branched FA or very long chain FA was confirmed by using gas chromatography in combination with mass spectrometry (GC/MS). The identification of fatty acids was performed on a LKB 2091 instrument by analysis of picolinyl ester derivatives (Christie, 1989).

Lipid classes of pike larvae were quantified by thin layer chromatography and flame ionization detection using Chromarods SIII and an latroscan Th-10 Mark IV analyser. The solvent migration time and operating conditions were as described previously by Peuchant *et al.*, (1984).

The detector responses of the latroscan were recorded and analysed by the Boreal Computer program (Flotec Software, Paris). Peak areas for each lipid class were converted into μg of lipid using calibration curves constructed from standard solutions.

Spearman's rank correlation coefficient was used for comparison of zooplankton fatty acid compositions with larval fatty acid profiles.

Differences between lipid classes were analysed by Student's t-test.

RESULTS

Larval growth

From all the captured larvae we could isolate one cohort which allowed us to determine: i) growth from the beginning of exogenous feeding overlaying zooplanktivorous phase; ii) the evolution of TAG and PL.FA from 8 days after the start of feeding until the insect feeding phase.

The growth of larvae (*fig.* 1) in length and weight showed no decrease and was quite fast. In 29 days pike larvae grew from 11.1±0.7 mm or 9.1±2.0 mg to 46±2.4 mm or 671.4±41.8 mg.

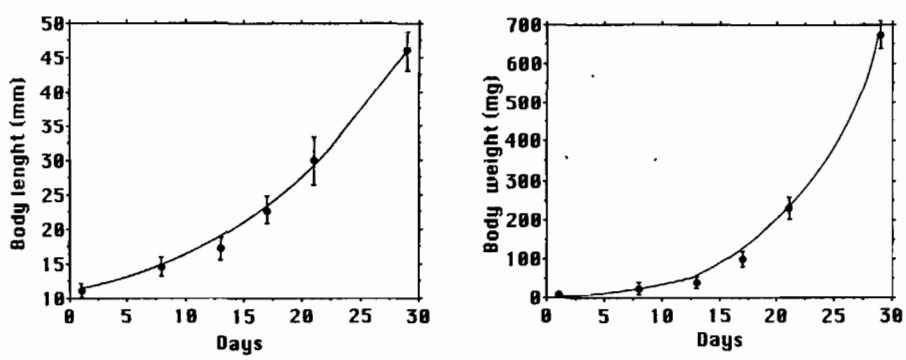

Figure 1. – Growth of pike larvae in a cohort overlaying samples collected from the start of exogenous feeding to the insect feeding phase. Growth in length and growth in wet weight are presented separately.

Food spectrum (*fig.* 2, *table* 1)

Eight days after the start of feeding, pike larvae (L = 14.5 mm) consumed essentially small Cyclopoids (Cn = 60.4 %) which were found in all the guts examined (Fp = 100 %). Three Cladocerans occurred in the diet as additional prey, one small species *Chydorus sphaericus* (Fp = 25 %) and two large items *Eurycercus lamellatus* and *Simocephalus vetulus* (Fp = 37 %). Pike only captured smaller sized Cladocerans (260 μm < L < 400 μm), (*fig.* 2).

Between day 8 and day 13, *E. lamellatus* (Cn = 42.4 %, Fp = 100 %) and *S. vetulus* (Cn = 36.42 %, Fp = 100 %) became of primary importance in the diet of larvae, while Cyclopoids accounted for only 1.22 % of prey eaten. The largest Cladocerans were then consumed.

From day 13 to day 21 *E. lamellatus* and *S. vetulus* were the major contributors to larval diet in numerical percentages, prey sizes (*fig.* 2) and frequency of occurrence (*table* 1). Cyclopoids occurred in the food spectrum as secondary prey present in very low percentages of prey eaten (Cn = 4.3 % on day 17, Cn = 5.5 % on day 21). Uncommon species like *Canthocampus* sp. and *C. sphaericus* were definitively ignored by the larvae.

On day 21 insect larvae (Chironomids, Ephemeroptera) appeared in a few stomachs (Fp = 20 %) and represented substantial percentages (Cn = 31.99 %) of total prey eaten (*fig.* 2, *table* 1)

On day 29, larvae were in the insectivorous phase (Fp = 100 %), with insects contributing most of the diet (Cn = 75 %). Very large *S. vetulus* (L = 2,800 μm) still accounted for 25 % of prey eaten but for only 20 % of the filled stomachs examined.

Table 1. – Frequency or occurrence (in percentage) of the major groups of food organisms in larvae.

	Day 8	Day 13	Day 17	Day 21	Day 29
Copepods					
Cyclopoids	100	20	37.5	50	0
Canthocamptus	12.5	5	0	0	0
Cladocerans					
S. vetulus	37	100	100	100	20
E. lamellatus	37	100	100	100	0
C. sphaericus	25	10	0	0	0
Insect larvae	0	0	0	20	100

Fatty acid composition of zooplankton total lipids

Zooplankton samples were fractionated into the 3 major groups encountered in larvae stomachs: *E. lamellatus, S. vetulus,* Cyclopoids. The sample taken on day 2 was only fractionated into Cyclopoids and Cladocerans. The distribution of fatty acids in Cyclopoids is shown in *table* 2. The saturated FA ranged from 35.70 to 40.94 % of the total FA; 16 : 0 dominated the saturates. The polyunsaturated FA constituted 28.80 to 47.91 % of total FA; 18 : 3 (n-3), 18 : 4 (n-3) and 22 : 6 (n-3) represented the major (n-3) FA. Of the (n-6) series FA, 18 : 2 (n-6) and 20 : 4 (n-6) were the most abundant.

The fatty acid composition of *E. lamellatus* was quite similar to that of *S. vetulus* (*table* 3). Saturates FA were dominated by 16 : 0 and 18 : 0 in both species. PUFA ranged from 24.65 to 46.61 % in *S. vetulus* and from 23.14 to 39.74 % in *E. lamellatus*, which

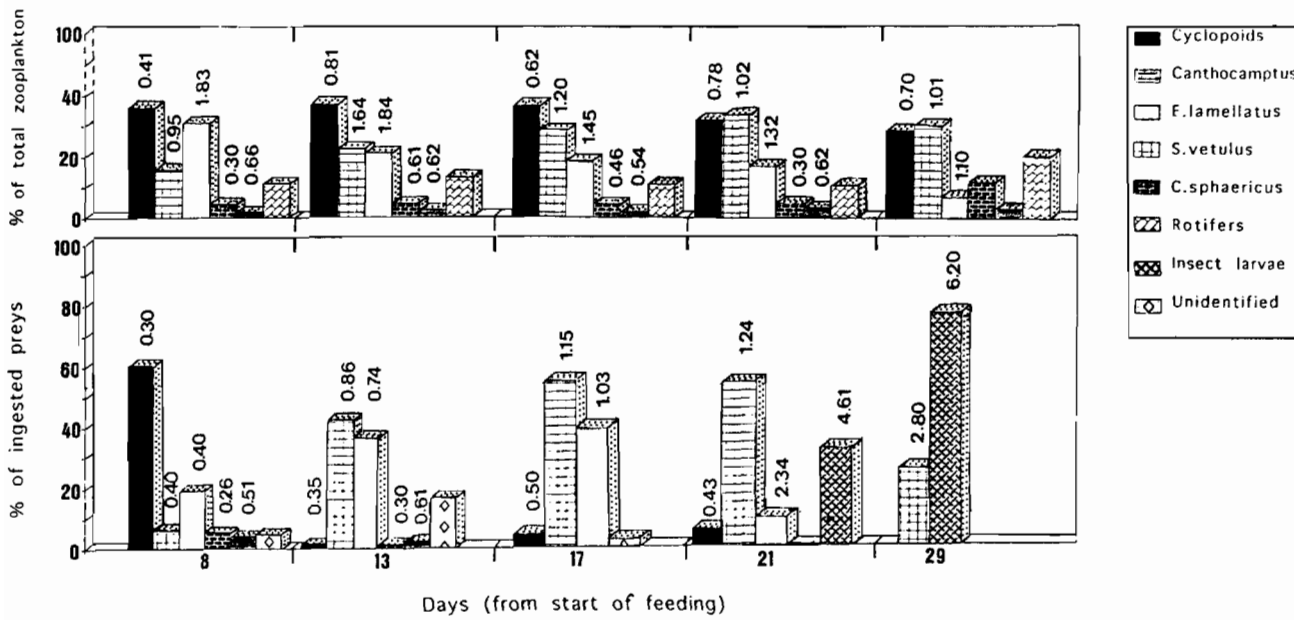

Figure 2. – Temporal changes in zooplankton groups (percentage of total numbers) encountered in vegetated littoral (upper illustration). Numbers are the mean size (mm) of the different items sampled.

Table 2. – Fatty acid composition of total lipid extracted from Cyclopoids sampled in vegetated littoral. Dara are weight percent of total FA and are results of a single analysis (tr indicates < 0.4 %; – indicates not detected. Br indicates branched FA; 16UFA indicates added percentage of 16:2 16:3 and 16:4 FA).

	Days (from start of feeding)			
	2	8	13	17
14:0	5.86	5.94	4.75	5.90
16:0	18.90	22.00	22.90	20.25
18:0	8.00	11.08	6.90	10.02
15Br	–	tr	–	–
15:0	1.84	1.17	1.51	2.09
17:0	1.10	0.75	0.80	1.30
Saturates	**35.70**	**40.94**	**36.86**	**39.56**
14:1	tr	–	–	–
15:1	tr	0.55	0.40	0.40
16:1	10.23	6.35	8.44	5.30
17:1	0.70	tr	tr	tr
18:1	9.72	14.31	15.30	6.00
20:1	tr	tr	0.65	tr
22:1	tr	tr	tr	tr
Monoenes	**20.93**	**22.03**	**26.21**	**11.70**
16UFA	3.84	1.83	2.10	2.22
18:2 (n-6)	4.43	2.61	2.45	3.60
18:3 (n-6)	tr	tr	tr	0.66
18:3 (n-3)	7.22	1.20	6.10	8.08
18:4 (n-3)	5.26	1.28	3.00	7.19
18:5 (n-3)	1.00	2.28	0.82	0.48
20:2 (n-6)	1.01	1.00	2.22	1.30
20:4 (n-6)	2.31	2.91	3.31	2.91
20:4 (n-3)	0.60	0.50	1.40	1.10
20:5 (n-3)	2.20	1.00	1.90	4.98
22:2 (n-6)	1.66	1.60	1.00	0.62
22:3 (n-6)	1.40	0.50	0.83	1.38
22:4 (n-3)	tr	0.84	0.81	1.20
22:5 (n-3)	2.16	2.25	2.56	1.56
22:6 (n-3)	4.08	9.00	7.60	10.63
Total (n-3)	**22.52**	**18.35**	**24.19**	**35.22**
Total (n-6)	**10.81**	**8.62**	**9.81**	**10.47**
PUFA	**37.17**	**28.80**	**36.10**	**47.91**
Unidentified	5.31	6.51	0.41	0.60

appeared to be less rich in PUFA than *S. vetulus*. 18:3 (n-3), 18:4 (n-3) and 20:5 (n-3) were the dominant (n-3) FA while 18:2 (n-6), 20:4 (n-6), 22:2 (n-6) and 22:3 (n-6) represented the major FA of the (n-6) series. The monoenes constitue approximately 20 to 25 % of the total FA.

There were notable differences between FA distributions in Cyclopoids and Cladocerans (*tables* 2 and 3). First, it may be noted that amounts of 20:5 (n-3) were greater than amounts of 22:6 (n-3) in both Cladocerans. Moreover, several unusual fatty acids occurred in both Cladoceran species and were either absent or present in considerably lower percentages in Cyclopoids. These FA were branched 15:0 and 17:0, 22:2 (n-6) and 22:3 (n-6) FA. In a previous study (unpubl. data) we suggested that branched FA and (n-6) long chain FA were originating in bacteria, detritus and benthic protozoa consumed by *E. lamellatus* and *S. vetulus* known for their detritivorous diet (Kankaala, 1988). Thus, the presence of 22:2 (n-6) and 23:3 (n-6) in addition to that of 18:2 (n-6) accounts for the substantial levels of (n-6) FA encountered in both Cladocerans as compared to the Cyclopoids samples.

Lipid class composition of larvae (*table* 4)

Amounts of phospholipids (mg/g of dry weight, D. W.) increased continuously throughout the experiment in parallel with the mean dry weights, this being a clear indication of good growth of the larvae.

From day 8 to day 13 there was a significant decrease in the triacylglycerol content of the larvae ($p < 0.01$), during the same period sterols and sterol esters declined significantly ($p < 0.01$ in both cases) coinciding with the reduced mean lipid content of larval dry weight. After day 13, neutral lipids stopped being metabolized, TAG increased significantly ($p < 0.01$) to day 17 and then continuously until we ceased sampling (day 29). During the same time sterol esters showed slight not always significant increases. Between day 13 and day 29 an increase in TAG was accompanied by a parallel increase of lipid content (mg/g of dry weight or mg/larva), such that at the end of the study the lipid content represented 13.1 to 13.4 a percentage of dry weight.

Fatty acid analysis of pike larvae

Triacylglycerols (*table* 5)

In the TAG reserve of larvae, there was a notable decrease in the percentage of saturates accompanied by an increased percentage of PUFA, between day 8 and day 29. Monoenes ranged from 23.74 to 29.20 % of TAG FA and were dominated by 16:1 and 18:1 isomers. From day 8 to 21, the fatty acid spectrum of TAG was close to that seen in Entomostraca. From day 13 to 21 the coefficient of Spearman's rank correlation indicated that the FA pattern of pike was always closer to that of both Cladocerans ($p = 0.0001$) than to that of the Cyclopoids ($p = 0.0008$ on day 13 and $p = 0.0002$ on day 17). On day 8 the FA spectrum of larvae was correlated to the 3 groups of Entomostraca ($p = 0.0001$ in all cases). On day 13, when Cladocera became the principal food items ingested by larvae, the unusual FA originating in *Eurycercus lamellatus* and *Simocephalus vetulus* e.g. branched 15:0 and 17:0, 22:2 (n-6) and 22:3 (n-6), were found in increased percentages (*table* 5). Increased proportions of 22:2 (n-6) and 22:3 (n-6) resulted in high amounts of (n-6) PUFA until the larvae definitively fed on insects at day 29.

Other FA such as 18:2 (n-6), 18:3 (n-3) and 18:4 (n-3) increased markedly after day 17 to maxima on day 29 – 22:6 (n-3) present initially in low level, declined continuously throughout the study, whereas 20:5 (n-3) and 20:4 (n-6) were present consistently in low percentages.

Table 3. – Fatty acid composition of total lipid extracted from *Eurycercus lamellatus* and *Simocephalus vetulus* sampled in vegetated littoral (Cladocerans indicates non-separated Cladocerans; other annotations as in *table* 2).

	Cladocerans	*Simocephalus vetulus*				*Eurycercus lamellatus*			
	2	8	13	17	21	8	13	17	21
12:0	tr	0.80	1.41	0.47	1.31	1.61	1.83	0.40	1.12
14:0	9.57	5.41	5.08	3.05	4.72	3.86	5.25	3.15	3.28
16:0	20.80	23.60	23.20	14.35	14.63	22.81	22.30	17.12	17.35
18:0	6.63	13.73	7.83	4.83	10.66	17.21	14.09	7.70	7.60
15Br	1.93	0.96	0.92	1.12	1.05	1.28	1.45	0.84	0.80
15:0	1.82	0.52	0.89	0.97	0.70	1.20	0.89	1.30	2.00
17Br	0.60	0.97	0.80	1.44	0.51	1.29	1.51	1.90	0.80
17:0	1.69	0.84	0.74	0.76	0.90	0.81	0.61	1.00	1.16
Saturates	**37.04**	**46.83**	**40.87**	**27.00**	**34.48**	**50.07**	**47.93**	**33.41**	**34.11**
14:1	0.44	–	tr	tr	tr	tr	0.87	tr	0.54
15:1	tr	tr	0.53	tr	0.50	0.62	tr	tr	0.46
16:1	8.22	7.36	9.62	10.03	9.45	4.66	5.37	7.75	7.98
17:1	0.60	0.72	0.84	tr	0.40	tr	0.60	0.81	0.93
18:1	16.33	12.90	14.61	10.27	10.50	11.50	13.57	14.05	15.70
20:1	tr	0.94	–	tr	tr	tr	tr	0.68	0.78
22:1	1.00	tr	1.05	tr	0.40	tr	tr	tr	–
24:1	tr	2.51	0.84	tr	tr	2.00	tr	tr	tr
Monoenes	**26.59**	**24.43**	**27.49**	**20.30**	**21.25**	**18.78**	**20.41**	**23.29**	**26.39**
16UFA	2.52	2.21	3.30	3.22	2.16	2.67	2.40	4.83	2.35
18:2 (n-6)	2.86	2.40	2.86	4.60	3.03	2.41	2.66	4.46	2.90
18:3 (n-6)	0.81	1.33	0.54	1.66	0.65	tr	tr	tr	0.43
18:3 (n-3)	7.46	2.81	4.12	8.18	5.95	1.24	1.63	5.67	5.21
18:4 (n-3)	2.58	2.69	3.00	7.09	5.30	0.50	0.62	2.04	1.68
18:5 (n-3)	1.00	1.65	0.83	0.58	1.10	1.16	1.88	1.23	1.02
20:2 (n-6)	0.71	1.09	0.69	1.29	1.13	0.82	1.83	1.23	1.02
20:4 (n-6)	1.14	0.43	0.71	1.37	2.10	0.91	0.51	1.73	2.43
20:4 (n-3)	0.62	0.40	0.48	1.16	0.62	1.14	1.13	0.71	0.91
20:5 (n-3)	4.59	1.18	4.05	7.98	7.22	3.01	4.16	5.34	5.60
22:2 (n-6)	4.70	5.67	5.39	4.62	6.60	6.73	7.44	5.26	6.54
22:3 (n-6)	1.23	1.03	2.33	2.38	2.97	1.71	2.79	4.91	3.96
22:5 (n-3)	tr	0.50	tr	tr	0.80	tr	tr	0.83	0.92
22:6 (n-3)	1.02	1.26	0.42	2.58	2.63	0.90	0.61	1.92	2.20
Total (n-3)	**17.27**	**10.49**	**10.90**	**27.47**	**23.62**	**7.95**	**10.03**	**17.32**	**17.13**
Total (n-6)	**11.45**	**11.95**	**12.52**	**15.92**	**16.48**	**12.58**	**15.23**	**17.59**	**17.28**
PUFA	**31.24**	**24.65**	**26.72**	**46.61**	**42.26**	**23.14**	**27.66**	**39.74**	**36.76**
Unidentified	4.01	3.14	4.00	4.89	1.32	6.21	3.10	3.20	2.34

Table 4. – Dry weights, lipids contents, and lipid class composition of pike larvae. Values are means (±SD) of 3-4 determinations (d.w. = dry weight; tr < 0.2 %.

	Days (from start of feeding)				
Measure of lipid	8	13	17	21	29
Mean dry weight (mg/larva)	4.5 (2.1)	7.5 (1.5)	11.2 (2.1)	31.0 (9.2)	164.2 (23.3)
Mean lipid content (mg/larva)	0.69	0.61	1.13	4.06	21.0
Lipid content (mg/g of d.w.)	153.0	81.8	100.9	130.9	127.9
Lipid class (mg/g of d.w.)					
Polar lipids	54.2 (8.3)	64.5 (3.6)	71.2 (2.8)	87.1 (6.8)	97.6 (9.9)
Triacylglycerol	32.9 (5.2)	7.4 (1.9)	12.0 (1.6)	18.8 (1.7)	25.2 (9.3)
Sterols	36.0 (3.7)	6.5 (2.4)	11.0 (2.2)	14.9 (0.7)	6.2 (0.3)
Free fatty acids	12.8 (4.1)	2.2 (1.8)	3.0 (1.0)	3.5 (0.7)	1.7 (0.5)
Sterol esters	12.0 (3.2)	0.9 (0.1)	1.6 (0.6)	1.7 (0.4)	1.9 (0.1)
Diacylglycerol	4.9 (0.8)	tr	1.7 (0.2)	1.9 (0.2)	1.3 (0.1)
Monoacylglycerol	tr	tr	0.4 (0.2)	1.0 (0.1)	0.2 (0.3)

Table 5. – Changes in the fatty acid composition of triacylglycerol reserves in pike larvae. Data are weight percent of total FA and are results of 1-3 analysis. VLCFA indicates very long chain fatty acids such as 25:0, 24:1 and low percentages of 24:3 and 24:4. Other annotations as in *table* 2.

	Days (from start of feeding)				
	8	13	17	21	29
12:0	1.00	1.95	1.01	tr	tr
14:0	5.80	5.02	3.18	2.44	2.02
16:0	24.20	18.10	19.20	19.44	21.50
18:0	11.16	7.70	6.63	5.25	3.82
15Br	0.40	1.50	0.93	0.52	tr
15:0	1.22	1.79	1.51	1.02	0.82
17Br	tr	1.63	1.31	0.40	–
17:0	1.35	0.88	0.81	0.91	0.65
Saturates	**45.13**	**38.57**	**34.28**	**29.98**	**28.81**
14:1	–	0.90	tr	tr	tr
15:1	0.50	0.80	0.60	tr	tr
16:1	7.80	8.81	9.10	10.67	10.70
17:1	tr	0.63	0.41	0.40	0.50
18:1	15.44	14.21	15.61	17.10	18.00
20:1	tr	1.74	tr	0.56	tr
22:1	tr	1.13	0.40	–	tr
Monoenes	**23.74**	**28.22**	**26.12**	**28.73**	**29.20**
16UFA	1.96	2.01	3.10	1.06	0.76
18:2 (n-6)	2.70	2.21	4.51	4.64	8.34
18:3 (n-6)	0.44	0.40	0.62	0.98	1.16
18:3 (n-3)	1.18	2.10	7.32	10.50	15.40
18:4 (n-3)	1.35	1.07	6.21	4.40	4.30
18:5 (n-3)	2.75	1.81	tr	tr	–
20:2 (n-6)	0.93	1.87	1.31	0.93	–
20:3 (n-6)	tr	0.67	0.41	tr	–
20:4 (n-6)	1.23	0.91	0.89	0.87	1.20
20:4 (n-3)	0.50	1.14	0.93	0.68	0.74
20:5 (n-3)	1.83	1.87	1.80	3.01	2.40
22:2 (n-6)	2.80	8.30	4.94	6.46	1.80
22:3 (n-6)	2.25	2.39	3.23	2.84	0.76
22:5 (n-3)	1.37	1.00	tr	tr	0.41
22:6 (n-3)	2.80	1.34	1.10	1.00	0.81
Total (n-3)	**11.78**	**10.33**	**17.36**	**19.59**	**24.06**
Total (n-6)	**10.35**	**16.75**	**15.91**	**16.72**	**13.26**
PUFA	**24.09**	**29.09**	**36.37**	**37.37**	**38.08**
VLCFA	3.88	2.18	2.20	2.50	2.87
Unidentified	2.67	1.30	0.81	1.17	0.57

Table 6. – Changes in the fatty acid composition of phospholipids in pike larvae. For annotations *see table* 5.

	Days (from start of feeding)				
	8	13	17	21	29
12:0	0.88	0.90	0.71	tr	tr
14:0	1.75	2.05	1.70	0.73	tr
16:0	27.60	20.60	22.20	23.90	20.80
18:0	13.60	11.24	10.21	7.78	8.50
15Br	tr	0.40	0.40	tr	tr
15:0	0.88	0.65	0.51	0.54	tr
17Br	tr	tr	tr	–	–
17:0	1.34	0.77	0.72	0.77	0.70
Saturates	**46.05**	**36.61**	**36.45**	**33.72**	**29.30**
14:1	tr	0.41	–	–	–
15:1	tr	tr	–	–	–
16:1	5.31	6.64	5.43	4.53	3.10
17:1	0.66	0.53	0.41	–	–
18:1	13.62	13.96	13.15	13.72	15.67
20:1	0.47	0.83	0.51	tr	tr
22:1	tr	tr	tr	–	–
Monoenes	**20.06**	**22.37**	**19.50**	**18.25**	**18.77**
16UFA	0.88	1.14	0.91	0.68	0.47
18:2 (n-6)	2.28	2.19	2.38	3.01	3.67
18:3 (n-6)	0.51	0.45	0.48	0.43	0.40
18:3 (n-3)	2.41	3.08	3.72	4.12	5.06
18:4 (n-3)	0.92	0.60	1.51	1.38	1.00
20:2 (n-6)	0.71	0.92	0.83	tr	tr
20:3 (n-6)	0.41	0.40	0.47	0.54	0.53
20:4 (n-6)	3.98	4.23	5.01	6.36	7.01
20:4 (n-3)	0.60	1.12	0.95	1.24	1.20
20:5 (n-3)	2.56	3.00	2.85	5.09	6.52
22:2 (n-6)	1.01	5.01	3.71	4.1	1.08
22:3 (n-6)	0.81	1.52	1.72	1.30	0.50
22:4 (n-3)	0.44	tr	tr	0.53	tr
22:5 (n-3)	2.70	2.11	2.31	2.80	3.40
22:6 (n-3)	9.46	10.20	12.71	14.10	17.38
Total (n-3)	**19.09**	**20.11**	**24.05**	**29.29**	**34.56**
Total (n-6)	**9.71**	**14.72**	**14.60**	**15.74**	**13.19**
PUFA	**29.68**	**35.97**	**39.56**	**45.71**	**48.22**
VLCFA	2.10	2.28	2.31	1.02	2.35
Unidentified	1.63	2.10	1.80	1.01	0.95

Phospholipids (*table* 6)

As it was described for TAG, PL showed decreasing proportions of saturated FA (mainly 16:0, 18:0) compensated by increasing percentages of PUFA. Monounsaturated FA were dominated by 18:1 with 18:1 (n-9) for the principal isomer.

18:2 (n-6), 18:3 (n-3) and 18:4 (n-3) were present in lower amounts in PL than in TAG, whereas the proportions of 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) were higher in phospholipids.

Unusual FA of zooplankton origin, especially branched FA, were incorporated little into PL. The percentages of total (n-6) PUFA in PL were lower than in TAG, contrasting to the situation for total (n-3) PUFA, which were higher in PL.

In both lipid classes of larvae we detected significant amounts of very long chain fatty acids (VLCFA) (*see tables* 5 and 6). Those compounds were identified by mass spectrometry as: 25:0 FA, 24:1 FA, 24:3 and 24:4 FA (both 24 PUFA were present in little more than traces).

DISCUSSION

In the present study the captured pike larvae were found to have a very good growth, as demonstrated by using the high specific growth rates (SGR). SGR is generally calculated from wet weights following this formula:

$$SGR = \frac{(\ln W_f - \ln W_0)}{(t_f - t_0)} \times 100$$

W_f = final weight, W_0 = initial weight,

$t_f - t_0$ = time.

Over 29 days our cohort exhibited a SGR of 15.3 % which is much higher, for larvae in the same range of

weights, than the SGR calculated from data reported for wild pike larvae by Franklin and Smith (1963) and Lejolivet (1988). Values of their SGR were respectively 11.9% and 11.4%.

It is of interest to note that between day 8 and 13 SGR was low (10.1%). This slow growth coincided with changes in the food sequence of larvae, accompanied by the significant ($p<0.01$) depletion of neutral lipids and a decreased lipid content of pike. The observed abandonment of Cyclopoid prey on day 13 had probably occurred gradually from day 8, in proportion to the increasing sizes of the Cyclopoid population. On day 13 the only Cyclopoids captured were 0.35 mm long while the mean size of the available Cyclopoids were 0.81 mm (*see fig.* 2). In the same way Lejolivet (1988) reported that small pike larvae showed poor ability to catch large Copepods (L>1 mm). The adult Cyclopoids generally show higher swimming speeds (0.9 $mm.s^{-1}$ – 3.0 $mm.s^{-1}$) than Cladocerans (Li and Li, 1979; Greene, 1983). For instance, *Simocephalus vetulus* was described as a very slow swimming Cladoceran (0.1 $mm.s^{-1}$) by Cooper *et al.* (1985).

However, according to Drost (1987) there is no evidence that larval pike neglect Cyclopoids due to their escape velocity. Thus this author calculated that 14-mm pike larvae create in the water flow a suction velocity sufficient to catch Copepods. In fact the failures in the capture attempts are ascribable to the Copepod's ability to jump away from the fish's mouth diameter, before the latter starts snapping. Nevertheless it seems obvious that a substantial decrease of reserve lipid of larvae can be attributed to insufficient food or inadequate prey. Thus, in herring larvae Fraser *et al.* (1987) established that endogenous TAG are catabolised when exogenously derived energy is insufficient to maintain the basal metabolism of larvae. Moreover, when starved, the adult pike first mobilize their perivisceral fats for energy provision (Ince and Thorpe, 1976).

As neither zooplankton densities (2,000-1,000 ind./l) nor zooplankton quality could explain the decrease of neutral lipids, we suggest that between day 8 and 13 pike larvae experienced difficulties in feeding on larger Cyclopoids and then captured progressively Cladocerans. But in pike<17.5 mm the digestibility of Cladocerans, in contrast to Copepods, is low, as long as the stomach is not completely developed (Shamardina, 1957; Ivanova et Lopatko, 1983). Therefore, it seems quite probable that changes in Cyclopoids sizes, added to low digestibility of *E. lamellatus* and *S. vetulus* (due to their large chitinous shell) induced the depletion of lipid reserves in larvae. Once the S-bend gut was formed, around day 13 with 17-18 mm pike (Frost, 1954), the amount of TAG started to increase again. Until the end of the experiment, the adequacy of food was then sufficient to ensure energy deposition and growth of the larvae. This is in accordance with Takeuchi and Watanabe (1979) and Bromley (1980) who reported that, in fish, the storage of neutral lipids is linked to the diet quality. On day 29 in 46 mm-pike, the distribution of the different lipid classes was already close to that described in adult pike by Kluytmans and Zandee (1973).

The fatty acid composition of fish is influenced by both food and water temperature (Farkas *et al.*, 1980). In a previous study (unpublished data) we established that between day 8 and day 13, high water temperatures (18°C maximum) encountered in the littoral area led to the decreased PUFA concentrations in both Cladocerans. Interestingly, during this period we observed low levels of PUFA in pike TAG and PL. After day 13, PUFA levels increased as water temperature decreased (10°C-12°C). Similar observations and the well known influences of temperature on fish lipids were reviewed by Greene and Selivonchick (1987).

The observed incorporation of zooplankton fatty acids into the TAG of pike is consistent with observations on marine fish larvae (Gatten *et al.*, 1983; Martin *et al.*, 1984, Pionetti *et al.*, 1986) and on juvenile of vendace in freshwater (Muje *et al.*, 1989). To illustrate the influences of the food FA on pike FA, the evolution of total PUFA and of four selected fatty acids are presented in *figure* 3. The patterns of TAG PUFA, TAG 18:3 (n-3) (*fig.* 3) and TAG 18:2 (n-6) (*table* 5) resemble only those of zooplankton between day 8 and day 17, after which and until the end of the experiment the profiles evolued in a different way as insects rich in 18:2 and 18:3 FA (Hanson *et al.*, 1985) began to modify the diet. Throughout the study, the profile of 22:2 (n-6) (*fig.* 3) clearly exhibits the influence of the different food sequences of the larvae. Found only in high levels in *E. lamellatus* and *S. vetulus*, this fatty acid increased slowly with the occurrence of Cladocerans in the gut, then it showed a profile close to that of Cladoceran 22:2 (n-6) and decreased markedly when insects dominated in the diet (day 21>day 29).

Accumulation of 18:2 (n-6) and 18:3 (n-3) into the TAG of pike larvae has been observed in a number of fish species such as carp (Farkas *et al.*, 1980), silver perch (Anderson and Arthington, 1989). These FA are generally deposited in an unaltered way. In contrast, dietary 18:2 (n-6) and 18:3 (n-3) can be elongated and desaturated into 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) and deposited in phospholipids in a number of freshwater fish in particular in rainbow trout (Watanabe, 1982).

In pike larvae we observed an accumulation of 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3) in polar lipids (*fig.* 3 *table* 6), a trend also being reported by Kluytmans and Zandee (1973) in adult pike. Without experimental diets or radiolabel study, it is difficult to firmly characterise the essential fatty acid requirements of larval pike and the desaturation/elongation pathways. Nevertheless, the results of this study are helpful

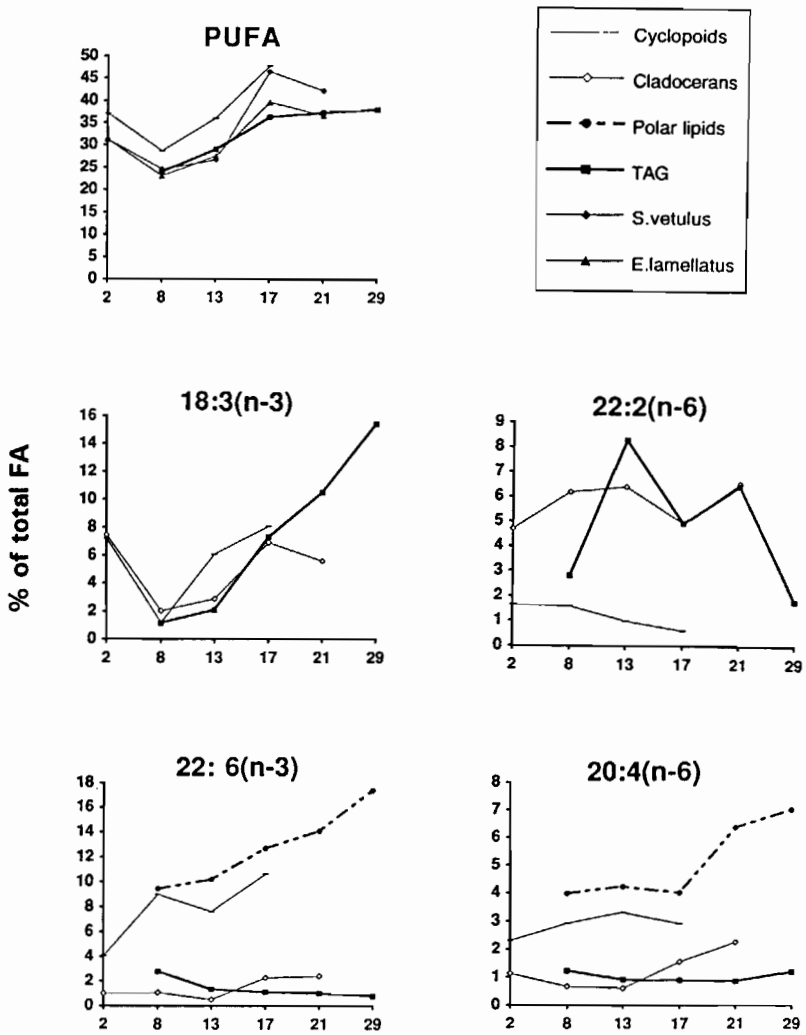

Figure 3. – Temporal changes in the relative proportions of PUFA in the pike TAG and in the total lipids of Cyclopoids, *S. vetulus* and *E. lamellatus*. Temporal changes in the relative proportions of: (*i*) 18:3 (n-3) FA and 22:2 (n-6) FA in pike TAG and total lipids of both Cladoceran and Cyclopoids; (*ii*) 20:4 (n-6) FA and 22:6 (n-3) FA in pike TAG and PL and total lipids of Cladocerans and Cyclopoids.

particularly with regard to eicosatetraenoic acid 20:4 (n-6) and docosahexaenoic acid 22:6 (n-3). The latter fatty acid has an important function in neural tissues of juvenile fish (Tocher *et al.*, 1992). In our survey it seems that neither Cladocerans (*table* 3) nor aquatic insects (Coste, 1985; Hanson *et al.*, 1985) were sufficiently rich in 22:6 (n-3) to ensure the high levels deposited in pike phospholipids.

Nevertheless, at the beginning of the study, as the larvae fed on Cyclopoids, there was clearly a substantial input of docosahexaenoic acid (*table* 2, *fig.* 3). It is likely that, from the start of feeding up to day 13, there was enough dietary 22:6 (n-3) deposited, maybe to the point of meeting the larval pike's requirements. More important, the selective retention of this fatty acid in fish (Henderson et Tocher, 1987) could argue in favour of its Cyclopoid origin.

After day 13, as Cladocerans dominated the diet we can wonder if a partial bioconversion of dietary 20:5 (n-3) into 22:6 (n-3) has not occurred. The significant amounts of 22:5 (n-3) found in PL and an active elongation system expressed by the very long chain FA detected (Henderson personal communication) could indicate a biosynthesis of 22:6 (n-3) from the dietary 20:5 (n-3) in pike larvae.

However, as the results are expressed in percentage, it is easy to be misled and the question of the possible elongation of dietary 20:5 (n-3) to 22:6 (n-3) remains unclear. On the other hand it seems obvious that bioconversion of 18:3 (n-3) into 20:5 (n-3) is highly unlikely. This because of the large dietary input of 20:5 (n-3).

Concerning 20:4 (n-6), the main precursor of eicosanoid biosynthesis in fish (Bell *et al.*, 1983), the results here might suggest, in the same way as 22:6 (n-3), that this fatty acid has a double origin: a dietary input from Cyclopoids (*fig.* 3) and a bioconversion of 18:2 (n-6) since intermediate products of the

20:4 (n-6) pathway were found in polar lipids, e.g. 18:3 (n-6) and 20:2 (n-6). In an experiment using labelled precursors performed by Kluytmans and Zandee (1974) on 400 g-pike, a little radioactivity was recovered in 18:2 (n-6) and 18:3 (n-6), consistent with radioactive units being used for bioconversion reactions of (n-6) fatty acids. Thus the elongation of 18:2 (n-6) could be possible in pike larvae, but conclusive evidence cannot be drawn because of the occurrence of a bioconversion enzymatic system more active in juvenile or adult fish than in larvae (Muje *et al.*, 1989). In conclusion, this study's results have shown that the FA composition of the triacylglycerols of wild pike larvae mainly resemble zooplankton lipids and reflect the changes in the feeding behaviour of larvae growing in littoral areas of ponds. Quantitative variations in the TAG content of pike larvae were linked to the nutritional status of larvae. According to Fraser *et al.* (1987) and Fraser (1989), the TAG content of these larvae probably indicates the sufficiency of the available food. The transfer of prey lipids could be monitored into reserve lipids with almost no FA transformations, and into phospholipids possibly with limited bioconversion of 18:2 (n-6) to 20:4 (n-6) and to a lesser extent 20:5 (n-3) to 22:6 (n-3). The accumulation, on the one hand of 20:5 (n-3) and 22:6 (n-3) and on the other of 20:4 (n-6) and 22:2 (n-6) into PL, suggest that pike larvae require both (n-3) FA and (n-6) FA. A hypothetical function of the deposited 22:2 (n-6) FA and 22:3 (n-6) FA, originating in zooplankton diet, remains unknown, and there is no known basis for the conversion of these fatty acids into eicosanoids in larval pike.

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