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Origins of carbon sustaining the growth of whitefish *Coregonus lavaretus* early larval stages in Lake Annecy: insights from fatty-acid biomarkers

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The hypothesis that diatom carbon (C) produced during the spring peak supported spring zooplankton production and, ultimately, the growth of *Coregonus lavaretus* early larval stages from March to May 2006 in Lake Annecy, France, was tested using gut content analyses and fatty acid biomarkers. Gut content results showed that *C. lavaretus* larvae from stages 1 to 4 preferentially fed on copepods with *Daphnia* sp. only a minor proportion of larval diet. The levels of diatom-marker fatty acids (C16:1n-7 and C20:5n-3) were high in *Daphnia* sp., but lower in both copepods and *C. lavaretus* larvae from stages 0 to 4. These results indicated that the spring diatom biomass was actually grazed by *Daphnia* sp., but, contrary to what was expected, the spring bloom was not the only C source supporting copepods secondary production and, consequently, the growth of *C. lavaretus* early larval stages. In contrast, levels of terrestrial fatty acid marker (C24:0) were low in *Daphnia* sp. but high in copepods and *C. lavaretus* larvae, indicating a significant contribution of terrestrial carbon to copepods and, ultimately, to the growth of *C. lavaretus* early larval stages.

Key words: copepods; *Daphnia*; diatoms; food web; lake; terrestrial carbon.

INTRODUCTION

Fish recruitment success is very strongly influenced by survival at the early larval stages (Bradford & Cabana, 1997). Thus, the origins of the carbon sources sustaining the growth of the early larval stages may be a crucial factor controlling fish dynamics. Recent studies in marine environment demonstrated that the survival and physiological conditions of larval fishes depend on both the specific composition (St John & Lund, 1996; Rossi *et al.*, 2006) and the timing of the local spring phytoplankton bloom (Beaugrand *et al.*, 2003; Platt *et al.*, 2003).

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In lakes, there is growing evidence that food webs can be fuelled by carbon (C) sources of various origins (phytoplankton-derived *v.* terrestrial-derived C; Carpenter *et al.*, 2005). The relative contributions of terrestrial-derived and phytoplankton-derived C to lake zooplankton, benthos and fish secondary production have been more thoroughly investigated recently, strongly benefiting from the development of stable-isotope and fatty-acid techniques (Karlsson *et al.*, 2003; Carpenter *et al.*, 2005; Perga *et al.*, 2006). Still, little attention has been dedicated to the identification of the carbon sources that could sustain the growth of fish early larval stages in lakes.

The whitefish *Coregonus lavaretus* (L.) constitutes a major fisheries resource in European subalpine lakes. In Lake Annecy, France, *C. lavaretus* catches by anglers and professional fishermen reach 20–30 t year^{-1}, *i.e.* 8–11 kg ha^{-1}, far above catches of Arctic charr *Salvelinus alpinus* (L.), perch *Perca fluviatilis* L. or brown trout *Salmo trutta* L. (SILA, 2007). *Coregonus lavaretus* spawn in late December and their eggs hatch in late February (Anneville *et al.*, 2007). In French subalpine lakes such as Lake Annecy, *C. lavaretus* feed essentially on zooplankton for its whole life (Perga & Gerdeaux, 2005; Anneville *et al.*, 2007). Phytoplankton blooms occurs in late winter to early spring, and diatoms largely dominate at this time of the year (SILA, 2007). Phytoplankton biomass is higher during the spring bloom than at any other period of the year (*e.g.* 2006; Fig. 1 from SILA, 2007). Thus diatom C produced during this spring bloom was expected to strongly support spring zooplankton production and, ultimately, the growth of *C. lavaretus* early larval stages from March to May in Lake Annecy. Hence, terrestrial-derived C contribution to *C. lavaretus* larvae growth during spring phytoplankton bloom should be negligible.

This hypothesis was tested using fatty-acid (FA) biomarkers. The fatty-acid trophic marker concept is based on the observation that C sources of different origins and natures lay down certain fatty-acid patterns that may be

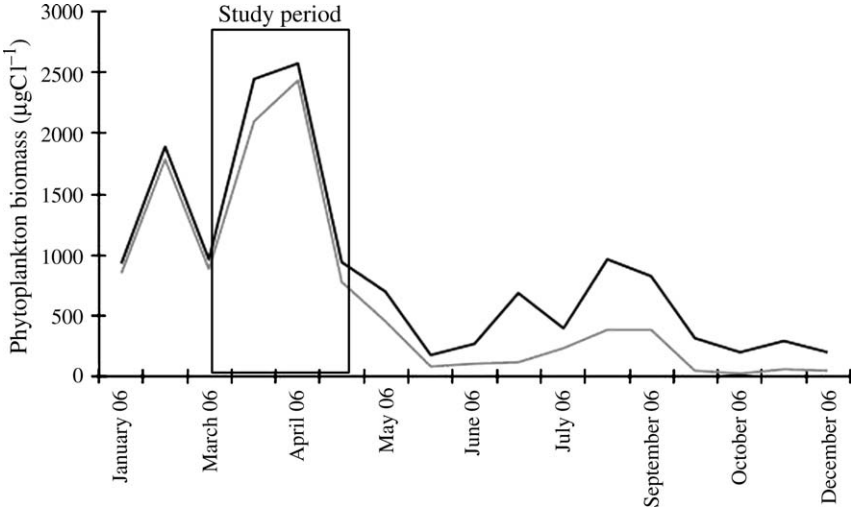

FIG. 1. Seasonal changes in total phytoplankton biomass (——) and diatoms biomass (——) in Lake Annecy in 2006 (from SILA, 2007).

transferred conservatively to, and hence can be recognized, in consumers (Dalsgaard *et al.*, 2003). Among these source-marker FAs, high levels of C16:1n-7 and of 20:5n-3 are typical of diatoms (Sargent *et al.*, 1987; St John & Lund, 1996; Parrish *et al.*, 2000). Chlorophytes are characterized by important concentrations of 16C and 18C polyunsaturated fatty acids (PUFA) (Ahlgren *et al.*, 1992; Sargent *et al.*, 1995). Cryptophytes and dinophytes contain also 18C n-3 PUFA and exhibit particularly high amounts of highly unsaturated fatty acids (HUFAs) such as eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) (Ahlgren *et al.*, 1990; Napolitano, 1998). Carbon processing through the microbial loop and subsequent zooplankton consumption of micro-organisms might be traced from bacterial FA [BAFA_odd-saturated and branched-chain FA, *i.e.* the sum of C15:0 and C17:0 and their iso-series and anteiso-series (Ederington *et al.*, 1995; Desvilettes *et al.*, 1997; Bec *et al.*, 2003; Perga *et al.*, 2006)]. In contrast, long-chain FAs, such as lignoceric acid (C24:0), are typical of terrestrial plants (Sun *et al.*, 2000). Hence, FA composition of zooplankton and fish larvae would provide information on the carbon sources that actually sustained zooplankton secondary production and, ultimately, *C. lavaretus* growth during the study period.

Furthermore, the availability of HUFA and especially EPA and DHA is of prime importance for larval development and fitness (Sargent *et al.*, 1999). Larval behaviour, growth rate and ability to feed as well as development of the brain and nervous systems are strongly dependant on levels of EPA and DHA provided by the diet (Ishizaki *et al.*, 2001). Therefore, the retention and accumulation of these essential FA (EFA) in the early larval stages are key factors in fish recruitment fluctuations (Bell & Sargent, 1996; St John *et al.*, 2001).

As EFAs are preferentially retained in zooplankton and fishes (Kainz *et al.*, 2004), they are not transferred conservatively between the food source and the prey, which could hence limit their reliability as fatty acid trophic markers. Therefore, in this study, only non-essential FAs (C16:1n-7, BAFA and C24:0) were employed as trophic markers. EPA and DHA, available for fish larvae, however, are strongly dependant on the primary C source at the base of the food chain and on the trophic pathways conveying this carbon up to larval fishes (St John *et al.*, 2001). EPA and DHA levels were thus additionally measured in order to document how zooplankton and larvae EPA and DHA content could be related to the C sources fuelling the food chain.

MATERIALS AND METHODS

STUDY SITE

Lake Annecy (45°54′ N; 06°08′ E) is located on the western border of the Alps, in the south-east part of France (altitude 446 m). Its catchment area is 302 km^2, its surface area 28 km^2 and its maximum depth is 65 m. It is a clear-water, oligotrophic lake, with an average total phosphorus concentration of 6 $\mu gP\ l^{-1}$ (1–2 $\mu gP\ l^{-1}$ from orthophosphates) and an average annual transparency of 7 m. Lake Annecy is subjected to a monthly

or bimonthly biomonitoring, which is carried out the Syndicat Intercommunal du Lac d'Annecy (SILA).

SAMPLING

In 2006, phytoplankton was sampled every 2 weeks during the routine lake biomonitoring (SILA, 2007). Integrated samples of 20 m depth were collected using a Pelletier bottle. Samples were preserved in lugol and counted under an inverted microscope according to the Ütermohl technique (Ütermohl, 1958). Counts were converted to g C l^{-1} according to Wetzel & Likens (2000). Algae with cell or colony size <20 μm were considered as nanophytoplankton, while those >20 μm were classified as microphytoplankton.

Zooplankton and *C. lavaretus* larvae sampling was carried out weekly from the end of February until the beginning of May. The larvae were collected early morning in the littoral zone of Lake Annecy using two rectangular 1 mm mesh nets (width = 1·5 m, height = 1·0 m and length = 5·0 m) towed on both sides of a motor boat. On the boat, larvae collected were maintained in lake water, and kept on ice to limit digestion and lipolytic degradation. At the laboratory, larvae were quickly measured and sorted according to their larval developmental stage (Luczynski *et al.*, 1988) and instantaneously frozen in a −80° C cryogenic freezer.

Zooplankton was sampled at a sampling station located near the area of larvae sampling. Zooplankton was collected with a 200 μm mesh-size zooplankton net with four vertical hauls. Samples intended for zooplankton counts were fixed using 5% formalin. Samples intended for fatty-acid analyses were preserved on ice until sorted in the laboratory.

Formalin-preserved zooplankton samples were counted under a dissecting microscope. Zooplankton samples intended for fatty-acid analyses were sorted according to three categories: *Daphnia* sp. and cyclopoids (*Cyclops prealpinus* and *Mesocyclos leuckartii*) of large (>500 μm) and small (<500 μm) size. Calanoids were not included in this study as they contributed to <5% of total crustaceans at the study time and were rare in *C. lavaretus* larvae gut contents (Anneville *et al.*, 2007).

STOMACH CONTENTS

Larval diets were evaluated by dissecting the entire gut contents. Prey were identified and counted at the group level under a microscope.

FATTY-ACID ANALYSIS

Fatty-acid analyses were performed from freeze-dried zooplankton and fish larvae samples (1–5 mg). Tricosanoic acid (C23:0) was added as an internal standard. Briefly, the lipids were extracted using a 4:2:1 chloroform:methanol:water mixture. The FAs, analysed as methyl esters (FAME), were prepared by saponification of the lipid extract ($NaOH–CH_3OH$ 2N) followed by esterification ($H_2SO_4–CH_3OH$ 2N). The FAME extract was subsequently analysed by gas chromatography (GC-2010; Shimadzu, Kyoto, Japan) on a Supelcowax 10 capillary column (30 m, 0·25 mm inner diameter and 0·25 μm film thickness) and measured by a flame ionization detector (FID). The FAME were identified by comparison of their retention times with known standards (37-component FAME mix, Supelco 47885-U; bacterial fatty acid methyl esters mix, Supelco 47080-U; Supelco, Bellefonte, PA, U.S.A.) and quantified with reference calibration curves derived from 2·5, 50, 100, 250, 500, 1000 and 2000 ng μl^{-1} solutions of the FAME standards.

As total FA absolute amounts vary substantially between zooplankton and fish larvae or between different larval stages as a result of changes in total lipid content, FAs were reported as per cent mass of the total identified FAs. Once reported as percentages, FA amounts are dependent on each other. For instance, an increased amount

from a single FA between two larval stages would necessarily result in a decreased percentage of the others even if their absolute amounts did not change. In order to avoid these pitfalls, source indexes were used that compare the amount of a marker FA to the amount of palmitoleic acid, which is quite ubiquitous in phytoplankton. Thus, the (C16:1n-7) : (C16:0) ratio has been used as an index of diatom C transfer in zooplankton and fish larvae (St John & Lund, 1996; Dalsgaard *et al.*, 2003). The (C24:0) : (C16:0) ratio was used to track terrestrial-derived C transfer and (BAFA) : (C16:0) to document the involvement of the microbial loop in transferring C from primary producers to zooplankton and, ultimately, *C. lavaretus* larvae.

DATA ANALYSIS

Differences in gut content compositions between larval stages were detected using χ^2-tests on contingence tables. General FA patterns were investigated using normalized principal component analysis (PCA). Variations in FA amounts or ratios between prey taxa and larval stages were indicated using non-parametric Kruskal–Wallis (KW) tests, as variances were not always homogenous between groups (prey taxa and larval stages). *Post hoc* multiple comparisons were run using Dunnett's test as it has no requirement for homogeneity of variances. Relationships between FA amounts or indexes were investigated using correlations and ANCOVA to detect potential interactions between the descriptors. All statistical tests were performed on S-Plus statistical software.

RESULTS

PHYTOPLANKTON BIOMASS, ZOOPLANKTON ABUNDANCE AND COMPOSITION

During the study period, diatoms contributed to 65–95% of phytoplankton carbon biomass, and were predominant in the microphytoplankton size fraction, contributing to 73–96% of its biomass [Fig. 2(a)]. Diatoms were less abundant in nanophytoplankton, representing 25–60% of its biomass [Fig. 2(b)]. Microphytoplankton prevailed over nanophytoplankton, representing between 80 up to 96% of total phytoplankton carbon biomass. *Cyclotella cyclopuncta* was the dominant diatom taxon within nanoplankton, while cryptophytes were essentially composed of *Rhodomonas minuta* and dinophytes of *Peridinium*

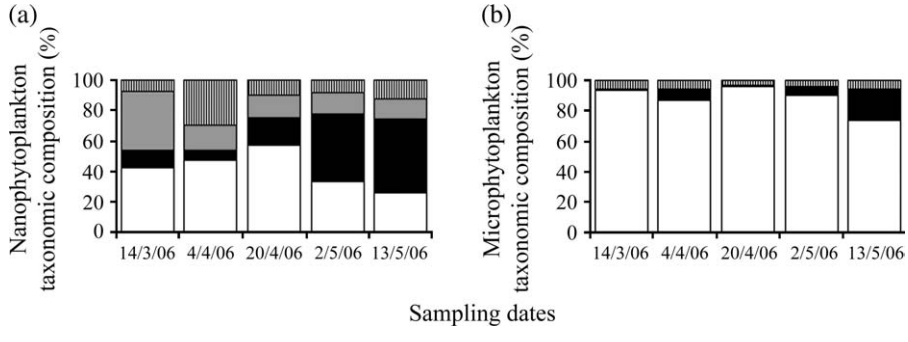

FIG. 2. Proportions of diatoms (□), dinophytes (■), cryptophytes (▒) and other algae (▥) in the (a) nanophytoplankton (<20 μm) and (b) microphytoplankton (>20 μm) in Lake Annecy at the sampling dates.

cunningtonii and *Gymnodinium* sp. Within microphytoplankton, dominant diatoms were essentially represented by *Asterionella formosa* and *Fragilaria crotonensis* and dinophytes by *Ceratium hirundinella*.

Copepods dominated zooplankton representing between 59 and 88% of crustacean abundance, while *Daphnia* sp. contributed to 3–33% over the study period (Fig. 3).

FISH LARVAE CAPTURES AND GUT CONTENTS

Stage 0 larvae could be caught for the first time in late February (21 February) and stage 1 larvae, corresponding to the first feeding stage, were captured for the first time on 15 March. Captures at the last sampling date (10 May) were still composed of larvae of stages 1–4. The sampling method used for this study was not efficient at catching *C. lavaretus* larvae of stages >4.

Only individuals for which >90% of prey items could be identified were kept in this analysis. As the number of prey items per individuals was low, the number of items counted for each prey taxon was summed for all individuals of the same larval stage, and diet composition was compared between larval stages. Diet composition changed significantly between stages (d.f. = 3, $P < 0{\cdot}001$). Copepods were the major prey taxa for *C. lavaretus* larvae at all stages, but the proportion of *Daphnia* sp. in guts increased regularly from stage 1 (<4%) to 4 (38%) (Fig. 4).

GENERAL FATTY-ACID PATTERNS

The amounts of the main FAs identified for the prey taxa and larval fish stages are given in Table I. In zooplankton and fish larvae, the ubiquitous C16:0 was always the dominant FA. Other major FAs were C20:5n-3 (EPA), C18:1n-9, C16:1n-7 and C18:3n-3 in *Daphnia* sp. 22:6n-3 (DHA), EPA and C18:2n-6 in small and large copepods and C18:1n-9, DHA and EPA in fish larvae. The DHA levels were very low in *Daphnia* sp. FAs included in the PCA

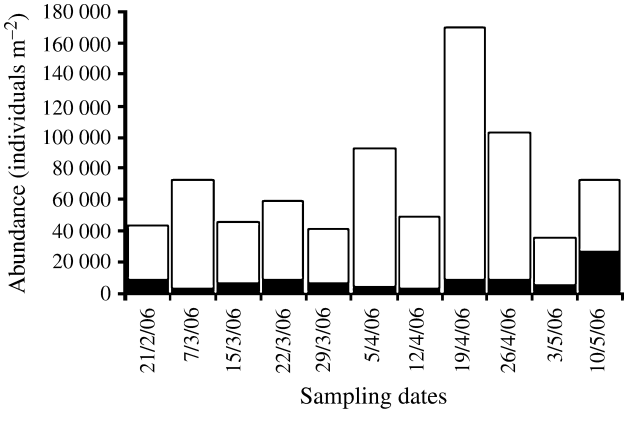

FIG. 3. Copepods (□) and *Daphnia* sp. (■) abundance in Lake Annecy at the sampling dates.

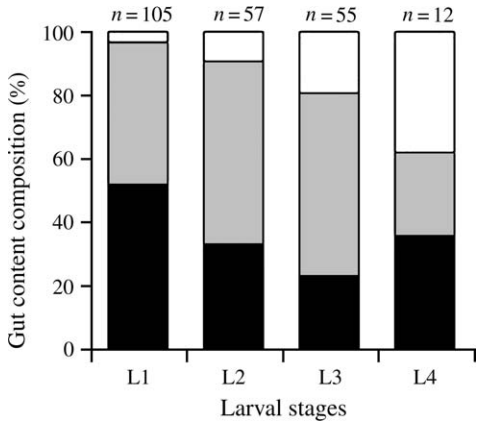

FIG. 4. Proportions of *Daphnia* sp. (□), small copepods (■) and large copepods (▒) in the gut contents of *Coregonus lavaretus* larvae from stage 1 (L1) to stage 4 (L4) for the study period.

analysis were targeted for their potential role as trophic markers or as EFA [BAFA, C16:1n-7, C24:0, C18:4n-3, C18:3n-3 and EPA (DHA was excluded as it was present at very low concentrations in *Daphnia* sp.)].

The two first components of the PCA accounted for 78% of total variability. C18:3n-3, C16:1n-7, C18:3n-4, C24:0 and EPA had the highest loadings on the first principal component (PC), with a positive correlation for C24:0 and a negative one for C18:3n-3, C18:4n-3, C16:1n-7 and EPA (Fig. 5). The BAFA had the highest loadings on the second PC. Samples scores on the first PC made it possible to distinguish *Daphnia* sp. with the most negative scores ($<-1{\cdot}5$), indicating that they were enriched in EPA and C16:1n-7. Copepods scores were more positive (from -2 to 2), illustrating they were depleted in these FAs and had a higher content in the terrestrial biomarker C24:0 relatively to *Daphnia* sp. Fish larvae had the most positive scores (>-1) and hence the lowest amount in EPA and diatom biomarkers and the highest content in the terrestrial biomarker. The second component essentially accounted for the variability in FA composition amongst larvae samples (Fig. 5).

FATTY-ACID COMPOSITION OF THE PREY TAXA

Analyses of individual fatty acids confirmed that *Daphnia* sp. were significantly enriched in EPA compared to copepods of both size-classes (Table II and Fig. 6). Values for the diatom index [(C16:1n-7) : (C16:0)] were also significantly higher in *Daphnia* sp. In contrast, copepods were enriched in DHA and had higher values for the terrestrial index compared to *Daphnia* sp. No differences in the bacterial index values amounts could be detected between prey taxa. *Post hoc* tests detected no significant differences in the amounts of all targeted FA or indexes values between copepods of the small and large size-classes.

Amounts of EPA and values for the diatom index were positively related for prey taxa ($r^2 = 0{\cdot}657$, $P < 0{\cdot}001$), such as the terrestrial and bacterial indices ($r^2 = 0{\cdot}46$, $P < 0{\cdot}001$; Fig. 7). The ANCOVA analyses revealed that both

TABLE I. Mean ± S.D. percentages of identified fatty acids (FA) in zooplankton prey and *Coregonus lavaretus* larvae

	C16:0	C16:1n-7	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	C20:4n-6	C20:5n-3	C24:0	C22:6n-3	C18:4n-3	BAFA
C_L	19·9 ± 1·2	4·8 ± 1·7	2·7 ± 0·1	10·0 ± 0·6	11·3 ± 0·6	6·7 ± 2·1	1·4 ± 0·3	13·8 ± 4·0	1·7 ± 0·2	19·2 ± 3·1	5·5 ± 4·2	2·9 ± 0·8
C_S	19·1 ± 0·6	6·0 ± 2·2	2·6 ± 0·2	7·6 ± 1·7	6·8 ± 2·0	7·0 ± 1·1	2·2 ± 0·6	17·9 ± 2·4	1·8 ± 0·2	19·7 ± 1·7	5·7 ± 2·0	3·4 ± 0·6
D	18·8 ± 0·9	10·5 ± 1·7	2·3 ± 0·4	13·3 ± 1·5	7·1 ± 0·6	10·6 ± 1·5	1·3 ± 1·1	22·1 ± 1·7	0·5 ± 0·1	1·3 ± 0·1	8·8 ± 2·2	3·1 ± 1·1
L0	18·9 ± 0·9	6·3 ± 0·6	2·1 ± 0·6	23·5 ± 2·8	6·1 ± 0·1	5·8 ± 2·3	2·0 ± 0·5	13·5 ± 1·6	2·8 ± 0·3	9·8 ± 1·5	6·6 ± 2·8	2·5 ± 0·6
L1	22·3 ± 1·5	3·5 ± 1·3	3·3 ± 0·8	19·0 ± 2·4	4·8 ± 1·0	3·5 ± 0·8	1·5 ± 0·6	14·8 ± 1·0	3·5 ± 0·4	17·0 ± 3·6	4·2 ± 0·8	2·6 ± 1·0
L2	22·1 ± 1·2	2·9 ± 1·6	3·6 ± 1·5	17·9 ± 4·2	4·9 ± 1·2	2·9 ± 0·1	1·6 ± 0·5	13·0 ± 1·4	3·0 ± 0·4	20·4 ± 6·7	3·7 ± 1·9	4·0 ± 1·8
L3	23·2 ± 1·1	2·6 ± 0·1	3·7 ± 0·3	14·5 ± 2·5	3·3 ± 1·8	3·1 ± 0·4	1·6 ± 0·2	13·5 ± 0·1	2·9 ± 0·2	25·6 ± 3·0	3·3 ± 0·5	2·6 ± 0·7
L4	24·9 ± 0·1	1·9 ± 0·1	4·1 ± 0·3	11·5 ± 2·7	4·1 ± 0·5	2·2 ± 0·2	2·0 ± 0·1	10·1 ± 0·1	2·3 ± 0·1	33·1 ± 2·0	1·7 ± 0·1	2·0 ± 0·1

BAFA, bacterial fatty acids; C_L, large-sized copepods; C_S, small-sized copepods; D, *Daphnia* sp.; L0–L4 *C. lavaretus* larvae from stage 0 to 4.

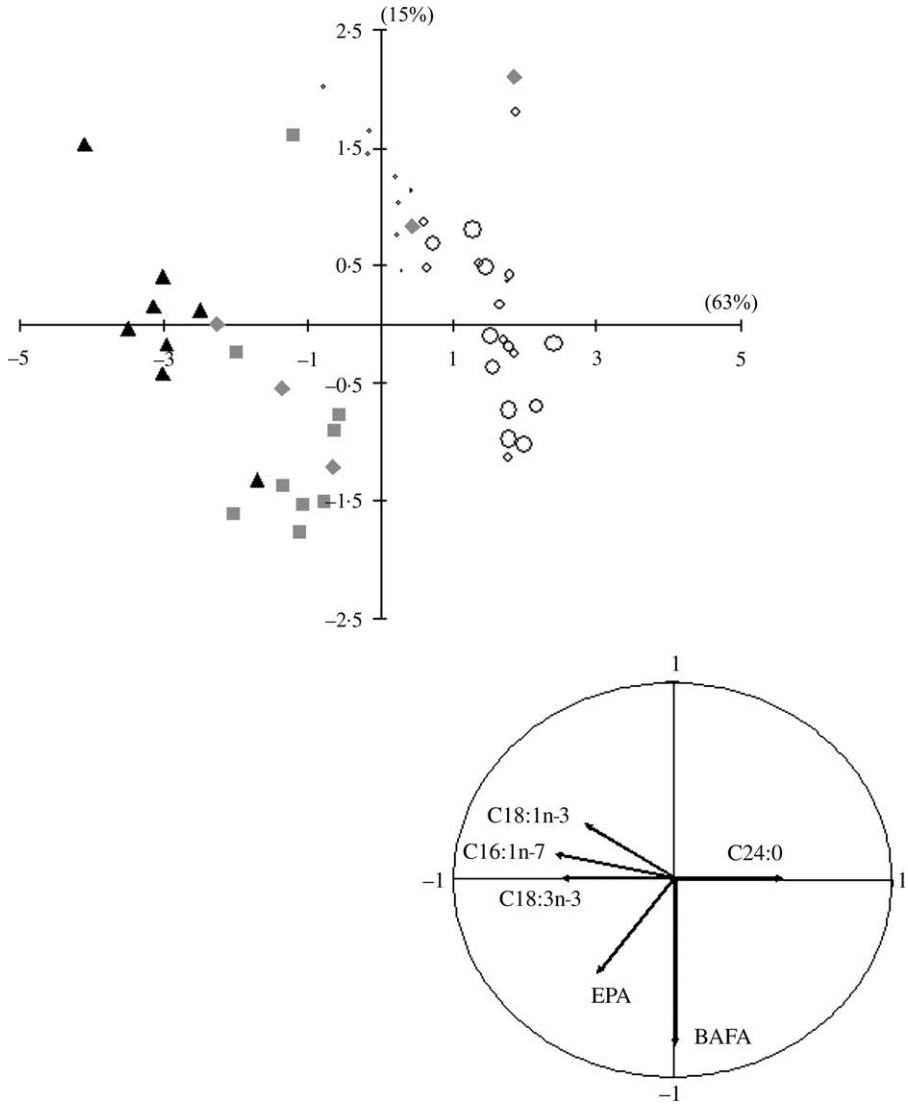

FIG. 5. Results from the principal component analyses on fatty-acid compositions of the prey [*Daphnia* sp. (▲) large-sized (◆) and small-sized (■) copepods] and *Coregonus lavaretus* larvae from stages 0 (‣), 1 (⊙), 2 (◯) and 3 plus 4 (⬭) (EPA, eicosapentaenoic acid; BAFA, bacterial fatty acids).

relationships depended on the considered prey taxa ($F_{2,20}$, $P = 0{\cdot}01$ and $F_{2,20}$, $P < 0{\cdot}001$ for diatom and terrestrial indices, respectively). DHA content was not related to any of the indices.

FATTY-ACID COMPOSITION OF *C. LAVARETUS* LARVAE

All targeted FA or indexes displayed significant changes during larval development, except for the bacterial index (Table II). The EPA relative amounts,

TABLE II. Differences in fatty acid (FA) per cent amounts and FA indices for zooplankton prey and larval stages of *Coregonus lavaretus*

	Between zooplankton prey taxa		Between *C. lavaretus* larval stages	
FA variations	KW χ^2 value	*P*	KW χ^2 value	*P*
EPA	16·3	<0·001	10·8	<0·05
DHA	15·0	<0·001	23·2	<0·001
Diatom index	12·5	<0·001	20·3	<0·001
Terrestrial index	14·0	<0·001	16·7	<0·001
Bacterial index	0·6	>0·05	4·0	>0·05

DHA, docoshexaenoic acid; EPA, eicosapentaenoic acid; KW, Krustal–Wallis test.

similar to the terrestrial, diatom indexes decreased from stage 0 to 4 (Fig. 6). For all FA or ratios, except EPA and DHA, the significant changes occurred between stages 0 (no feeding) and 1, while FA amounts or ratios remained similar from stages 1 to 4. In contrast, the significant decrease in EPA occurred between stages 3 and 4. For the latest stages, the amounts of EPA such as the values for all the indices were similar to those in copepods. For DHA, amounts increased continuously from stages 0 to 4, with significantly higher DHA relative amounts in L4 than those of the prey taxa (KW, d.f. = 4, $P = 0{\cdot}01$).

DISCUSSION

The major marine fish stocks in the world are located in upwelling regions in which the injection of nitrate from deep water layers stimulates high diatom productions. These systems are characterized by short food chains in which the diatom C is transferred to large copepods and then finally to fish larvae (St John *et al.*, 2001). These diatom-based food chains are thought to enhance the physiological condition of larval fishes, such as North Sea cod *Gadus morhua* L. (St John & Lund, 1996) or anchovy *Engraulis encrasicolus* (L.) (Rossi *et al.*, 2006) and thus recruitment. As spring primary production in Lake Annecy is largely dominated by diatoms, consistent with what has been observed in marine areas, diatoms should be the ultimate carbon source sustaining the growth of *C. lavaretus* early larval stages.

ZOOPLANKTON FOOD WEB

In Lake Annecy in 2006, although the spring diatom bloom reached relatively high biomasses that lasted for nearly 3 months, no clear peak in zooplankton abundance could be detected. High (C16:1n-7) : (C16:0) ratios in *Daphnia* sp., however, attested they were actually grazing on diatoms. Yet *Daphnia* sp. remained at a low level in the crustacean zooplankton population at this period of time, and *C. prealpinus* was strongly dominant. Copepods are omnivorous and tend to become carnivorous when they get older (Kerfoot & Kirk, 1991). Therefore, it would be expected that small copepods would graze

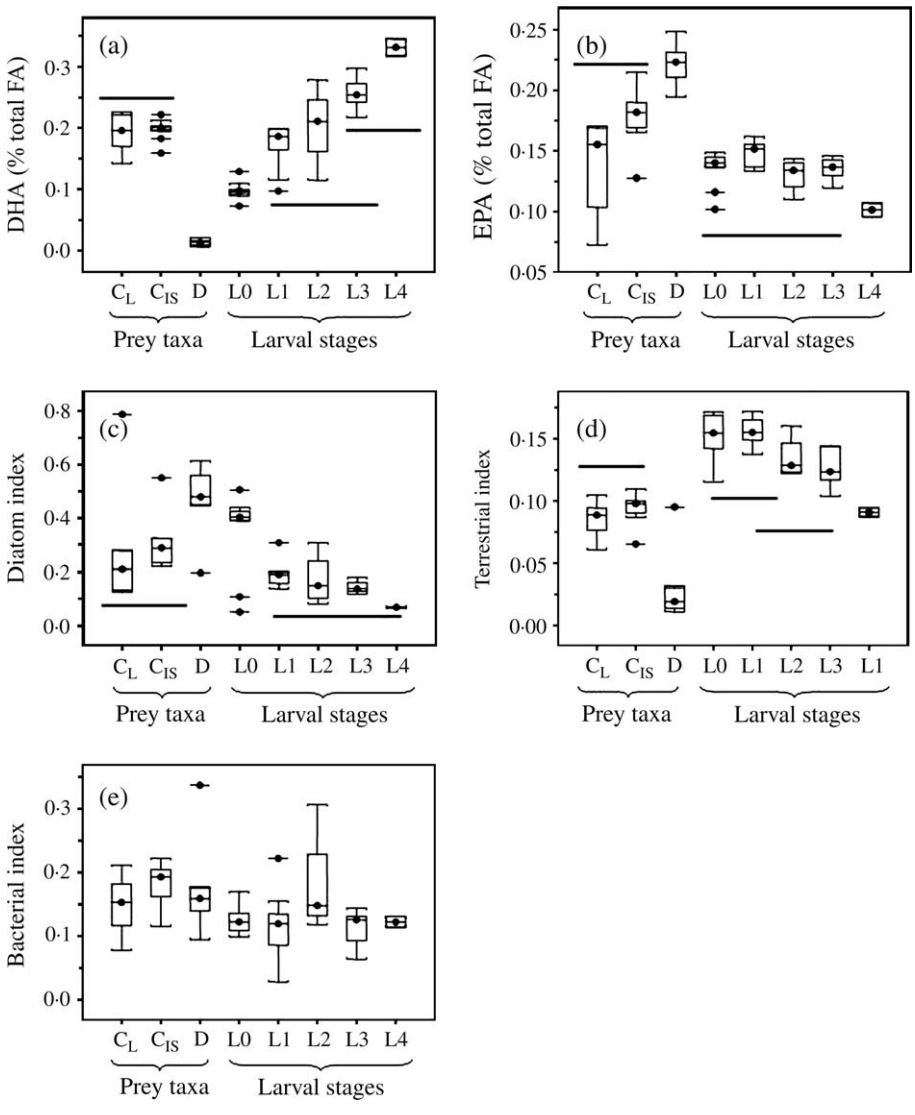

FIG. 6. Boxplot representations (the box represents the inter-quartile range, the bar with the box the median value, ● the mean, ⊥ 95% CI and the outside points are outliers) of the values of (a) per cent amounts of docosahexaenoic (DHA) and (b) eicosapentaenoic acid acid (EPA) and (c) diatom, (d) terrestrial and (e) bacterial indices in the zooplankton prey [*Daphnia* sp. (D) large-sized (C_L) and small-sized (C_S) copepods] and *Coregonus lavaretus* larvae from stage 0 (L0) to stage 4 (L4). The horizontal bars indicate homogeneous groups of means.

on diatoms. For the larger ones, the diatom biomarker C16:1n-7 could have been expected to be transferred from the copepods' prey, which would have been grazing on diatoms. In contrast, low values for the C16:1n-7:C16:0 ratio in copepods from both size-classes suggested that copepod secondary production only moderately benefited from direct grazing on diatoms. The relatively low reliance of copepods of both size-classes on diatom-derived C may explain why the

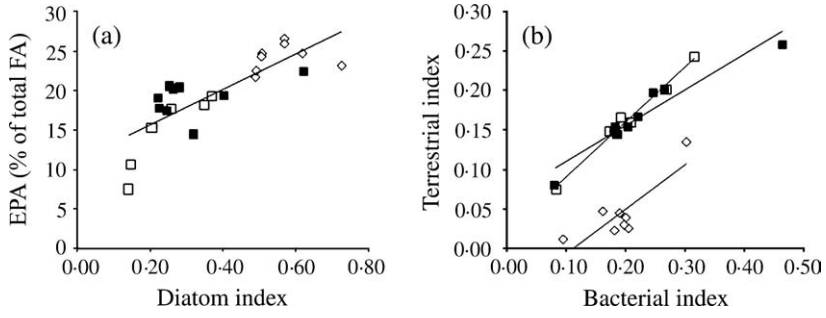

FIG. 7. Relationships between (a) the value of the diatom index and eicosapentaenoic acid (EPA) content and (b) the value of the terrestrial and bacterial indexes, in *Daphnia* sp. (◇), large-sized (□) and small-sized (■) copepods. The curves were fitted by: (a) $y = 12{\cdot}3x + 16{\cdot}3$ and (b) *Daphnia* sp. $y = 0{\cdot}35x - 0{\cdot}03$ and large-sized and small-sized copepods $y = 0{\cdot}29x + 0{\cdot}04$.

high diatom primary production did not result in a peak in copepod abundance in the spring, although other factors, such as temperature or fish predation, might be involved. High (C24:0) : (C16:0) ratios in copepods compared to *Daphnia* sp. would attest of their significant reliance on terrestrial-derived carbon. The positive correlation between BAFA and C24:0 would suggest that terrestrial-derived and bacterial-derived C were co-transferred either because copepods grazed on terrestrial particles on which bacteria grew or because copepods switched to the microbial loop (bacterivorous protists) and terrestrial particles when phytoplankton C was limiting.

The spring bloom in Lake Annecy in 2006 was largely dominated by microplanktonic species, and especially *A. formosa* and *F. crotonensis*, which are large, colonial diatoms (>40 μm). This size belongs to the range of edible particles for *Daphnia* sp. (Burns, 1968) but might be beyond the range of size of grazable particles for copepods nauplii and copepodites (Kerfoot & Kirk, 1991). Only nanoplanktonic diatom species might have been grazed by copepods, while *Daphnia* sp. could benefit from diatom production as well in the microplankton size fraction.

The positive correlation between the diatom index and EPA levels in crustacean zooplankton suggested that diatoms were the source of EPA in this food chain (Pohl & Zurheide, 1979). Depletion in diatom biomarkers in copepods suggested that lower levels of EPA in copepods compared to *Daphnia* sp. were the consequences of diet differences between the two taxa and the copepods' lower reliance on diatoms, rather than from differences in accumulation rates of this FA (Farkas *et al.*, 1981). In contrast, differences in DHA amounts between *Daphnia* sp. and copepods cannot be related to feeding differences. Indeed, *Daphnia* sp. do not accumulate DHA despite its occurrence in their diet (Brett *et al.*, 2006). In addition, this pattern, observed for DHA, might also occur for other FAs. Nevertheless, knowledge is clearly lacking on that point as, so far, experiment studies on zooplankton (and especially copepods) fed known diets are scarce and mainly focused on EFA. For instance, *Daphnia* sp. fed terrestrial organic matter would not retain C24:0 in their polar lipids (M. Brett, pers. com.). This result would not impair the present study as it

would only suggest that terrestrial C contribution to *Daphnia* sp. was even underestimated during the diatom spring peak. It yet stresses the necessity for further experiment studies comparing copepods and *Daphnia* sp. ability to retain non-essential and source-specific FAs.

CARBON SOURCES SUSTAINING THE GROWTH OF *C. LAVARETUS* LARVAE

In this survey, the FA composition of *C. lavaretus* larvae fairly matched that of their food source, especially for non-essential FA (C16:1n-7, C24:0 and BAFA), thus validating the approach by FA trophic biomarkers. The observed incorporation of zooplankton FAs into *C. lavaretus* larvae lipids was consistent with observations previously done on other freshwater fishes, *e.g.* pike *Esox lucius* L. larvae (Desvilettes *et al.*, 1994) and Vendace *Coregonus albula* (L.) 0+ years of age (Muje, 1989).

The FA composition at stage 0 was substantially different from that at older stages. At stage 0, larvae do not have exogenous feeding and stage 0 larvae (L0) FAs are provided by hydrolysis of the yolk-sack lipids. Then, their FA composition reflects that of the yolk sac (Desvilettes *et al.*, 1994). Relative richness in C24:0 in L0 suggested that terrestrial-derived C contributed significantly to the constitution of yolk reserves, in addition to phytoplankton-derived C sources.

In accordance with previous studies, from stages 1 to 4, *C. lavaretus* larvae preferentially fed on copepods (Anneville *et al.*, 2007) which were shown to be relatively poor in diatom-derived and enriched in terrestrial-derived FA. Consistently, FA profiles of larvae were impoverished in diatom-derived FAs. In larval fish from stages 1 to 4, the terrestrial-derived C24:0 and the diatom-derived C16:1n-7 represented similar percentages of total FAs (2–3%). Values for the diatom index in *C. lavaretus* larvae at stage 4 were fairly similar to those of copepods, their major prey. Values for the terrestrial index were already high in L0 stages. They then decreased from L0 to L4 but remained significant, consistently with larvae feeding preferentially on C24:0-rich copepods. Hence, the growth of *C. lavaretus* early larval stages was only partially supported by diatom spring production peak but, for significant part, by terrestrial-derived C.

As copepods were a poor EPA source, *C. lavaretus* larvae were depleted in EPA. This is in contrast with previous studies showing that larvae tend to retain preferentially EPA (Desvilettes *et al.*, 1994) as an essential fatty-acid for fish larval growth. One explanation might be that, in spite of their relative deficiency in EPA, copepods provided enough EPA to meet the larvae's requirements for their development and growth in Lake Annecy at this time. In contrast, as copepods are DHA rich, larvae showed increasing DHA amounts. In stage 4, DHA reached higher amounts in larvae than in their prey. This pattern confirmed that larvae preferentially accumulate DHA (Desvilettes *et al.*, 1994). Some of this excess DHA, however, may be provided by the desaturation of dietary FA [*e.g.* C22:5n-3 (Desvilettes *et al.*, 1994) or EPA (Ballantyne *et al.*, 2003; Zheng *et al.*, 2005)], although enzymatic bioconversion systems might be more active in juvenile and adult fishes than in larvae (Muje, 1989). It would be crucial, in a subsequent study, to identify the major DHA sources for copepods and ultimately *C. lavaretus* larvae. Cryptophytes,

dinophytes, both abundant in the nanophytoplankton size fraction at the study time, and heterotrophic protists are all candidates (Ahlgren *et al.*, 1990; Napolitano, 1998; Bec *et al.*, 2006).

Contrary to the initial hypothesis, the growth of *C. lavaretus* early larval stages was not only supported by the phytoplankton production peak, but terrestrial-derived C contributed significantly to larval fish growth. This is, however, a 1 year study. Further research is required to study how such a pattern varies between years and how it may affect *C. lavaretus* recruitment. In 2006, this pattern resulted from *C. lavaretus* larvae feeding preferentially on copepods, while secondary production was supported by both terrestrial-derived and diatom-derived C at this time of the study year. Although this assumption would require further analyses, however, the diatom production peak in spring could eventually contribute indirectly to the copepods' secondary production if dissolved organic carbon, produced from diatom exudates or senescence, stimulates bacterial growth. Recycled diatom organic matter could then be transferred to copepods through grazing on the microbial loop and then contribute indirectly to the copepods' secondary production. Unfortunately, such a pathway might not be easily traced from fatty acid profiles.

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