



Polyunsaturated Fatty Acids in Neutral Lipids and Phospholipids of Some Freshwater Insects

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ABSTRACT. The fatty acid compositions of total neutral lipids and total polar lipids from eight species of freshwater insects were determined: stonefly nymphs (Plecoptera), beetle larvae (Coleoptera), Chironomidae (Diptera), water boatmen (Corixidae and Notonecta; Heteroptera) and mayfly nymphs (*Ecdyonurus venosus*, *Caenis*, *Ephemera*; Ephemeroptera). In addition, the compositions of individual phosphoglycerides were determined for four of the species (Plecoptera, Corixidae, *Ecdyonurus venosus* and *Ephemera*). Saturated and monounsaturated fatty acids together represented up to 85% of the fatty acids of total neutral lipids with 16:0 (18–31%) being the most abundant saturated fatty acid and 16:1n-7 (10–28%), 18:1n-9 (6–12%) and 18:1n-7 (3–12%) the most abundant monounsaturates. Polyunsaturated fatty acids (PUFA) accounted for between 16% and 33% of the total fatty acids of neutral lipids, with 20:5n-3 (4–12%), 18:3n-3 (3–30%) and 18:2n-6 (1–8%) all being major components. Arachidonic acid, 20:4n-6 (0.4–1.0%) and 22:6n-3 were, respectively, minor and insignificant components of total neutral lipids. PUFA were major fatty acids (34–56% of the total) in total polar lipids and in phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. The major PUFA present were 20:5n-3 (14–27%) and 18:3n-3 (6–23%). The most abundant n-6 PUFA, especially in phosphoglycerides from Corixidae, was 18:2n-6 (3–11%). Arachidonic acid, 20:4n-6, was present in all phosphoglycerides accounting for 1–4% of the total fatty acids, except in the phosphatidylinositol of Corixidae where it accounted for 12% of the total. 22:6(n-3) was not present in significant amounts in any phosphoglyceride in any species. 18:1n-9 (8–20%) and 18:1n-7 (2–14%) were the most abundant monounsaturated fatty acids, especially in phosphatidylethanolamine. 16:0 was abundant in phosphatidylcholine (11–21%), and 18:0 (17–23%) was abundant in phosphatidylserine. The results are discussed in relation to the functions and origins of PUFA in freshwater insects. COMP BIOCHEM PHYSIOL 114B, 161–170, 1996.

KEY WORDS. Freshwater, insects, lipids, phospholipids, polyunsaturated fatty acids

INTRODUCTION

We have previously reported that the major polyunsaturated fatty acids (PUFA) in total lipids from a range of freshwater insects are 20:5n-3, 18:3n-3, 18:2n-6 and 20:4n-6 and commented on the implications of the findings for the requirements of freshwater fish for both n-6 and n-3 PUFA series and the extent to which freshwater fish naturally convert C₁₈ PUFA to C₂₀ and C₂₂ PUFA (2). The study was prompted by the relative lack of information on the origins and transformations of PUFA in freshwater ecosystems as compared with marine ecosystems (22). However, the n-3 PUFA profiles of insects are of interest not only because of

their nutritional value to insectivores including fish but also because of how these profiles have evolved in relation to the fundamental roles of PUFA in insect cell membranes.

There is evidence that the fatty acid profiles of insects are taxonomically related (28) and are dependent on diet (12,13). It is also known that some insect species can convert C₁₈ PUFA to their C₂₀ products (14,25) and that the elongation and desaturation pathways involved are not different from those occurring in mammals (10,25). However, the *de novo* synthesis of linoleic and linolenic acid from oleic acid has also been reported (4) in some insects, implying a potential release from a nutritional dependence on dietary PUFA. The fatty acid compositions of terrestrial insects have been determined to elucidate functional feeding groups in ecological investigations (7,8,15), and fatty acids also play a significant role in the various life stages of insects (1,6).

In the present study, we determined the fatty acid compositions of total neutral lipids, total polar lipids and individ-

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Abbreviations—PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; TLC, thin-layer chromatography.

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TABLE 1a. Neutral lipid fatty acid composition (weight %) of freshwater insects*

Fatty acid	Plecoptera	Coleoptera	Chironomidae	Corixidae
14:0	2.5 ± 0.5	2.7 ± 0.6	2.7 ± 0.6	1.4 ± 0.4
16:0	21.3 ± 3.3	23.0 ± 6.5	22.1 ± 4.9	21.6 ± 1.8
18:0	3.7 ± 1.2	8.8 ± 0.9	6.1 ± 1.7	3.8 ± 0.6
Total saturates†	29.9 ± 4.1	39.6 ± 6.5	32.6 ± 7.5	30.3 ± 0.8
16:1n-7	18.7 ± 1.5	13.2 ± 3.9	24.5 ± 4.8	9.7 ± 0.9
18:1n-9	14.0 ± 0.6	12.0 ± 0.2	10.0 ± 4.5	8.3 ± 1.2
18:1n-7	9.8 ± 1.6	9.4 ± 1.1	7.4 ± 0.6	3.4 ± 1.2
24:1	0.4 ± 0.7	—	0.6 ± 0.6	tr
Total monoenes‡	43.7 ± 3.1	35.3 ± 2.5	42.9 ± 1.6	21.9 ± 1.5
18:2n-6	3.6 ± 0.5	8.2 ± 3.9	4.9 ± 2.0	7.4 ± 0.2
18:3n-6	tr	tr	tr	0.7 ± 0.6
20:4n-6	0.8 ± 0.2	0.8 ± 0.6	0.8 ± 0.5	1.0 ± 0.4
Total n-6§	4.7 ± 0.4	9.7 ± 4.1	6.2 ± 2.4	9.4 ± 1.1
18:3n-3	6.2 ± 1.6	9.7 ± 1.6	4.4 ± 2.2	29.7 ± 5.3
18:4n-3	0.9 ± 0.6	0.7 ± 0.6	0.5 ± 0.4	2.2 ± 0.8
20:5n-3	10.5 ± 4.2	5.9 ± 1.6	7.5 ± 5.4	3.9 ± 2.2
22:5n-3	—	0.5 ± 0.8	0.2 ± 0.4	—
22:6n-3	tr	tr	tr	tr
Total n-3	18.0 ± 6.3	17.0 ± 1.5	13.1 ± 8.2	36.3 ± 2.1
Total PUFA¶	26.3 ± 7.1	31.4 ± 6.4	23.5 ± 10.1	47.2 ± 0.4
n-3/n-6	3.8 ± 1.3	1.9 ± 0.8	2.0 ± 1.1	3.9 ± 0.7
C ₂₀ PUFA	11.5 ± 4.4	7.4 ± 2.4	8.7 ± 6.0	5.3 ± 2.7
C ₁₈ PUFA	11.2 ± 1.7	19.4 ± 5.0	10.7 ± 3.8	40.5 ± 4.3
C ₂₀ /C ₁₈ PUFA	1.0 ± 0.3	0.4 ± 0.2	0.8 ± 0.5	0.1 ± 0.1

*Values are percent by weight ± SD from three samples of freshwater insects.

†Includes 15:0, 17:0 and 20:0.

‡Includes 20:1n-9, 20:1n-7 and 22:1n-9.

§Includes 20:2n-6, 20:3n-6 and 22:5n-6.

||Includes 20:4n-3.

¶Includes 16:2, 16:3, 16:4 and 18:2n-9.

tr = value < 0.05%.

ual phosphoglycerides of several species of freshwater insects. Our aims were to illuminate the dietary origins of PUFA and their cell membrane functions in freshwater insects.

MATERIALS AND METHODS

Experimental Animals

The insects analysed were netted in the river Allan and its tributaries (Stirlingshire, Scotland) and in Airthrey Loch (Stirlingshire, Scotland) in August 1993, identified and pooled to provide three samples where possible, according to genera and species: stonefly nymphs (Plecoptera), beetle larvae (Coleoptera), Chironomidae (Diptera), water boatmen (Corixidae and Notonecta; Heteroptera), mayfly nymphs (*Ecdyonurus venosus*, *Caenis*, *Ephemerella*; Ephemeroptera). Samples were stored at -80°C for less than 3 months before analysis.

Lipid Extraction and Analysis

The insects were homogenised in 10 volumes of chloroform-methanol (2:1, v/v) using a Polytron tissue disrupter

and total lipid extracted by the method of Folch *et al.* (11). Polar lipids were separated from neutral lipids by loading 1 mg of total lipid on to a 3-cm origin of a 20 × 20 cm thin layer chromatography (TLC) plate and developing with hexane-diethyl ether-acetic acid (80:20:2, v/v/v). The areas of silica corresponding to polar and neutral lipids were identified by spraying the lateral edges of the plate with 1% iodine in chloroform and scraped from the plate. The recovered silica was eluted into a glass tube through a prewashed filter paper with 5 ml of chloroform-methanol (2:1, v/v). The solvent was evaporated under nitrogen and the samples transmethylated overnight in 2 ml of 2% sulfuric acid in methanol (plus 1 ml toluene for neutral lipid samples) at 50°C. The methyl esters were extracted twice in 5 ml hexane-diethyl ether (1:1, v/v) after neutralization with 2 ml of 6% KHCO₃. The combined organic phases were dried under nitrogen. The methyl esters were redissolved in 0.5 ml chloroform-methanol (2:1, v/v) and purified by TLC (hexane-diethyl ether-acetic acid, 90:10:1, v/v/v) before separation and quantification by gas-liquid chromatography (Carlo Erba Vega 6000, Fisons Ltd, Crawley, U.K.) using a 30 m × 0.32 mm capillary column (Omegawax, Supelco Ltd., Saffron Walden, Essex, U.K.). Hydrogen was

TABLE 1b. Neutral lipid fatty acid composition (weight %) of freshwater insects*

Fatty acid	Notonecta	<i>Ecdyonurus v.</i>	<i>Caenis</i>	<i>Ephemerella</i>
14:0	2.9 ± 0.7	3.4 ± 0.3	3.9 ± 0.6	4.5 ± 0.3
16:0	18.3 ± 4.0	30.5 ± 2.3	29.2 ± 2.8	27.5 ± 6.2
18:0	6.6 ± 0.7	2.7 ± 0.8	2.1 ± 0.4	2.3 ± 0.5
Total saturates†	29.8 ± 5.3	37.4 ± 2.5	36.5 ± 3.8	36.1 ± 5.5
16:1n-7	17.3 ± 1.3	27.6 ± 2.7	27.3 ± 3.8	24.8 ± 2.1
18:1n-9	12.3 ± 3.3	7.8 ± 3.5	6.4 ± 0.0	6.9 ± 1.7
18:1n-7	6.9 ± 1.1	11.2 ± 1.5	11.6 ± 0.6	7.6 ± 0.6
24:1	—	0.1 ± 0.1	tr	tr
Total monoenes‡	37.1 ± 3.1	46.7 ± 2.4	45.4 ± 4.6	39.4 ± 1.3
18:2n-6	6.0 ± 0.6	1.3 ± 0.1	1.5 ± 0.0	1.8 ± 0.6
18:3n-6	tr	0.1 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
20:4n-6	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.7 ± 0.4
Total n-6§	7.0 ± 0.7	1.8 ± 0.1	2.5 ± 0.1	2.9 ± 1.0
18:3n-3	11.3 ± 1.2	2.6 ± 0.0	2.6 ± 0.1	3.9 ± 1.5
18:4n-3	1.0 ± 0.5	0.8 ± 0.3	1.1 ± 0.2	1.9 ± 0.6
20:5n-3	9.9 ± 5.1	6.3 ± 0.4	8.5 ± 0.7	11.6 ± 2.3
22:5n-3	tr	tr	tr	tr
22:6n-3	tr	0.1 ± 0.2	tr	tr
Total n-3	22.6 ± 6.2	10.1 ± 0.0	12.2 ± 0.8	17.6 ± 4.4
Total PUFA¶	32.8 ± 6.5	15.5 ± 0.3	17.7 ± 0.5	24.0 ± 6.2
n-3/n-6	3.2 ± 0.7	5.5 ± 0.4	4.9 ± 0.5	6.3 ± 0.9
C ₂₀ PUFA	10.9 ± 5.2	6.9 ± 0.6	9.2 ± 0.5	12.5 ± 2.7
C ₁₈ PUFA	18.5 ± 1.9	4.9 ± 0.3	5.5 ± 0.1	8.0 ± 2.7
C ₂₀ /C ₁₈ PUFA	0.6 ± 0.2	1.4 ± 0.2	1.7 ± 0.1	1.6 ± 0.3

Footnotes as described in Table 1a except values for *Ecdyonurus v.* and *Caenis* are mean ± SD of two determinations.

used as a carrier gas and temperature programming was from 50 to 150°C at 35°C/min and then to 225°C at 2.5°C/min. Individual methyl esters were identified by comparison with known standards.

Individual phosphoglycerides were also isolated from the most abundant specimens (Plecoptera, Corixidae, *Ecdyonurus venosus* and *Ephemerella*) by loading 2 mg of total lipid on to a 2-cm origin of a 20 × 20-cm TLC plate and eluting with methyl acetate–isopropanol–chloroform–methanol–0.25% KCl (25:25:25:10:9, v/v/v/v/v). Individual phospholipids were identified by spraying the lateral edges of the plate with 1% iodine in chloroform, recovered from silica and transmethylated as described previously (2).

Materials

TLC plates, coated with silica gel 60, were obtained from Merck (Darmstadt, Germany). All solvents were of high-performance liquid chromatography grade and obtained from Rathburn Chemicals (Walkerburn, Peebleshire, Scotland).

RESULTS

Fatty Acid Compositions of Neutral Lipids

The fatty acid compositions of total neutral lipids are shown in Tables 1a and 1b. With the exception of Corixidae, the

abundance of fatty acids in neutral lipids was monounsaturated (35–47%) > saturated (30–40%) > PUFA (16–33%). Corixidae were atypical in having elevated PUFA (47%) and depressed monounsaturates (22%). Palmitic acid, 16:0, was the most abundant saturated fatty acid with 14:0 and 18:0 being present in much lower amounts. The abundance of the monounsaturated fatty acids was generally 16:1n-7 > 18:1n-9 > 18:1n-7. The major PUFA were 20:5n-3 and 18:3n-3, with 22:6n-3 being present in negligible or undetectable amounts. 20:5n-3 was the major PUFA in Plecoptera, Chironomidae and the Ephemeroptera, whereas 18:3n-3 was particularly abundant in Corixidae. Linoleic acid, 18:2n-6, was the principal n-6 PUFA present and lower, but significant amounts of 20:4n-6, were present in all cases. The n-3/n-6 ratio always exceeded unity and was highest in Ephemeroptera.

Fatty Acid Compositions of Total Polar Lipids

The fatty acid compositions of total polar lipids are presented in Tables 2a and 2b. All the freshwater insects analysed showed a very similar pattern, the abundance of the fatty acids being PUFA (34–56%) > monounsaturates (17–37%) > saturates (20–25%). Corixidae were again atypical in having the highest level of PUFA (56%), due to their high level of 18:3n-3 (23%), and the lowest level of monounsaturates (17%). n-3 PUFA were always more abundant than n-6 PUFA with the n-3/n-6 PUFA ratio varying from

TABLE 2a. Polar lipid fatty acid compositions (weight %) of freshwater insects*

Fatty acid	Plecoptera	Coleoptera	Chironomidae	Corixidae
14:0	tr	0.7 ± 0.5	1.2 ± 0.9	tr
16:0	10.6 ± 0.4	11.1 ± 0.3	14.7 ± 2.0	11.0 ± 0.8
18:0	7.7 ± 1.2	10.1 ± 6.5	8.1 ± 1.7	12.4 ± 1.0
Total saturates†	19.6 ± 1.5	24.1 ± 6.7	24.9 ± 5.5	25.2 ± 1.0
16:1n-7	6.1 ± 1.3	5.3 ± 2.5	14.9 ± 11.8	2.4 ± 1.1
18:1n-9	19.6 ± 1.0	10.4 ± 5.4	11.0 ± 1.9	11.7 ± 1.0
18:1n-7	8.7 ± 0.3	6.1 ± 2.6	10.2 ± 2.7	2.4 ± 0.6
24:1	tr	2.8 ± 4.3	1.2 ± 1.1	0.5 ± 0.8
Total monoenes‡	34.8 ± 1.5	24.8 ± 3.0	37.4 ± 13.8	17.4 ± 1.2
18:2n-6	6.0 ± 0.6	5.6 ± 4.0	8.0 ± 1.5	11.0 ± 0.4
18:3n-6	tr	tr	tr	0.7 ± 0.1
20:4n-6	2.9 ± 0.2	1.6 ± 1.1	1.8 ± 1.2	3.0 ± 0.7
Total n-6§	9.4 ± 0.9	7.6 ± 4.6	10.1 ± 2.8	15.3 ± 0.8
18:3n-3	9.1 ± 0.3	10.1 ± 8.1	5.5 ± 2.3	22.7 ± 3.0
18:4n-3	0.8 ± 0.2	0.7 ± 0.3	0.5 ± 0.2	1.7 ± 0.1
20:4n-3	tr	0.4 ± 0.5	0.9 ± 1.5	0.6 ± 0.0
20:5n-3	22.3 ± 1.5	15.0 ± 10.8	14.1 ± 9.2	14.1 ± 1.0
22:5n-3	0.6 ± 1.0	—	—	tr
22:6n-3	tr	—	—	tr
Total n-3	33.1 ± 0.9	26.1 ± 18.1	21.0 ± 12.6	39.4 ± 2.2
Total PUFA	44.0 ± 0.5	36.0 ± 20.8	34.4 ± 17.0	56.0 ± 0.5
n-3/n-6	3.5 ± 0.4	3.4 ± 0.8	1.9 ± 0.9	2.6 ± 0.3
C ₂₀ PUFA	25.5 ± 1.2	17.4 ± 10.7	16.9 ± 11.6	18.2 ± 1.7
C ₁₈ PUFA	16.1 ± 0.2	17.5 ± 10.9	15.4 ± 4.8	36.7 ± 2.6
C ₂₀ /C ₁₈ PUFA	1.6 ± 0.1	1.0 ± 0.1	1.0 ± 0.5	0.5 ± 0.1

*Values are percent by weight ± SD from three samples of freshwater insects.

†Includes 15:0, 17:0 and 20:0.

‡Includes 20:1n-9, 20:1n-7 and 22:1n-9.

§Includes 20:2n-6, 20:3n-6 and 22:5n-6.

||Includes 16:2, 16:3, 16:4 and 18:2n-9.

tr = value < 0.05%.

1.9 to 6.8. Palmitic acid, 16:0, was again the most abundant saturated fatty acid except for the two water boatmen genera (Corixidae and Notonecta) that contained comparable amounts of 16:0 and 18:0. Stearic acid, 18:0, was the second most abundant saturated fatty acid in all the other specimens examined. The monoenes were mostly 18:1n-9 and 18:1n-7, the latter being dominant only in *E. venosus* and *Ephemerella*. Palmitoleic acid, 16:1n-7, was particularly high and variable in the Chironomidae. Linolenic acid (18:3n-3) and 20:5n-3 accounted for the majority of the PUFA with 20:5n-3 the major PUFA in all species except Corixidae in which 18:3n-3 accounted for 22.7% of the total fatty acids. Linoleic acid, 18:2n-6, was the most abundant n-6 PUFA followed by 20:4n-6. Docosahexanoic acid, 22:6n-3, was not detectable in other than traces in any of the species analysed.

The C₂₀/C₁₈ PUFA ratio was calculated to evaluate the ability of the freshwater insects either to accumulate C₂₀ PUFA from their diets or to form C₂₀ PUFA from C₁₈ PUFA precursors. The C₂₀/C₁₈ PUFA ratio was always greater than unity, except in Corixidae where high levels of 18:3n-3 were present. For all species analysed, the C₂₀/C₁₈ ratio

was higher in polar lipids (Tables 2a & 2b) than in neutral lipids (Tables 1a & 1b).

Fatty Acid Compositions of Phosphoglycerides

The fatty acid compositions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) in Plecoptera, Corixidae, *E. venosus* and *Ephemerella* are recorded in Tables 3–6.

In Ephemeroptera, PUFA were the most abundant fatty acids in PC, with n-3 PUFA always more abundant than n-6 PUFA and principally represented by 20:5n-3 and 18:3n-3, with 22:6n-3 being present only in trace amounts. In PC from Plecoptera, monoenoic fatty acids were more abundant than PUFA. Linoleic acid, 18:2n-6, was consistently the most abundant n-6 fatty acid in PC, PE, and PI of both Ephemeroptera and Plecoptera, with the exception that 20:4n-6 was most abundant in PS. In PE, monoenoic fatty acids were the most abundant fatty acids in Plecoptera and Ephemeroptera, although the principal PUFA were similar to PC. In PS, total amounts of PUFA and monoenoic fatty acids were similar in *Ephemerella* but PUFA were

TABLE 2b. Polar lipid fatty acid compositions (weight %) of freshwater insects*

Fatty acid	Notonecta	<i>Ecdyonurus v.</i>	<i>Caenis</i>	<i>Ephemerella</i>
14:0	0.8 ± 0.6	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.2
16:0	12.3 ± 2.7	12.4 ± 2.3	11.9 ± 1.1	12.9 ± 0.4
18:0	10.4 ± 0.8	7.0 ± 1.2	7.2 ± 0.3	7.4 ± 0.8
Total saturates†	25.1 ± 2.5	20.9 ± 3.5	20.5 ± 1.6	21.4 ± 1.1
16:1n-7	5.6 ± 1.4	10.1 ± 1.4	10.2 ± 1.7	5.6 ± 0.6
18:1n-9	15.5 ± 1.5	7.8 ± 1.1	13.5 ± 4.7	11.0 ± 1.2
18:1n-7	7.4 ± 1.1	14.1 ± 1.5	10.6 ± 4.6	13.9 ± 1.8
24:1	0.6 ± 0.8	0.6 ± 0.6	0.5 ± 0.7	0.1 ± 0.1
Total monoenes†	31.2 ± 2.6	32.7 ± 2.6	34.8 ± 1.2	30.7 ± 3.0
18:2n-6	6.3 ± 1.1	3.7 ± 0.3	3.3 ± 0.0	4.3 ± 0.5
18:3n-6	tr	0.2 ± 0.0	0.6 ± 0.1	0.5 ± 0.1
20:4n-6	1.5 ± 0.6	1.7 ± 0.3	1.4 ± 0.2	1.9 ± 0.6
Total n-6§	8.2 ± 1.6	5.8 ± 0.8	5.3 ± 0.2	7.0 ± 1.1
18:3n-3	8.4 ± 1.7	10.0 ± 1.3	6.9 ± 0.2	10.4 ± 1.8
18:4n-3	1.0 ± 0.8	1.6 ± 0.3	1.7 ± 0.1	2.4 ± 0.3
20:4n-3	0.5 ± 0.7	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:5n-3	21.6 ± 2.5	25.3 ± 3.6	27.1 ± 0.1	25.8 ± 1.1
22:5n-3	tr	0.1 ± 0.1	tr	0.1 ± 0.1
22:6n-3	tr	tr	0.2 ± 0.1	0.1 ± 0.1
Total n-3	31.8 ± 2.0	37.2 ± 5.2	36.2 ± 0.0	38.8 ± 0.8
Total PUFA¶	41.9 ± 4.3	44.7 ± 6.2	43.1 ± 1.5	46.9 ± 2.2
n-3/n-6	3.9 ± 0.6	6.4 ± 0.1	6.8 ± 0.2	5.7 ± 0.9
C ₂₀ PUFA	23.9 ± 2.0	27.3 ± 4.0	28.7 ± 0.3	28.0 ± 0.5
C ₁₈ PUFA	16.8 ± 2.3	16.1 ± 2.2	13.1 ± 0.8	18.0 ± 2.4
C ₂₀ /C ₁₈ PUFA	1.4 ± 0.2	1.7 ± 0.2	2.2 ± 0.1	1.6 ± 0.2

Footnotes as described in Table 2a except that values for *Caenis* were mean ± SD of two determinations.

more abundant in Plecoptera and *E. venosus*. Conversely in PI, total amounts of PUFA and monoenoic fatty acids were similar in *E. venosus*, but PUFA were the most abundant fatty acids in Plecoptera and *Ephemerella*. Linoleic acid, 18:2n-6, and 20:4n-6 were equally represented in the PS of all the species and also in the PI of *E. venosus*.

The phosphoglycerides of Corixidae contained high levels of PUFA, up to 68.7% in PC, mainly due to high levels, up to 30%, of 18:3n-3. Monounsaturated fatty acids were always present in lower percentages in this group, and in PE, saturated fatty acids were more abundant compared with all the other species examined. Linoleic acid, 18:2n-6, was also present in higher levels in all the phosphoglycerides of Corixidae than in any other species. α -Linolenic acid, 18:3n-3, was the most abundant n-3 PUFA in PC and PE of Corixidae, and in the PS and PI 18:3n-3 was present in levels comparable with 20:5n-3.

Palmitic acid, 16:0, was the most abundant saturate in PC. Although 18:0 was dominant in PS, 16:0 and 18:0 were present in comparable amounts in PE and PI. Monounsaturates were highest in PE (up to 40.4%). In Plecoptera, PC, PE, and PS had a high content of 18:1n-9, whereas, in Ephemeroptera, 16:1n-7 and 18:1n-7 accounted for the majority of the monounsaturates in PC and PI, whereas 18:1n-7 was also abundant in PE and PS.

The C₂₀/C₁₈ PUFA ratios in the individual phosphoglycerides from the four species are consistently higher in PS and PI than in PC and PE.

DISCUSSION

The present study establishes that the abundance of C₂₀ PUFAs in the total lipids of the eight insect species analysed in our previous study (2) is due to the preponderance of these fatty acids in polar rather than neutral lipids. This is clearly shown in the C₂₀/C₁₈ PUFA ratios of polar and neutral lipids and is in accord with the high content of C₂₀ PUFA reported in other aquatic insects (13). C₂₀ PUFA are common in some specialised tissues from terrestrial insects, including the retina of the butterfly *Deilephila elpenor* (32) and spermatophores of the cricket *Teogryllus commodus* (27), although they were not detected (17) or present only in small amounts (23) in phospholipids from the whole insect bodies. It is probable that C₂₀ PUFA are ubiquitous in insects even if they are concentrated in specialised tissues in the terrestrial species studied earlier.

One reason for the presumed ubiquity of C₂₀ PUFA in insects is their role as precursors of eicosanoids. The three C₂₀ PUFA, 20:3n-6, 20:4n-6 and 20:5n-3, are all eicosanoid precursors in vertebrates. Of these, 20:4n-6 is the major eicosanoid precursor in mammals and many invertebrates.

TABLE 3. PC fatty acid compositions (weight %) of freshwater insects*

Fatty acid	Plecoptera	Ephemera	Ecdyonurus v.	Corixidae
14:0	2.5 ± 0.5	0.6 ± 0.2	0.7 ± 0.1	tr
16:0	21.3 ± 3.3	15.5 ± 2.1	14.3 ± 1.2	11.3 ± 0.5
18:0	3.7 ± 1.2	4.9 ± 0.5	5.8 ± 0.7	4.6 ± 0.3
Total saturates†	29.9 ± 4.1	21.6 ± 2.4	21.4 ± 2.1	17.3 ± 0.9
16:1n-7	18.7 ± 1.5	7.7 ± 0.7	11.5 ± 0.1	3.5 ± 0.7
18:1n-9	14.0 ± 0.6	5.8 ± 1.2	4.3 ± 1.0	8.0 ± 0.4
18:1n-7	9.8 ± 1.6	8.9 ± 1.7	10.4 ± 1.4	2.0 ± 0.6
24:1	0.4 ± 0.7	—	tr	tr
Total monoenes‡	43.7 ± 3.1	22.6 ± 2.9	26.6 ± 2.0	14.0 ± 1.2
18:2n-6	3.6 ± 0.5	5.1 ± 0.9	3.7 ± 0.2	10.5 ± 0.3
18:3n-6	tr	1.0 ± 0.2	tr	0.6 ± 0.5
20:4n-6	0.8 ± 0.2	2.4 ± 0.7	1.8 ± 0.2	3.1 ± 0.9
Total n-6§	4.7 ± 0.4	8.7 ± 1.5	6.2 ± 0.6	15.0 ± 1.5
18:3n-3	6.2 ± 1.6	11.4 ± 2.3	10.6 ± 1.5	29.9 ± 6.1
18:4n-3	0.9 ± 0.6	3.4 ± 0.2	2.0 ± 0.2	2.4 ± 0.2
20:4n-3	tr	tr	tr	0.9 ± 0.0
20:5n-3	10.5 ± 4.2	30.6 ± 1.0	29.8 ± 1.1	18.3 ± 2.2
22:6n-3	tr	tr	tr	0.9 ± 0.4
Total n-3	18.0 ± 6.3	45.7 ± 2.9	42.9 ± 3.2	52.9 ± 3.8
Total PUFA¶	26.3 ± 7.1	55.8 ± 4.7	52.1 ± 4.0	68.7 ± 1.6
n-3/n-6	3.8 ± 1.3	5.3 ± 0.7	6.9 ± 0.4	3.6 ± 0.7
C ₂₀ PUFA	11.7 ± 4.4	33.5 ± 1.6	32.2 ± 1.6	22.9 ± 3.2
C ₁₈ PUFA	10.9 ± 2.2	21.2 ± 3.1	17.9 ± 1.6	43.7 ± 5.8
C ₂₀ /C ₁₈ PUFA	1.0 ± 0.2	1.6 ± 0.2	1.8 ± 0.1	0.5 ± 0.1

*Values are percent by weight ± SD from three samples of freshwater insects.

†Includes 15:0, 17:0 and 20:0.

‡Includes 20:1n-9, 20:1n-7 and 22:1n-9.

§Includes 20:2n-6, 20:3n-6 and 20:5n-6.

||Includes 22:5n-3.

¶Includes 16:2, 16:3, 16:4 and 18:2n-9.

tr = value < 0.05%.

However, the housefly *Musca domestica* contains higher levels of 20:3n-6 than 20:4n-6 and converts 20:3n-6 to eicosanoids more efficiently than 20:4n-6 (30). Similarly, 20:3n-6 is an efficient substrate for prostaglandin biosynthesis in the cricket *Teleogryllus commodus* (24). Conversion of both 20:4n-6 and 20:5n-3 to prostaglandins has been noted in the waxmoth (26). The testicular phospholipids of the lepidopteran *Pieris brassicae* accumulate 20:3n-6 and 20:5n-3 (18), both of which are potential eicosanoid precursors. The fatty acid compositions of all the phosphoglycerides presented in this study establish that 20:5n-3 is the most abundant potential eicosanoid precursor in the freshwater insects studied, with 20:4n-6 being present in much lower amounts than 20:5n-3. The only exception to this is the PI from Corixidae, where 20:4n-6 is present in comparable amounts to 20:5n-3 and in much higher amounts than in PI from the other species analysed. These findings by themselves do not establish that eicosanoids are produced mainly from 20:5n-3 in the insects studied here, because it is well established that, whereas 20:5n-3 is generally the most abundant eicosanoid precursor in many marine animals (22), 20:4n-6 is commonly preferred to 20:5n-3 as an eicosanoid precursor

in fish, molluscs and amphibians (3,16). It is noteworthy, however, that 20:4n-6 in marine animals is commonly concentrated in PI (20,22) as it is in Corixidae.

As well as being essential for the formation of eicosanoids, C₂₀ PUFA are essential for maintaining the structural integrity of biological membranes, clearly reflected in the present analyses in the abundance of C₂₀ PUFA in the polar lipids in general, and in the major membrane phosphoglycerides, PC and PE, in particular. This structural role for C₂₀ PUFA is generally fulfilled in higher vertebrates by 20:4n-6 but in aquatic animals, whether vertebrate or invertebrate, 20:5n-3 is very often the dominant C₂₀ PUFA (22) and the insects studied here clearly fall into this category. The dominance of 20:5n-3 as a C₂₀ PUFA is particularly clear in marine animals where 20:5n-3 is commonly accompanied by very substantial amounts of 22:6n-3 (20,22), which is essentially absent from the insects studied here and from many other freshwater insects studied previously (12,13). It has traditionally been considered that the abundance of 20:5n-3 and 22:6n-3 in cell membrane phospholipids of aquatic animals reflects an adaptation to low environmental temperatures, and this has already been proposed

TABLE 4. PE fatty acid compositions (weight %) of freshwater insects*

Fatty acid	Plecoptera	Ephemera	Ecdyonurus v.	Corixidae
16:0	7.5 ± 1.1	11.9 ± 1.5	11.2 ± 0.7	8.6 ± 1.1
18:0	8.8 ± 1.5	9.3 ± 1.3	9.4 ± 1.0	23.4 ± 1.3
Total saturates†	18.4 ± 1.9	22.2 ± 2.7	21.9 ± 2.3	32.6 ± 2.2
16:1n-7	4.3 ± 0.1	3.3 ± 0.7	6.7 ± 0.9	1.7 ± 0.3
18:1n-9	29.6 ± 1.1	18.0 ± 2.2	13.3 ± 1.1	15.2 ± 3.9
18:1n-7	8.9 ± 1.0	18.4 ± 1.4	20.7 ± 0.7	4.1 ± 0.8
24:1	—	0.3 ± 0.6	tr	—
Total monoenes‡	43.2 ± 1.3	40.4 ± 2.9	40.7 ± 2.0	21.5 ± 3.1
18:2n-6	4.5 ± 0.4	3.6 ± 0.2	3.4 ± 0.1	12.9 ± 1.3
18:3n-6	tr	tr	tr	0.5 ± 0.1
20:4n-6	2.9 ± 0.2	1.8 ± 0.4	1.7 ± 0.2	1.7 ± 0.5
Total n-6§	7.6 ± 0.7	5.8 ± 0.5	5.6 ± 0.4	15.5 ± 1.9
18:3n-3	9.4 ± 0.2	7.9 ± 1.8	9.0 ± 1.0	17.0 ± 1.5
18:4n-3	0.6 ± 0.0	1.8 ± 0.2	1.3 ± 0.0	0.8 ± 0.0
20:4n-3	—	tr	tr	0.4 ± 0.1
20:5n-3	20.1 ± 2.1	20.3 ± 1.0	20.0 ± 1.8	10.3 ± 1.7
22:5n-3	tr	0.2 ± 0.3	0.6 ± 0.8	0.3 ± 0.3
22:6n-3	—	0.7 ± 0.7	tr	tr
Total n-3	30.2 ± 2.3	30.9 ± 1.1	31.1 ± 3.2	28.9 ± 1.8
Total PUFA	38.4 ± 2.2	37.4 ± 1.7	37.4 ± 4.1	45.9 ± 4.1
n-3/n-6	4.0 ± 0.4	5.3 ± 0.3	5.6 ± 0.3	1.9 ± 0.2
C ₂₀ PUFA	23.1 ± 2.1	22.4 ± 0.8	22.1 ± 2.0	12.7 ± 2.3
C ₁₈ PUFA	14.6 ± 0.6	13.6 ± 1.6	14.0 ± 1.2	31.8 ± 1.6
C ₂₀ /C ₁₈ PUFA	1.6 ± 0.1	1.7 ± 0.3	1.6 ± 0.1	0.4 ± 0.1

*Values are percent by weight ± SD from three samples of freshwater insects.

†Includes 14:0, 15:0, 17:0 and 20:0.

‡Includes 20:1n-9, 20:1n-7 and 22:1n-9.

§Includes 20:2n-6, 20:3n-6 and 22:5n-6.

||Includes 16:2, 16:3, 16:4 and 18:2n-9.

tr = value < 0.05%.

to account for the abundance of 20:5n-3 (and also 20:4n-6) in the range of aquatic insects studied by Hanson *et al.* (13). However, it has more recently been proposed that the uniqueness of C₂₀ an C₂₂ PUFA in determining the special structural and functional properties of biomembranes reflects much less the phase transition temperatures ("melting points") of these fatty acids, which in the cases of 20:4n-6, 20:5n-3 and 22:6n-3 are far below 0°C, and much more their unique conformations arising from their basic methylene-interrupted *cis*-dienoic structures (20). This proposition is consistent with C₂₀ and C₂₂ PUFA, whether of the n-6 or n-3 series, being abundant in a wide range of animals, both poikilothermic and homeothermic, over a wide range of ambient temperatures. It is particularly relevant to the marked abundance of 22:6n-3, with its unique conformational and structural properties, in the highly specialised sperm, neural and retinal cell membranes of vertebrates (21). It is notable in this context that the insects studied here, and the range of aquatic insects studied previously (12,13), effectively lack 22:6n-3. It can be inferred that the role fulfilled in vertebrate neural and retinal tissue (and other body tissues) by 22:6n-3 is readily fulfilled in insect neural and retinal tissue by 20:5n-3, which may account for

the abundance of C₂₀ PUFA in the retina of the butterfly (17) and the spermatophores of the cricket (27) referred to earlier.

There is now accumulating evidence that the 20:5n-3 and 22:6n-3 abundant in marine invertebrates and vertebrates are directly derived from unicellular phototrophs and heterotrophs at the base of the marine food web (21). The situation for freshwater insects, and freshwater invertebrates in general, is much less clear. However, a dietary origin of some, if not most of their C₁₈ and C₂₀ PUFA is probable if not certain. Thus, higher levels of C₂₀ PUFA are present in carnivorous terrestrial insects than related phytophagous species (29), consistent with a substantial dietary origin. It is likely, therefore, that higher proportions of C₂₀ PUFA are present in aquatic species that are predatory. It is not always easy to locate every insect species in a specific feeding group, because this is influenced by the general food availability in an ecosystem at a certain period of time and not solely related to feeding mechanisms. However, according to the functional feeding classification proposed by Cummins and Klux (9), the insect species considered here are mostly carnivorous. Perlid nymphs (Plecoptera, suborder Archiperlaria), common in British rivers, are largely carnivorous, prey-

TABLE 5. PS fatty acid compositions (weight %) of freshwater insects*

Fatty acid	Plecoptera	Ephemera	Ecdyonurus v.	Corixidae
14:0	tr	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.4
16:0	7.6 ± 1.5	11.3 ± 6.1	8.9 ± 2.0	8.3 ± 2.2
18:0	19.9 ± 2.5	17.3 ± 1.5	16.7 ± 0.2	23.2 ± 3.1
Total saturates†	29.3 ± 2.6	30.4 ± 7.9	28.3 ± 2.5	34.3 ± 1.2
16:1n-7	3.7 ± 1.6	4.8 ± 2.9	6.0 ± 1.1	2.2 ± 1.0
18:1n-9	19.5 ± 1.9	13.6 ± 0.9	10.8 ± 0.4	14.5 ± 2.2
18:1n-7	8.4 ± 1.2	12.7 ± 1.1	13.5 ± 1.2	3.1 ± 0.8
24:1	—	0.4 ± 0.3	0.3 ± 0.3	—
Total monoenes‡	32.3 ± 4.1	31.6 ± 4.1	31.2 ± 2.7	20.3 ± 3.8
18:2n-6	3.8 ± 0.3	3.1 ± 0.4	2.7 ± 0.3	8.0 ± 1.4
18:3n-6	0.3 ± 0.2	tr	tr	0.4 ± 0.3
20:4n-6	4.3 ± 0.7	2.2 ± 0.9	2.8 ± 0.7	3.2 ± 2.4
Total n-6§	8.5 ± 0.8	6.0 ± 0.9	6.3 ± 0.8	12.1 ± 3.8
18:3n-3	7.3 ± 0.2	5.9 ± 0.9	5.2 ± 0.4	16.8 ± 1.3
18:4n-3	0.3 ± 0.3	2.2 ± 2.1	1.0 ± 0.2	1.0 ± 0.4
20:4n-3	tr	0.6 ± 0.6	0.4 ± 0.1	0.5 ± 0.2
20:5n-3	21.7 ± 2.7	22.2 ± 4.0	27.1 ± 4.6	14.6 ± 1.2
22:5n-3	tr	tr	0.4 ± 0.3	0.3 ± 0.3
22:6n-3	tr	—	—	tr
Total n-3	29.8 ± 2.3	31.0 ± 4.9	34.0 ± 4.7	33.3 ± 2.2
Total PUFA	39.3 ± 1.3	39.2 ± 5.5	42.8 ± 7.1	47.2 ± 4.9
n-3/n-6	3.5 ± 0.5	5.3 ± 1.0	5.4 ± 0.1	2.9 ± 0.8
C ₂₀ PUFA	26.2 ± 2.8	25.4 ± 4.2	30.7 ± 5.4	18.8 ± 3.5
C ₁₈ PUFA	11.8 ± 0.6	12.1 ± 2.2	9.8 ± 1.7	26.8 ± 0.9
C ₂₀ /C ₁₈ PUFA	2.2 ± 0.4	2.1 ± 0.3	3.2 ± 0.8	0.7 ± 0.1

*Values are percent by weight ± SD from three samples of freshwater insects.

†Includes 15:0, 17:0 and 20:0.

‡Includes 20:1n-9, 20:1n-7 and 22:1n-9.

§Includes 20:2n-6, 20:3n-6 and 22:5n-6.

||Includes 16:2, 16:3, 16:4 and 18:2n-9.

tr = value < 0.05%.

ing chiefly on Ephemeroptera nymphs and Chironomid larvae (19). They are usually classified as predators or shredders of coarse particulate organic matter, including leaves, needles and nonwoody material with associated fungi and bacteria (31). Among diptera, only a few species have phytophagous larvae, whereas all the others are either saprophagous or predatory. Coleoptera larvae are also saprophagous, feeding on decaying organic matter, and in this habitat fungi may also form an important part of the diet (5). Notonectidae are markedly predatory, attacking small fish and tadpoles, whereas Corixidae are microphagous, feeding on algal cells and diatoms. Ephemeroptera are collector/gatherers of fine particulate organic matter, or scrapers. These very varied foods clearly offer a wide range of dietary PUFA to insects, and although little is known of their lipid composition, it is probable that they present abundant amounts of all of the PUFA present in the insects analysed in the present study. This proposition is supported by the presence of substantial amounts of 20:5n-3, 18:3n-3 and 18:2n-6, and also significant amounts of 20:4n-6 in the insects' neutral lipid, whose fatty acid composition will reflect that of dietary lipid much more directly than cell membrane phospholipid.

There are two final aspects to be considered in elucidat-

ing the origin of insect PUFA, the first being the extent to which insects are capable of converting 18:2n-6 and 18:3n-3 derived from the diet to 20:4n-6 and 20:5n-3, respectively. Evidence that these processes occur to some extent in the insects studied here is the preponderance of C₂₀ PUFA relative to C₁₈ PUFA in polar lipid and the phosphoglycerides as compared with the neutral lipid. Thus, the preponderance of C₁₈ PUFA in the neutral lipid is likely to reflect direct dietary input, whereas the preponderance of C₂₀ PUFA in the phosphoglycerides is likely to reflect the conversion in tissues of C₁₈ PUFA assimilated in the diet to C₂₀ PUFA. Although values obtained from C₂₀/C₁₈ PUFA ratios do not prove absolutely the existence of Δ6 and Δ5 desaturase activities, this ratio has been used in conjunction with direct measurement of desaturase activities using radioactive precursors to confirm the existence of C₂₀ PUFA synthesis in a number of insect species (25). The second aspect is the extent to which the ability to form C₁₈ PUFA *de novo* from 18:1n-9, which has been reported for some species of insects (4), extends to other insect species. As noted earlier, this property has the important potential to release those insect species concerned from a nutritional dependence on dietary PUFA. Until these two aspects of

TABLE 6. PI fatty acid compositions (weight %) of freshwater insects*

Fatty acid	Plecoptera	Ephemera	Ecdyonurus v.	Corixidae
14:0	0.3 ± 0.2	0.7 ± 0.3	1.2 ± 1.1	0.5 ± 0.4
16:0	11.0 ± 1.0	13.9 ± 3.1	14.6 ± 8.3	12.5 ± 2.3
18:0	13.5 ± 3.6	14.4 ± 4.1	12.5 ± 2.2	15.8 ± 3.2
Total saturates†	28.2 ± 2.2	31.4 ± 6.3	31.2 ± 7.3	30.1 ± 5.5
16:1n-7	4.8 ± 2.6	4.1 ± 2.1	14.0 ± 5.2	2.8 ± 0.7
18:1n-9	9.5 ± 0.7	5.6 ± 1.0	5.5 ± 1.9	8.1 ± 1.1
18:1n-7	6.6 ± 0.6	8.8 ± 1.1	11.0 ± 1.9	3.0 ± 0.7
24:1	0.2 ± 0.4	0.4 ± 0.5	—	tr
Total monoenes‡	22.7 ± 3.2	19.3 ± 2.8	30.9 ± 9.2	14.4 ± 2.2
18:2n-6	4.7 ± 2.7	4.0 ± 2.6	1.0 ± 0.3	8.6 ± 3.8
18:3n-6	tr	0.3 ± 0.2	tr	tr
20:2n-6	0.2 ± 0.1	0.6 ± 0.3	0.1 ± 0.1	1.0 ± 1.2
20:3n-6	—	—	—	1.7 ± 2.5
20:4n-6	3.0 ± 0.8	0.9 ± 0.4	0.9 ± 0.6	11.6 ± 1.9
Total n-6	8.0 ± 2.4	5.7 ± 2.4	2.1 ± 0.8	23.1 ± 4.3
18:3n-3	9.1 ± 4.0	12.9 ± 11.5	6.6 ± 5.7	14.9 ± 6.1
18:4n-3	0.7 ± 0.2	1.5 ± 0.6	0.4 ± 0.3	0.3 ± 0.4
20:4n-3	—	tr	0.3 ± 0.2	0.4 ± 0.5
20:5n-3	28.8 ± 7.0	26.5 ± 8.8	25.5 ± 11.4	15.1 ± 3.3
22:5n-3	0.2 ± 0.4	0.3 ± 0.4	tr	tr
22:6n-3	0.4 ± 0.6	0.3 ± 0.4	—	tr
Total n-3	39.4 ± 3.4	41.6 ± 2.5	33.0 ± 14.2	30.9 ± 3.6
Total PUFA§	48.7 ± 3.3	49.2 ± 5.5	37.8 ± 15.8	55.4 ± 7.6
n-3/n-6	5.3 ± 2.1	8.5 ± 4.2	17.3 ± 10.8	1.4 ± 0.2
C ₂₀ PUFA	32.1 ± 7.6	28.1 ± 9.2	26.8 ± 11.8	29.9 ± 8.6
C ₁₈ PUFA	14.7 ± 6.7	18.9 ± 13.3	9.4 ± 6.5	24.7 ± 10.4
C ₂₀ /C ₁₈ PUFA	2.6 ± 1.4	2.3 ± 1.8	3.4 ± 1.9	1.4 ± 0.7

*Values are percent by weight ± SD from three samples of freshwater insects.

†Includes 15:0, 17:0 and 20:0.

‡Includes 20:1n-9, 20:1n-7 and 22:1n-9.

§Includes 16:2, 16:3, 16:4 and 18:2n-9.

tr = value < 0.05%.

the PUFA nutritional biochemistry of insects are elucidated, our understanding of the sources and functions of PUFA in insects is likely to continue to lag substantially behind that for vertebrates.

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