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SEASONAL VARIATIONS OF TRIACYLGLYCEROLS AND FATTY ACIDS IN *FUCUS SERRATUS*

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Key Word Index—*Fucus serratus*; Phaeophyta; brown alga; triacylglycerols; fatty acids; seasonal variations.

Abstract—The triacylglycerols and fatty acids of *Fucus serratus* were analysed during the course of a year. The amount of triacylglycerols was maximal in July (19.2% total lipids), but minimal in February (6.6%). Triacylglycerols accumulated more in summer (2.8 mg g^{-1} dry wt) and autumn (2.6 mg g^{-1} dry wt) than spring (0.7 mg g^{-1} dry wt) and winter (0.5 mg g^{-1} dry wt). In the total lipids, the dominant fatty acids were palmitic (16:0, 24.1%), oleic (18:1, 22.4%) and arachidonic (20:4, 14.4%), but in the triacylglycerols, palmitic 16:0 (22.8%), oleic 18:1 (36.4%) and linoleic acids (18:2, 16.4%) were most abundant. C_{16} fatty acids were predominant in winter and C_{18} ones in summer and autumn. The ratios 16:0/18:1 and 20:4/20:5 appeared to be important indicators of the status of the seaweed according to variation of environmental conditions. We conclude that, although the emersion of *F. serratus in situ* is more effective during winter and spring, correlating with good growth and productivity of thalli, synthesis of triacylglycerols is activated during the summer. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The brown algae (seaweeds) are very important for the littoral biomass and the ecological distribution of primary production. Like higher plants, they contain two types of lipids, polar lipids (PL), essentially located in membranes, and triacylglycerols (TAG) as storage lipids [1–4]. The membrane lipids of some members of the Phaeophyta (*Pelvetia canaliculata*, *Fucus serratus* and *F. platycarpus*) have been studied for analytical purposes and their relationships to photosynthetic complexes [5–9]. However, few triacylglycerols have been reported until now [10].

The fatty acid composition of lipids has been shown to be affected by many factors, such as light, salinity, mineral ions, heavy metals (Cu, Cd and Pb), pollution, herbicides, infection of fungi and of bacteria, habitat and environmental conditions *in situ* as well as *in vitro* [11–25]. Although they result from a large pool of very diverse environmental factors, the seasonal parameters of the environment stay within a relatively narrow range and are mostly repetitive from year to year. Following our previous study on Cyanobacteria [26], we thought these environmental conditions might have some influence on the lipids of brown algae, especially on their storage lipids. In order to examine this factor, we have monitored changes in triacylglycerol content and fatty acid composition of *F. serratus* according to season. On the one hand, we expected that, *in situ* and according to season, changes in light regime and growth speed would affect TAG level relative to total lipids (TL), while the changes in temperature would affect primarily fatty acid composition. Such changes would indicate the physiological status of the algae, i.e. rich in storage lipids at the end of the growing period.

Among the brown macroalgae present on the Britanny coast, *F. serratus* was selected because it exhibits various periods of important growth and population extension throughout the year. Moreover, the industrial use of *Fucus* is now under development and the recovery of by-products, such as unsaturated lipids, might be of interest. Since brown algae contain the highly valued eicosapentaenoic acid [27, 28], like red algae and some marine bacteria [29–34], it was of interest to examine its variations in lipids throughout the year [35].

RESULTS AND DISCUSSION

We have analysed TL and separated triacylglycerols (TAG) of *F. serratus* according to season (Fig. 1 and Tables 1–2). On a dry weight basis TL were more

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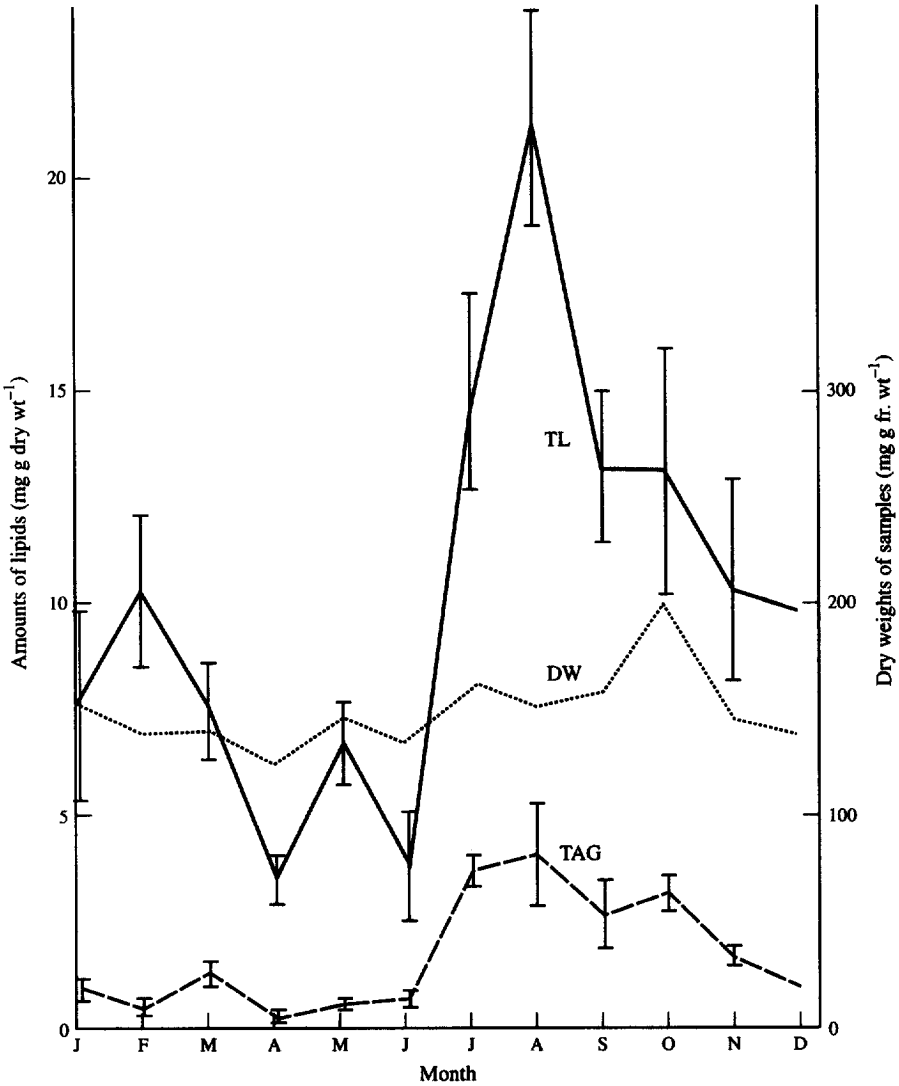

Fig. 1. Annual evolution of quantities of total lipids (TL), triacylglycerols (TAG) and dry weight in *Fucus serratus*. Values are means + s.d. (n = 14–16).

abundant in August (21.3 mg g^{-1}, 2.1%) and lower in April (3.5 mg g^{-1}, 0.4%). The ratio between the maximum and minimum was 6.1 and the annual mean amount was *ca* 10.1 mg g^{-1}. Dry weight was minimal in April (127 mg g^{-1} fresh wt) and maximum in October (192 mg g^{-1} fresh wt). This variation in dry weight would increase the difference in the lipid amounts between maxima and minima when expressed as fresh weight.

We found that TL in *F. serratus* accounted for 0.4–2.1% of its dry weight. This is close to the values recorded by Levring *et al.* for the same species [36] (3%) and also to those found in *F. vesiculosus* and *F. ceranoides* (4% and 5%, respectively) [37]. The latter data were obtained in August and must be compared with our corresponding results (2.1%). This also shows that lipids of algae are generally accumulated less than those of higher plants [38, 39] and some Bryophytes [24].

The amount of storage lipids varied a lot according to season. The amount of TAG was maximal in August (4.1 mg g^{-1} dry wt, 0.4%) and minimal in April (0.2 mg g^{-1} dry wt, 0.02%) (Table 2). However, the relative amount of TAG to TL was maximal (24.7%) in July and minimal (4.9%) in February. Thus, the synthesis of lipids was most active in summer, while it was less in spring (Fig. 2). The highest TAG content was in summer (2.8 mg g^{-1} dry weight, 0.3%) and the lowest in spring (0.74 mg g^{-1} dry weight, 0.07%) (Fig. 2). These results are similar to those obtained by Pham Quang and Laur [5], who showed that the amount of PL (representing counterpart of TAG among TL) was more abundant in spring and winter than in summer and autumn. These authors [13] also showed that total PL in comparison with TL in Fucales varied according to the time of harvest for *P. canaliculata* (50–58% of TL), *F. vesiculosus* (52–54% of TL) and *F. serratus* (50–52% of TL). We can estimate that, from our studies of *F.*

Table 1. Seasonal variations in percentage of fatty acids, amounts of total lipids (%) and dry weight in *F. serratus in situ*

	Composition of fatty acids and dry weight of samples									
Month	16:0	16:1	18:1	18:2	18:3	18:4	20:4	20:5	TL±SD (mg g^{-1} dry wt)	Dry wt (mg g^{-1} fr. wt)
J	23.9	2.9	20.3	9.9	7.9	9.3	12.2	13.7	7.5±2.4	146
F	22.1	2.4	14.3	11.7	8.7	10.6	14.4	15.8	10.4±1.8	140
M	23.6	3.0	17.3	11.3	9.7	9.5	12.3	10.3	7.7±1.0	140
A	27.8	3.5	19.6	13.1	5.2	7.5	12.8	9.9	3.5±0.6	127
M	26.9	2.0	19.5	10.0	7.4	9.3	15.0	10.2	6.7±0.1	146
J	29.2	tr	26.8	9.0	5.6	5.7	14.3	8.7	4.8±1.3	135
J	26.0	1.9	23.5	9.9	7.0	6.0	16.4	9.2	15.0±2.3	163
A	18.9	3.1	32.8	9.5	5.0	4.0	16.1	7.1	21.3±2.6	152
S	20.0	3.4	34.1	14.2	5.0	4.6	13.5	6.3	13.2±1.8	158
O	21.2	3.2	31.4	8.3	5.8	5.3	15.6	9.1	13.1±2.9	192
N	22.9	1.3	18.0	8.1	9.1	10.5	16.0	11.5	10.3±3.1	145
D	26.5	9.5	11.4	8.4	7.6	11.1	13.6	11.9	7.8	129

Period of extraction of lipids: July 86–June 87.
TL: total lipids; tr: traces (<0.02 mg g^{-1} dry wt).

serratus, that the amount of PL varies from 75 to 95%. Finally, the annual mean amount of TAG was 1.8 mg g^{-1} dry wt (Table 2); the ratio between the annual means of TL and TAG was 5.6. This means that TAG are accumulated (18%) in the cytosol, namely, the counterpart 82% of TL is PL that consists of membrane lipids of thylakoids and cell organelles. Thus, it makes conspicuous the seasonal variations of lipids in *F. serratus*.

The different fatty acids were not evenly distributed amongst the lipids. In the TL, the major fatty acids were palmitic (16:0, 24.1%), oleic (18:1, 22.4%) and arachidonic (20:4, 14.4%). The relative amounts of 16:0 in TL were less in summer and autumn than in spring and winter (Table 1). From August to October, the amount of 18:1 in TL was more abundant than 16:0, but it decreased in December down to 11.4% of TL. With regard to the absolute quantities of fatty acids, 16:0 was maximal in July and August, while 18:1, 18:3 and 20:4 were maximal in August.

In TAG, 16:0, 18:1, 18:2 (linoleic acid) and 20:4 were dominant throughout the year (22.8, 36, 164 and 9.2%, respectively, year-mean values), but varied widely with season (Table 2). The major acid, 18:1, was abundant from May to November (up to 44%) but became as low as 11% in December. The dominant fatty acids (16:0, 18:1 and 20:4) were higher in summer and autumn, and the absolute quantities of all the fatty acids were maximal in July and August.

The quality of fatty acids in TL and in TAG did not vary but the quantity varied considerably according to season, as found in previous studies [5, 17, 38]. When the fatty acids of *F. serratus* are considered according to their carbon numbers, we can observe that the content of C_{18} (18:1 + 18:2 + 18:3 + 18:4) is dominant (TL: 47%; TAG: 59%), while C_{16} (16:0 + 16:1)

Table 2. Seasonal variations in percentage of fatty acids, amounts of triacylglycerols (%) and dry weight in *F. serratus in situ*

	Composition of fatty acids and dry weight of samples									
Month	16:0	16:1	18:1	18:2	18:3	18:4	20:4	20:5	TAG±SD (mg g^{-1} dry wt)	Dry wt (mg g^{-1} fr. wt)
J	17.6	4.8	33.4	23.2	4.8	tr	7.1	9.3	1.0±0.2	146
F	25.4	6.3	24.4	33.2	5.2	tr	13.2	8.5	0.5±0.2	140
M	19.4	6.4	27.8	18.2	12.3	4.9	7.2	7.0	1.3±0.3	140
A	31.7	0.9	30.7	19.0	3.5	1.5	8.5	4.8	0.2±0.4	127
M	27.6	tr	42.9	20.0	2.9	1.3	3.5	2.1	0.6±0.1	146
J	25.6	tr	43.7	13.5	2.2	1.3	8.5	5.2	0.7±0.2	135
J	19.4	2.2	41.6	16.2	3.7	1.8	11.5	3.8	3.7±0.4	163
A	16.4	2.9	44.7	14.0	3.2	1.8	12.5	3.6	4.1±1.2	152
S	17.3	4.2	46.8	14.9	4.2	2.9	10.7	2.5	2.7±0.8	158
O	14.8	2.8	44.2	13.2	3.7	1.7	15.7	4.4	3.2±0.4	192
N	18.4	1.5	44.9	17.7	5.2	0.7	9.5	3.0	1.7±0.2	145
D	40.2	34.3	11.1	6.4	2.4	tr	2.8	2.9	1.5	129

TAG: triacylglycerols; tr: traces (< 0.02 mg g^{-1} dry wt).

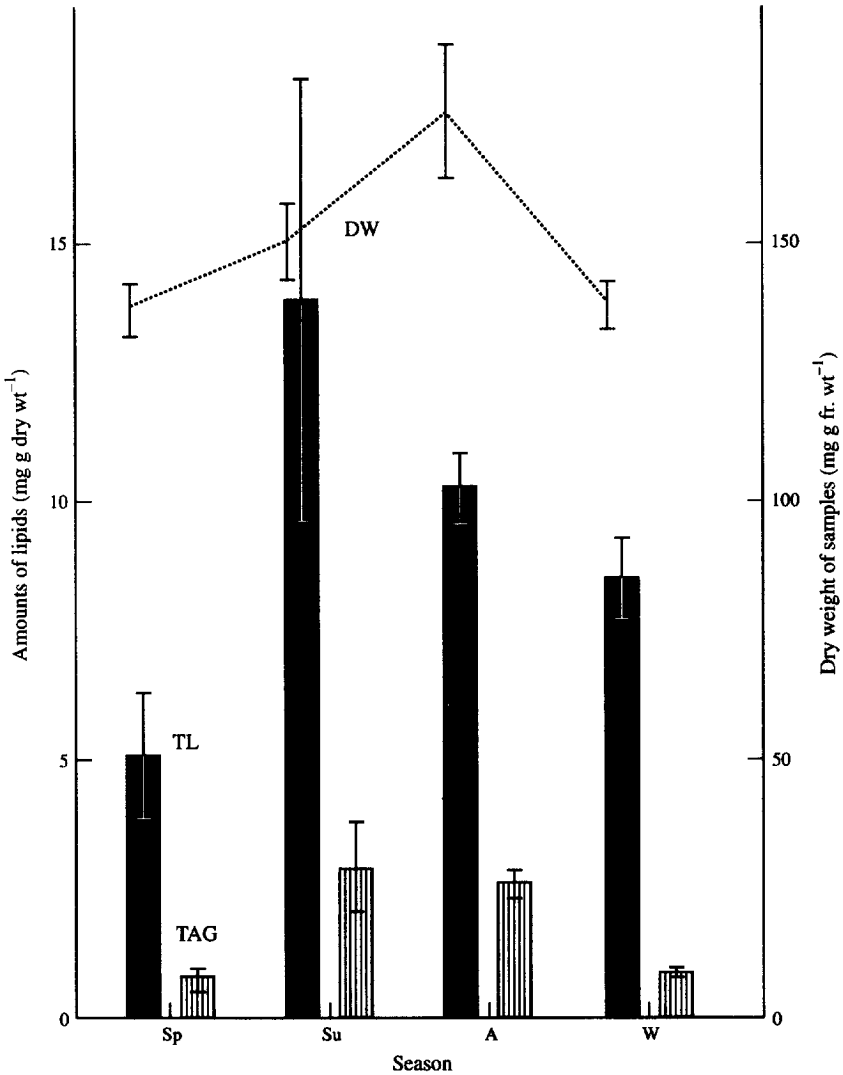

Fig. 2. Seasonal evolution of quantities of total lipids, triacylglycerols and dry weight in *Fucus serratus*. Sp: spring; Su: summer; A: autumn; W: winter.

is 27% in TL and 28% in TAG. The mean of saturated fatty acid (SFAs) in TL (*ca* 12%) is similar to that of the leaves of red clover (11.6%) [39, 40], but higher than that in beans (7.1%) and wheat (8.2%) [41]. Among algae, the SFA content of TL in *F. serratus* (12%), however, is lower than that of red (*Ceramium rubrum* 33.3%) [42] or green algae (*Valonia utricularis* 38.5%) [6]. Much more interesting are the ratios between 16:0 and 18:1 (Fig. 3), and 20:4 and 20:5 (Fig. 4), because they can be important as indicators of adaptation to environmental conditions. The 16:0/18:1 ratio varied from 0.6 to 2.3 in TL and from 0.3 to 3.6 in TAG; it is lower from August to October. On the contrary, the 20:4/20:5 ratio, which was from 0.9 to 2.3 in TL and from 0.8 to 4.3 in TAG, was highest in August for TL (2.3) and September for TAG (4.3). Hence, the degree of unsaturation of fatty acids was higher in spring and winter corresponding to the cold seasons. Such an augmentation of the 16:0/18:1 ratio indicates unfavourable conditions for elongation of fatty acids, while the increase of the 20:4/20:5 ratio indicates a lower fluidity of cellular membranes [10].

The polyunsaturated fatty acids (PUFAs) belong to the C_{20} series, which are typical of marine algae rather than of higher plants [43–49]. In *F. serratus*, they are the most abundant among PUFAs, while the corresponding C_{18} compounds PUFAs are low. In particular, 20:4 (14.4% of total fatty acids) is more abundant than that of 20:5 (10.4%). These two PUFAs are much more important in PL (glyco- and phospholipids) than in TAG. Such results are in agreement with the analyses performed by Smith and Harwood [17, 38] on only one stage of *F. serratus* development, which showed that these fatty acids are mostly present in phosphatidylcholine and phosphatidylethanolamine. Jamieson and Reid [50] observed that 20:5 was most abundant in monogalactosyl diglycerides and digalactosyl diglycerides. Our data fit very well with the general observation that membrane lipids, especially those from photosynthetic membranes, are the most

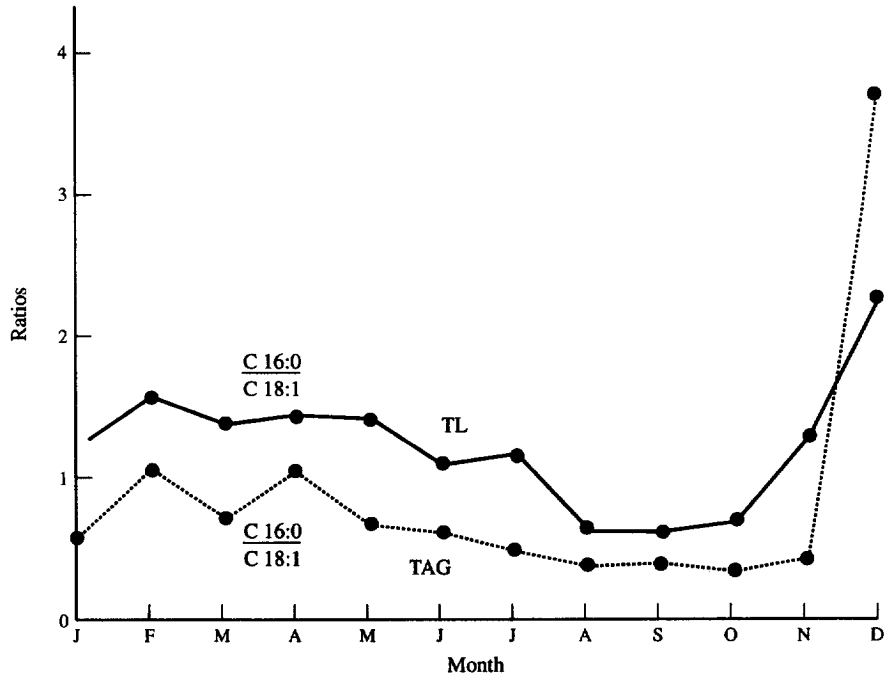

Fig. 3. Seasonal evolution of 16:0/18:1 ratios in *Fucus serratus*.

unsaturated lipids in the cell with regard to their role in physiological activities.

The amounts of lipids and their fatty acid composition in *F. serratus* might depend on temperature, light, the nitrogen status and the variation of salinity in seawater. When the emersion of *F. serratus* is important, especially, during summer, the saturation of TAG would appear as a possible result of adaptation to environment. During this period, photosynthetic activity and fixation of CO_2, are stimulated by the duration of emersion [51], the concentration of CO_2 and HCO_3^- [52, 53] and the intensity of light [54]. With regard to

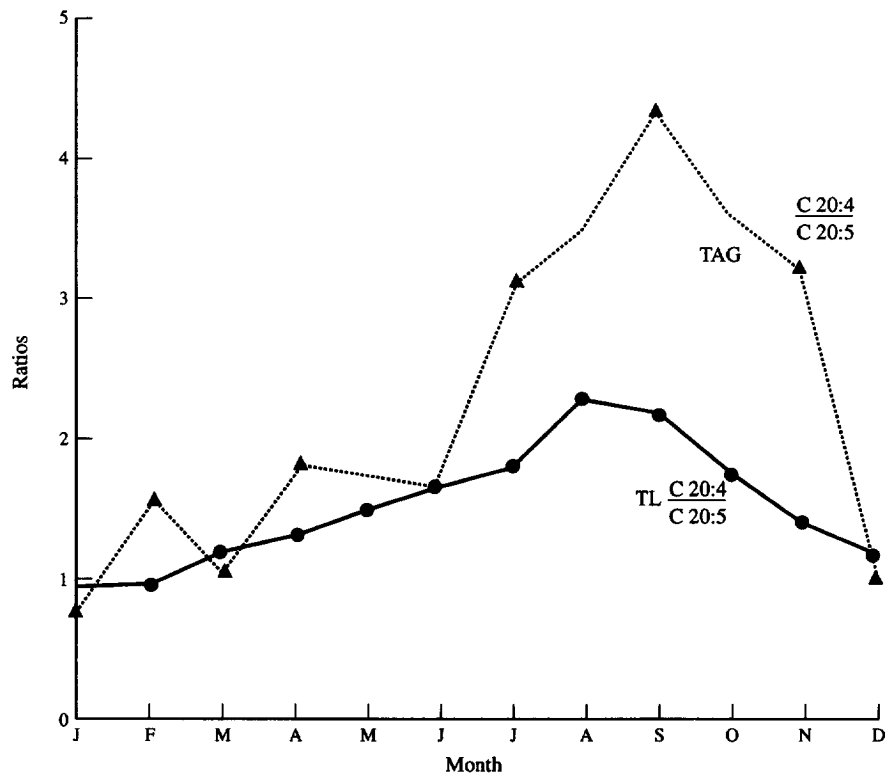

Fig. 4. Seasonal evolution of 20:4/20:5 ratios in *Fucus serratus*.

the industrial and chemical utilization of TAG and C_{20} fatty acids [55], it appears beneficial to get the intertidal algae adapted to alternating conditions, emersion and submersion, and ecophysiological conditions occurring during the summer.

EXPERIMENTAL

Plant material. Samples were harvested at Roscoff (48° 43′ N and 3° 58′ W) in France, at low tide once a week (September 1986 to August 1987). Samples used were *ca* 25–35 cm long without fertile parts. The estimated age of the algae was *ca* 5–6 months [56].

Extraction of lipids. Fragments of thalli (1 g fr. wt) were washed with H_2O and dried on filter papers. TL were extracted by the method of ref. [57]. Samples in 4 ml of H_2O were heated at 100° for 5 min. They were then ground with MeOH (10 ml) in an Ultraturrax. After addition of 2 vol. of $CHCl_3$ (2 × 5 ml) and agitation, 4 ml 1% NaCl soln was added. After centrifugation (5000 rpm × 10 min), TL were recovered in the $CHCl_3$ layer, which was then evapd under N_2 in order to avoid oxidation of lipids. Lipids were dissolved in C_6H_6–EtOH (4:1) and preserved frozen at −20°.

Separation of TAG by TLC. Extracted lipids (300–500 μl) were sepd by TLC on silica gel according to ref. [58], using petrol–Et_2O–HOAc (70:30:0.4). Lipids were visualised using 0.01% primuline in Me_2CO–H_2O, (4:1) and observed under UV light (350 nm). Lipids were subsequently extracted from the adsorbent using 1 ml MeOH.

Analysis of fatty acids. TL, as well as TAG sepd by TLC, were methylated using NaOMe (1%) and 1.2 NHCl–MeOH with 17:0 as int. standard. Samples were heated at 50° for 5 min. Fatty acid Me esters were then extracted by addition of heptane (1 ml) and H_2O (0.5 ml). Fatty acid Me esters were identified using GC on a capillary column coated with polyethylene glycol 20 M (25 cm × 0.32 mm) with He as carrier gas.

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