Freshwater Biology (2006) **51,** 2029–2040 doi:10.1111/j.1365-2427.2006.01633.x

Effects of artificial ultraviolet-B radiation on growth and fatty acid composition of duckweed (*Lemna minor*)

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SUMMARY

1. Duckweed (*Lemna minor*), collected either in summer or early fall was exposed under laboratory conditions to control (photosynthetically active and UV-A radiation) or experimental (control plus UV-B radiation) conditions.
2. Growth and survival were determined by counting the number of green, and brown/white fronds following 1–5 or 11 days of irradiation. Growth of duckweed was impaired by exposure to UV-B radiation in the fall experiment but not in the summer.
3. Fatty acid compositions were analysed following 5 or 11 days of irradiation and a recovery period of 0, 5, 29 or 40 h. Concentrations of the major fatty acids, palmitic, linoleic (LA) and α-linolenic (ALA) acids were similar in the summer and fall duckweed collections, but the summer samples had higher concentrations of the desaturation products of LA and ALA.
4. UV-B exposure had small, but significant, and contrasting effects on duckweed fatty acid concentrations. In the summer experiment, duckweed exposed to UV-B had slightly lower concentrations of major fatty acids than control duckweed, while the reverse was true in the fall experiments.
5. These minor effects of UV-B on concentrations of LA and ALA would be unlikely to have a major impact on the supply of these essential fatty acids from duckweed to freshwater food webs.

Keywords: duckweed, fatty acids, growth, *Lemna minor*, ultraviolet-B radiation

Introduction

The decline in stratospheric ozone has resulted in an increase in ultraviolet-B (UV-B) transmittance. Many studies have been conducted on the possible biological effects on higher plants of enhanced UV-B exposure. Although the results are varied, the most commonly documented impacts of UV-B exposure include damage to DNA, proteins and lipids (Kramer *et al*., 1991; Jordan, 1993; Britt, 1996), alteration in growth and morphology (Sullivan *et al*., 1994; Gonzales *et al*., 1996; Keiller & Holmes, 2001; Jayakumar *et a*l. 2002) and decreased rates of photosynthesis (Mackerness, Jordan & Thomas, 1997; Jayakumar *et al*., 2002; Keiller, Mackerness & Holmes, 2003). Damage to eukaryotic DNA may be photoenzymatically repaired by exposure to ultraviolet-A (UV-A) and photosynthetically active radiation (PAR) (Grad, Burnett & Williamson, 2003).

Kramer *et al.* (1991) found that UV-B exposure decreased the ratio of unsaturated : saturated fatty acids in cucumber (*Cucumis sativus*) and increased lipid peroxidation, and they suggested that membrane lipids may be a target of UV-B damage. Moorthy & Kathiresan (1998) reported a decrease in unsaturated fatty acids and an increase in saturated fatty acids in the thylakoid membranes of *Rhizophora apiculata* Blume in response to UV-B exposure. UV-B exposure has also been shown to induce numerous changes in plant morphology, including increased leaf

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thickness (Sullivan *et al*., 1994), decreased foliage area (Cen & Bornman, 1990; Keiller & Holmes, 2001) and decreased growth rates (Jayakumar *et al*., 2002).

In contrast to the many studies of the effects of UV-B radiation on terrestrial plant species, considerably less information is available regarding the impact of UV-B on aquatic plant species. We were particularly interested in possible effects of UV-B on the production of (n−6) and (n−3) polyunsaturated fatty acids (PUFA) by aquatic plants. Plants produce the C18 parent molecules of both the (n−6) and (n−3) families of fatty acids, namely linoleic acid [LA, 18 : 2(n−6)] and α-linolenic acid [ALA, 18 : 3(n−3)] (Brett & Müller-Navarra, 1997). With few exceptions, animals are unable to insert double bonds into the (n−6) or (n−3) positions in the acyl chain, but many are able to elongate and further desaturate the C18 parent molecules. Among the products of elongation and desaturation are three highly unsaturated fatty acids, arachidonic acid [20 : 4(n−6), ARA], eicosapentaenoic acid [EPA, 20 : 5(n−3)] and docosahexaenoic acid [DHA, 22 : 6(n−3)]. These fatty acids are prominent in membrane phospholipids, with DHA being particularly important in neural tissue. ARA and EPA also serve as precursors for eicosanoids, a diverse family of chemical messengers. Either the products of elongation and desaturation or the C18 parents can be considered essential in animal diets (Cook, 1985; Arts, Ackman & Holub, 2001). Any impairment by UV-B of production of (n−6) and (n−3) fatty acids by primary producers is thus relevant to understanding the implications of increased UV-B irradiation for aquatic communities.

The Lemnaceae, commonly known as duckweeds, are small free-floating flowering plants found in freshwater ponds and streams throughout temperate latitudes (Landolt, 1986). *Lemna minor* can proliferate rapidly to cover the surface of ponds, restricting light penetration to photosynthetic organisms lower in the water column. Duckweeds are important aquatic macrophytes as a food source for mammals, birds, fish and gastropods (Landolt, 1986) in wastewater and effluent treatment (Hasar, 2002; Tripathi & Upadhyay, 2003), and as feed for animals and fish (Bairagi *et al*., 2002). As a floating mat, duckweed is subject to full sun exposure, yet little is known about the sensitivity of these species to UV-B. The purpose of this investigation was to determine the effects of elevated levels of artificial UV-B radiation on *L. minor*. The parameters studied were growth, health of the fronds and fatty acid content.

Materials and methods

Duckweed

Lemna minor was collected from a shallow pond at Fort Whyte Centre, Winnipeg, MB to establish indoor cultures on 17 May 2004 (experiment 1) and 15 September 2004 (experiments 2a and 2b). Duckweed was cultured in aquaria containing dechlorinated water supplied with nutrients in the form of fish fertiliser (Alaska® Fish Fertilizer (Alaska Fish Fertilizer Co., Renton, WA, U.S.A.): 5% N, 2% P_2O_5, 2% K_2O, 40% organic matter). The plants were maintained at room temperature on a 14-h light (08:00–22:00) : 10-h dark cycle.

Incubation chambers

Each incubation chamber consisted of a platform suspended under UV-A (Philips F40 T12/BL), UV-B (Philips TL 40W/12RS, peak emission approximately 312 nm) (Philips Electronics Ltd, Markham, ON, Canada) and PAR (GE F48T12 CWHO) (General Electric Co., Mississauga, ON, Canada) light bulbs. The UV-B bulb was loosely wrapped with preburned cellulose acetate to filter out the shorter wavelengths that do not normally reach the surface of the earth (Grad *et al*., 2003). UV-A and PAR irradiances under our laboratory conditions were substantially lower while the UV-B irradiance used in these experiments was higher than outdoor conditions (measured 26 July 2004 in Winnipeg, MB, latitude 49.9°N, longitude 97.23°W) (Table 1). Moreover, our UV-B bulb emits a higher proportion of more damaging, shorter

Table 1 PAR, UV-A and UV-B irradiances used in laboratory exposures of duckweed compared to natural levels in Winnipeg

	Irradiance	
Radiation	Lab	Outside
PAR (W m^{-2})	14.2	403
UV-A (W m^{-2})	14.2	39.8
UV-B (μW cm^{-2})	19.8	15.5

UV-B measurements are integrated over the UV-B range and do not imply equivalent spectral compositions in laboratory and natural exposures.
PAR, photosynthetically active radiation.

wavelength photons than would be experienced under natural conditions (Dattilo *et al.*, 2005). The fact that laboratory levels of PAR and UV-A (3.5% and 36% respectively) were much lower than natural exposure is important because the combination of PAR and UV-A (designated photorecovery radiation, PRR) can mitigate adverse effects of UV-B by stimulating photoenzymatic repair (Grad *et al.*, 2003). Thus, our results reflect responses to conditions involving exposure to more biologically damaging UV-B radiation and lower PRR than that experienced in natural environments.

Irradiances were determined with a model PMA2100 light meter (Solar Light Co. Inc., Glenside, PA, U.S.A.) equipped with a biologically weighted integrated sensor for UV-B (PMA2104) (280–320 nm) and with integrated sensors for UV-A (PMA2111) (320–400 nm) and PAR (PMA2132) (400–700 nm).

Incubation conditions

In all experiments, control (PAR + UV-A) dishes were covered with Mylar® (type D from Grafix® Plastics, Grafic Art Systems Inc., Cleveland, OH, U.S.A.) to reduce exposure to UV-B wavelengths (Williamson *et al.*, 2001) and experimental dishes were covered with preburned cellulose acetate to exclude the shorter wavelengths of UV-B radiation (Grad *et al.*, 2003). All plants received PAR 14 h day^{-1} (08:00–22:00) and either UV-A (control) or UV-A and UV-B (experimental) for 6 h day^{-1} (11:00–17:00). Dishes were rotated periodically, both within and among chambers, to ensure equal exposure. Additional medium was added to the dishes as required to maintain constant culture volume.

In experiment 1, 20 healthy fronds were transferred from the stock culture to small Petri dishes containing WC′ (Wright and Chu's) media (10 control and 10 experimental dishes for each of days 1–5) and placed in the incubation chambers. Following the final day of irradiation, each group of fronds received a recovery period of 40 h of 14 h PAR : 10 h dark prior to sampling to allow sufficient time for any adverse effects of UV-B to become apparent. After the 40-h recovery period, plants were removed from the incubation chambers. The total number of healthy (green) fronds and the number of 'unhealthy' fronds, defined as greater than one half the surface appearing brown or white, were counted. Fronds were then air dried in the dark for 72 h and weighed. For fatty acid analysis in experiment 1, larger dishes of duckweed were incubated in the same manner as those used for measurement of growth. Because of the large number of incubates in this experiment and the restricted size of the chamber, this experiment was run in several iterations during late July and August 2004.

In experiment 2, 20 healthy fronds were transferred from the stock culture to small Petri dishes containing WC′ media and placed in the incubation chambers. Duckweed was exposed to 5 days of control or experimental radiation beginning 30 October 2004 (experiment 2a), or 11 days of radiation beginning on 26 November 2004 (experiment 2b). In experiments 2a and 2b, the numbers of green and brown/white fronds were recorded daily for each dish immediately prior to UV-B or control irradiation. For fatty acid analysis in experiment 2, larger dishes of duckweed were sampled at 0, 5, 29 and 40 h post-UV-B at the end of the experiment. During the recovery periods, the plants were on a 14-h PAR : 10-h dark cycle.

For analysis of growth data, numbers of green or brown/white fronds were log transformed and used as the dependent variable in an ANCOVA model. As some of the dishes had zero brown/white fronds, the log transformation was done on the number of brown/white fronds +1. Treatment (control or experimental) was the independent variable and day of incubation was the covariate. Day 1, where the number of fronds in each dish was fixed at 20, was excluded from the ANCOVA. Data were pooled for experiments 2a and 2b and month of the experiment was an additional independent variable in the ANCOVA.

Fatty acid analysis

Duckweed taken from the large dishes was washed with approximately 1 L of double distilled water (ddH_20) and given several rinses. Excess water was removed under gentle aspiration during which the duckweed was mixed gently to ensure uniform removal of water. Duplicate or triplicate samples (0.5 g) were placed in 5 mL isopropanol containing 0.01% butylated hydroxytoluene (BHT; Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) and 0.5 mL of internal standard (triheptadecanoin; Sigma – approximately 0.5 mg mL^{-1} $CHCl_3$) was added. Preliminary work showed heptadecanoic acid to comprise < 0.5% of total fatty acid in our duckweed. Samples were stored at −20 °C under N_2.

Samples were combined with a 1 mL of isopropanol (+BHT) rinse of the storage tubes and ground in a glass homogeniser. After grinding, the homogenate was placed into a digestion tube and the homogeniser was rinsed with a further 2 mL of isopropanol (+BHT), which was also added to the digestion tube. Solvents were evaporated at 60 °C with a stream of N_2. The samples were resuspended in 2 mL of 4% H_2SO_4 in methanol, incubated at 80 °C for 1 h and then cooled to 4 °C. The digests were diluted with 2 mL of ddH_20 and vortexed, after which 2 mL hexane was added. Digests were vortexed again and the phases were separated using a clinical centrifuge. Supernatants were stored under N_2 at −20 °C.

The organic extracts were cleaned on silica gel columns prepared in petroleum ether and washed with 10 mL of dichloromethane : methanol (19 : 1) (Wiegand *et al.*, 2004) followed by 5 mL of hexane. The sample was applied to the column, followed by 3 mL of hexane. $CHCl_3$ (1 mL) containing 0.2% BHT was added to the eluate which was diluted with chloroform to tube volume (15 mL) and stored under N_2 at −20 °C. Integrity of the fatty acid methyl esters (FAME) was verified by thin layer chromatography (Wiegand & Idler, 1982).

Fatty acid methyl esters were then analysed by capillary gas chromatography (Moodie *et al.*, 1989) and the data analysed using Varian Star 5.52 software (Varian Inc., Palo Alto, CA, U.S.A.). Duplicate chromatograms were obtained for each sample. Fatty acids of interest were identified on the basis of comparison of retention times with a secondary standard (cod liver oil) and by spiking with authentic standards. Duckweed fatty acid contents were determined by comparison of peak areas with that of the internal standard. Fatty acid data were analysed either by full factorial two-way analysis of variance (ANOVA) with treatment and sample time as independent variables and duckweed fatty acid contents as dependent variables or by linear regression. The software package used was SPSS 10.1 (SPSS Inc., Chicago, IL, U.S.A.).

Results

Growth and health of fronds

In experiment 1, the growth of fronds was similar in control and experimental groups. There were no significant differences in the number of green fronds between the control plants and plants exposed to UV-B for 1–5 days (Fig. 1a). Numbers of brown/white fronds were higher in incubates that received 5 days of control radiation than in UV-B-treated incubates, resulting in a significant treatment effect (Fig. 1b, Table 2). The covariate, incubation duration, was significant for both the green and the brown/white fronds. The dry mass of fronds was unaffected by treatment (data not shown).

In experiments 2a and 2b, UV-B treatment inhibited growth of duckweed (Fig. 2a,b). Growth rates in the laboratory differed between experiment 2a (October) and experiment 2b (November) (Table 3). Numbers of brown/white fronds were significantly higher in the UV-B-treated dishes in both experiments 2a and 2b (Fig. 2c,d, Table 3).

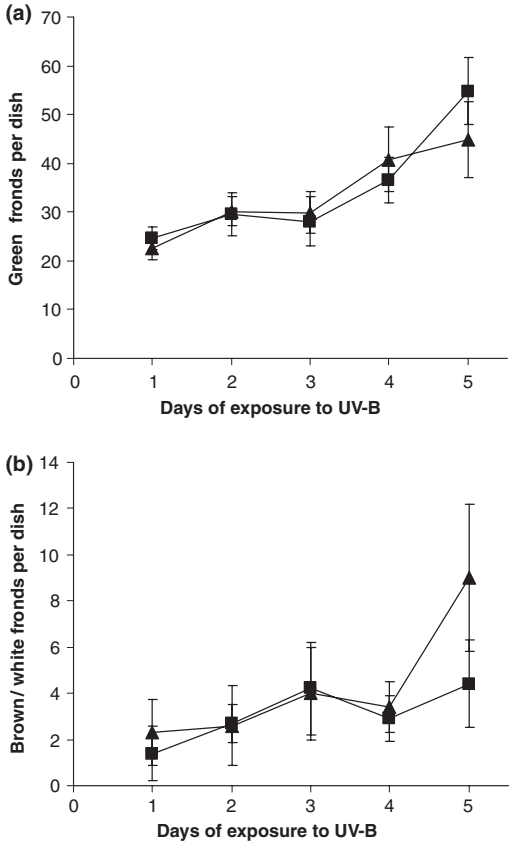

Fig. 1 Numbers of green (a) and brown/white (b) fronds (±SE) in duckweed exposed to control (PAR + UV-A) radiation (triangles) or experimental (control plus UV-B) radiation (squares) for 1–5 days (experiment 1). Fronds were counted after 40 h recovery following exposure to control or experimental radiation ($n = 10$ for each observation). PAR, photosynthetically active radiation.

Table 2 ANCOVA analysis of log-transformed numbers of green and brown/white fronds in experiment 1

		Green fronds		Brown/white fronds	
Source	d.f.	*F*	*P*	*F*	*P*
Day (covariate)	1	217	<0.001	49.2	<0.001
Treatment	1	0.333	0.565	4.464	0.037
Error	97				
Total	99				

Growth rates are not directly comparable between experiments because in experiment 1, fronds were counted 40 h after final exposure to UV-B, while in experiment 2, fronds were counted at the onset of exposure each day.

Fatty acid concentrations

Concentrations of three major fatty acids, palmitic acid (PA, 16 : 0), LA [18 : 2(*n*−6)] and ALA [18 : 3(*n*−3)] were similar in control incubates from the summer and fall duckweed collections (Fig. 3). In contrast, concentrations of the two major Δ^6 desaturation products of LA and ALA, 18 : 3(*n*−6) and 18 : 4(*n*−3) respectively, were much higher in the summer collection than in the fall. Other fatty acids routinely found in duckweed included stearic acid (18 : 0) and monounsaturated C16 and C18 fatty acids.

In experiment 1, mean levels of PA, LA and ALA were generally higher in control duckweed than in those exposed to UV-B (Fig. 4). For each fatty acid two-way ANOVA revealed significant treatment effects (Table 4). 'Day' effects were significant for PA and LA as were day × treatment interactions. Contents of the three major fatty acids did not vary according to linear regression models in this experiment.

Contents of one monounsaturated fatty acid, palmitoleic acid [PlA, 16 : 1(*n*−7)] increased dramatically over the latter portion of the experiment in control plants and, to a lesser extent, in those exposed to

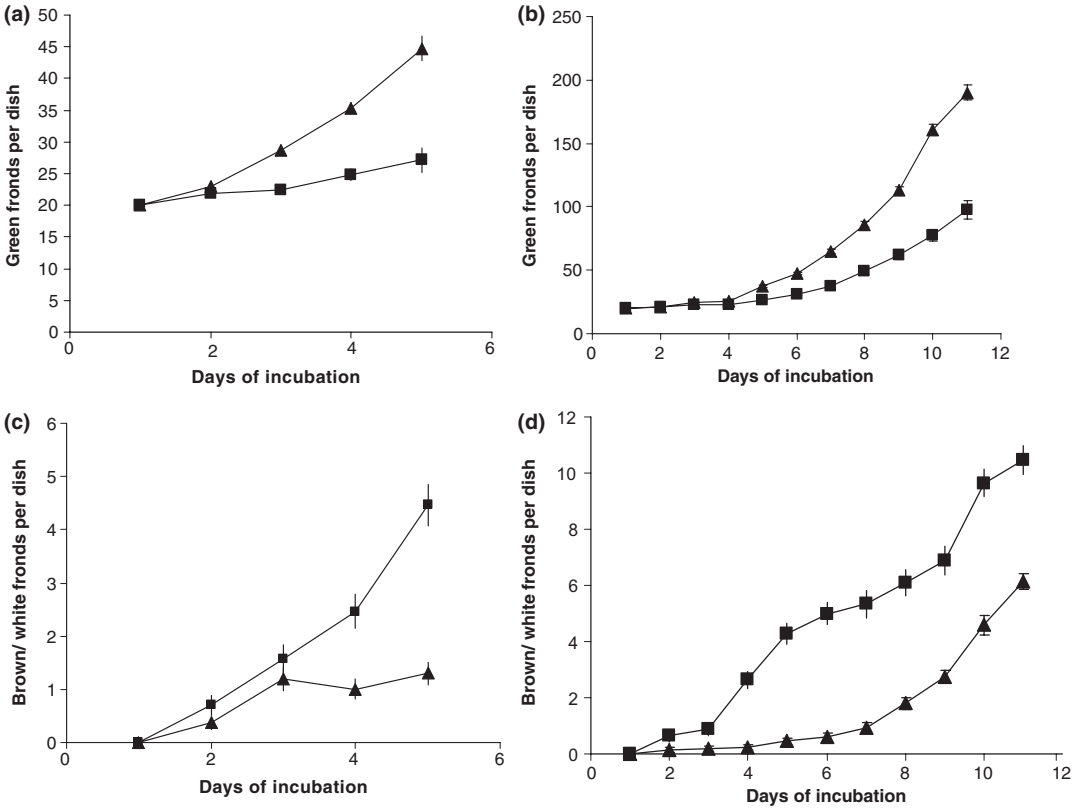

Fig. 2 Numbers of green (a,b) and brown/white (c,d) fronds (±SE) in experiments 2a (a,c) and 2b (b,d). Triangles, control incubations; squares, experimental incubations. Fronds were counted daily, immediately prior to exposure to control or experimental radiation ($n = 29$ or 30 for each observation).

Table 3 ANCOVA analysis of log-transformed numbers of green and brown/white fronds in experiment 2a (October) and experiment 2b (November)

		Green fronds		Brown/white fronds	
Source	d.f.	*F*	*P*	*F*	*P*
Day (covariate)	1	3324	<0.001	1084	<0.001
Experiment (2a, 2b)	1	15.7	<0.001	41.7	<0.001
Treatment	1	337	<0.001	286	<0.001
Experiment × treatment	1	11.2	0.001	27.7	<0.001
Error	825				
Total	829				

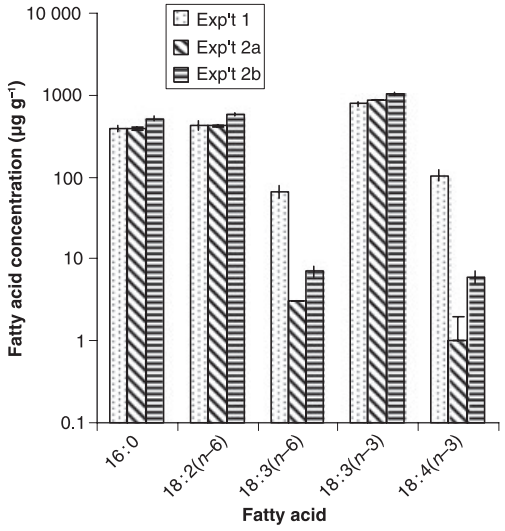

Fig. 3 Comparison of concentrations of major fatty acids and of 18 : 3(*n*−6) and 18 : 4(*n*−3) (±SD) in duckweed 40 h after 5 days of control radiation (experiments 1 and 2a), and 40 h after 11 days of control radiation (experiment 2b) ($n = 2$ or 3 for each observation).

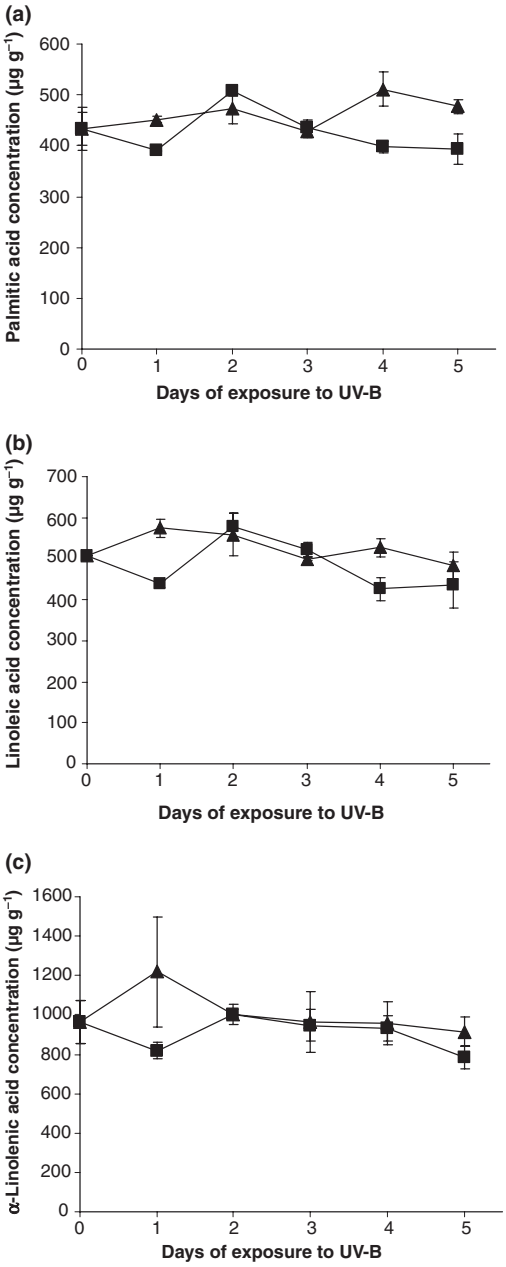

Fig. 4 Concentrations of palmitic acid (a), linoleic acid (b) and α-linolenic acid (c) (±SD) in duckweed exposed to control or experimental radiation for up to 5 days in experiment 1. Samples were taken after a 40 h recovery period. Triangles, control incubations; squares, experimental incubations ($n = 2$ or 3 for each observation).

UV-B (Fig. 5). This result was not observed in other minor fatty acids in the summer duckweed (data not shown).

In experiment 2, we investigated the response of the duckweed during the 40 h immediately post-UV-B treatment. The 40-h sample in the 5-day trials of experiment 2a (October) was thus most comparable to the 5-day sample in experiment 1. Results for PUFA were similar for the two trials of experiment 2. In both cases, fatty acid contents were higher in UV-B-exposed fronds than in controls (Fig. 6). In all cases, there were significant effects of both treatment and time, and in no case in either experiment was the time × treatment interaction significant (Table 5).

In experiment 2a, concentrations of three minor fatty acids decreased significantly with time during the 40-h recovery period in both control and experimental duckweed, with the exception of oleic acid

Table 4 Two-way ANOVA analysis of major fatty acid contents in experiment 1

		Fatty acid					
		Palmitic acid 16 : 0		Linoleic acid 18 : 2(*n*−6)		α-Linolenic acid 18 : 3 (*n*−3)	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Day	4	9.67	<0.001	9.51	<0.001	1.92	NS
Treatment	1	29.6	<0.001	14.6	0.001	6.99	0.016
Day × treatment	4	13.2	<0.001	5.91	0.003	2.50	NS
Error	18						
Total	27						

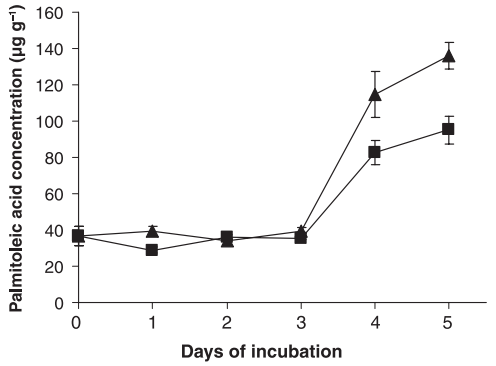

Fig. 5 Concentrations of palmitoleic acid [16 : 1(*n*−7)] (±SD) in duckweed exposed to control or experimental radiation for up to 5 days in experiment 1. Samples were taken after a 40 h recovery period. Triangles, control incubations; squares, experimental incubations ($n = 2$ or 3 for each observation).

[18:1(*n*−9)] in control fronds (Table 6). In that case, there was decrease from 74 to 24 µg g^{-1} in the first 5 h of the recovery period, followed by an increase to about 36 µg g^{-1} in the latter two samples. While PlA [16 : 1(*n*−7)] conforms tightly to a linear regression model in experiment 2a, the other minor fatty acids conform less strongly (Table 6). Examination of scatter plots of minor fatty acids from experiment 2a showing declines during the recovery period, demonstrates that the majority of the decline, in both control and experimental duckweed, occurred during the first 5 h of the recovery period (data not shown).

In experiment 2b (November), there was a linear increase in the contents of the minor fatty acids in the control samples during the recovery period (Table 6). Only PlA showed a similar response in the experimental fronds.

Discussion

The duckweed samples were collected from the same pond population in May for experiment 1 and in September for experiment 2. The PAR, UV-A and UV-B irradiances used in the lab for both experiments were similar. Comparing absolute growth rates between experiments 1 and 2 is difficult however, given that the plant material was collected at different times and therefore, the plants were acclimatised to very different natural irradiation conditions.

Artificial UV-B exposure did not affect growth of summer duckweed but, using identical incubation conditions, significantly reduced growth in both experiments using duckweed collected in the fall. Summer duckweed also had much higher contents of the Δ^6 desaturation products of LA and ALA. This was surprising as the advantages of production of more highly unsaturated PUFA would presumably be greater in the fall in order to enhance homeoviscous adaptation to cooler temperatures. Summer duckweed would have experienced higher natural UV-B irradiance in the source pond than the fall samples and was clearly more resistant to UV-B. The mechanism by which this resistance occurs in duckweed has not been determined. In general, mechanisms of such resistance involve shielding by pigments (Day & Vogelmann, 1995) and repair of the damage caused by UV-B (Britt, 1996). Determining whether the resistance shown by the summer duckweed is a true seasonal effect or merely a stochastic one is the subject of ongoing investigation.

Unlike duckweed, freshwater algae contain substantial quantities of more highly unsaturated fatty acids, including EPA [20 : 5(*n*−3)] and DHA [22 : 6(*n*−3)] (Wang & Chai, 1994; Weers & Gulati, 1997). In algae exposed to UV-B, the most adverse effects of exposure were frequently on the production of the more highly unsaturated (*n*−3) PUFA, 16 : 4(*n*−3), EPA, and DHA (Goes *et al.*, 1994; Wang & Chai, 1994; de Lange & van Donk, 1997). In some cases, UV-B exposure reduced contents of these fatty acids by 90% or more, and also reduced the contents of ALA in some species. Reduction in algal (*n*−3) PUFA production as a result of UV-B exposure can disrupt the assembly of chloroplast membranes and thereby affect growth (Goes *et al.*, 1994). Compared to the situation in algae, effects of UV-B on production of the major duckweed fatty acids are much more subtle.

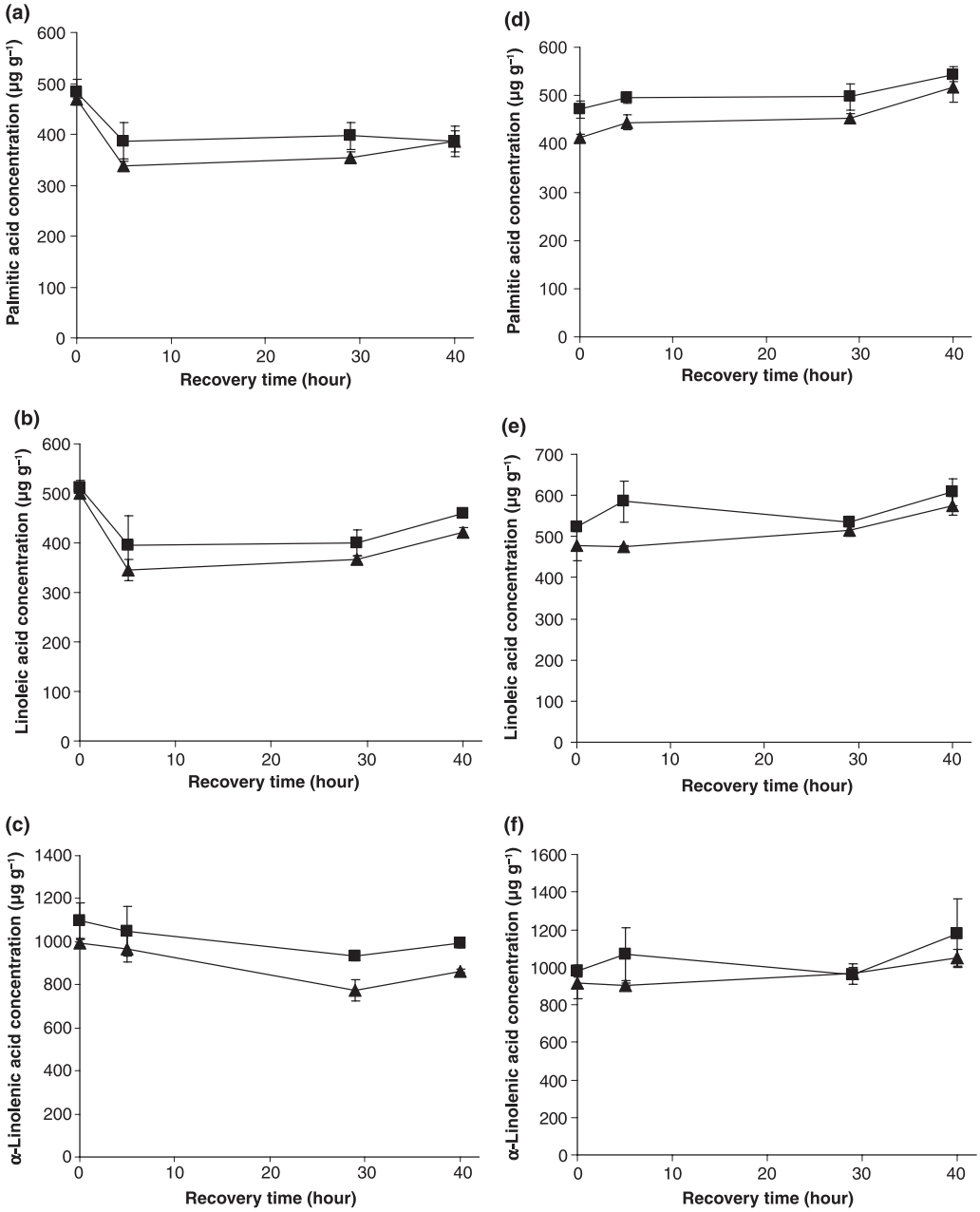

Fig. 6 Concentrations of palmitic acid (a,d), linoleic acid (b,e) and α-linolenic acid (c,f) (±SD) in duckweed exposed to control or experimental radiation in experiments 2a (a–c) and experiment 2b (d–f). Duckweed was irradiated for 5 days in experiment 2a and 11 days in experiment 2b. Samples were taken at intervals during the 40 h after final exposure to control or experimental radiation. Triangles, control incubations; squares, experimental incubations ($n = 2$ or 3 for each observation).

In both experiments 1 and 2, there were significant effects of both sampling time and UV-B treatment on contents of the three principal fatty acids. While sampling time had a significant influence on major fatty acid contents, for the majority of cases, the relationships did not fit linear regression models. The sampling time effects may have been due in part to the fact that the samples consisted of both young and older fronds. As the activity of enzymes involved in fatty acid synthesis declines with tissue age (Konishi *et al.*, 1996), the concentrations of individual fatty acids could be influenced by the relative abundance of older and younger fronds. As well as rates of synthesis, turnover of existing fatty acids will

Table 5 Two-way ANOVA analysis of major fatty acid contents in experiments 2a (October) and 2b (November)

		Fatty acid					
		Palmitic acid 16 : 0		Linoleic acid 18 : 2(*n*–6)		α-Linolenic acid 18 : 3(*n*–3)	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Experiment 2a							
Recovery time	3	28.4	<0.001	42.3	<0.001	16.1	<0.001
Treatment	1	8.35	0.012	14.9	0.002	31.4	<0.001
Time × treatment	3	1.52	NS	0.649	NS	0.577	NS
Error	14						
Total	21						
Experiment 2b							
Recovery time	3	23.9	<0.001	11.4	<0.001	3.81	0.033
Treatment	1	34.7	<0.001	20.2	<0.001	4.95	0.042
Time × treatment	3	0.726	NS	2.56	NS	0.873	NS
Error	15						
Total	22						

Table 6 Linear regression analysis of changes in concentrations of three minor fatty acids in duckweed during the 40 h recovery period after control or UV-B exposure for 5 days (experiment 2a) or 11 days (experiment 2b)

Experiment	Fatty acid	Treatment	Intercept $t = 0$ h μg g^{-1}	Slope μg g^{-1} h^{-1}	d.f.	*F*	r^2	*P*
2a	16 : 1(*n*–7)	Control	35.5	−0.442	1,10	22.1	0.689	0.001
	16 : 1(*n*–7)	Experimental	33.9	−0.392	1,8	20.2	0.716	0.002
	18 : 0	Control	30.0	−0.341	1,10	12.4	0.554	0.006
	18 : 0	Experimental	31.9	−0.340	1,8	11.2	0.582	0.010
	18 : 1(*n*–9)	Control			1,10	2.395		NS
	18 : 1(*n*–9)	Experimental	58.2	−0.778	1,8	9.43	0.541	0.015
2b	16 : 1(*n*–7)	Control	32.3	+0.970	1,9	104	0.920	<0.001
	16 : 1(*n*–7)	Experimental	50.9	+1.16	1,10	43.3	0.812	<0.001
	18 : 0	Control	22.6	+0.227	1,9	12.6	0.583	0.006
	18 : 0	Experimental			1,10	0.632		NS
	18 : 1(*n*–9)	Control	24.1	+0.612	1,9	21.2	0.702	0.001
	18 : 1(*n*–9)	Experimental			1,10	0.191		NS

influence concentrations measured at particular times. The fatty acid content expressed on a per unit mass basis will also be affected to a minor extent by variability in mass of other cellular contents. For example, in another *Lemna* species, the total of starch and protein content was found to vary between about 7% and 11% of wet weight (Farooq *et al*., 2000).

Effects of UV-B treatment on major fatty acid concentrations in the duckweed of experiments 1 and 2 were contrasting. In experiment 1, UV-B treated duckweed had lower major fatty acid concentrations while the reverse was true in the two trials of experiment 2. In both cases, the differences, while significant, were small in magnitude and thus unlikely to have major impact on the dietary value of duckweed from the perspective of fatty acid concentrations alone. Our results are consistent with experiments on some terrestrial plants where exposure to UV-B had only small, if any, effects on fatty acid profiles (Kramer *et al*., 1991; Predieri *et al*., 1995). In those studies, UV-B did cause an increase in concentrations of malondialdehyde, an indicator of fatty acid peroxidation. It remains to be determined whether that metabolic response to UV-B exposure or others, such as induction of synthesis of protective flavonoids or other phenolic compounds (Rozema *et al*., 1997), also occur in duckweed. Metabolic responses that adversely influence the palatability

or digestibility of aquatic plants can be expected to alter the passage of essential fatty acid to higher trophic levels, regardless of fatty acid contents of the plant.

In all experiments, rapid changes in concentrations of minor fatty acids were observed. These fatty acids were present in about 1/10 of the amounts of the major fatty acids and showed much higher proportional change during the experiments than did the three major fatty acids. Our results are similar to findings in algae where turnover of the less unsaturated fatty acids can be very rapid (Goes *et al*., 1994; Wang & Chai, 1994). The rapid changes in concentrations could be caused by changes in rates of synthesis, rates of subsequent desaturation of newly synthesised oleic acid to LA or ALA, rates of catabolism for energetic purposes or a combination of all three.

In experiment 1, the most important change in the minor fatty acids was the increase in levels of PlA in the latter 2 days of the incubations, an increase that was attenuated by UV-B. Fatty acid synthesis will normally produce PA and/or stearic acid as primary end products. PlA can be produced as a Δ^9 desaturation product of palmitate, although stearic acid is the more common substrate for this desaturation (Harwood, 1988). The increase in PlA is likely the accumulation of a metabolic reserve, as this fatty acid is not further desaturated in significant amounts. Accumulation of a reserve, along with high resistance to UV-B and the presence of 18 : 3(n−6) and 18 : 4(n−3) indicate a higher level of fitness of the summer duckweed, compared to that collected in the fall.

In experiment 2, significant changes in concentrations of minor fatty acids were generally similar in control and UV-B-treated duckweed, indicating a cause other than UV-B radiation. During recovery periods following control exposure or UV-B exposure, duckweed received PAR but no UV-A radiation. The absence of UV-A thus cannot be eliminated as a possible contributor to the observed changes in minor fatty acid levels. In experiment 2a, minor fatty acid concentrations dropped during the first 5 h of recovery and then largely stabilised whereas in experiment 2b concentrations, most notably of PIA, increased throughout the recovery period. The reason for this discrepancy between experiments 2a and 2b in the dynamics of minor fatty acids is unclear.

In conclusion, summer duckweed was more resistant to artificial UV-B radiation than fall duckweed collected from the same pond, although further study will be required to determine if this is a true seasonal effect or merely a stochastic effect. The summer duckweed also had much higher concentrations of the Δ^6 desaturation products of LA and ALA. UV-B had significant, but contrasting effects on concentrations of PUFA that are essential in animal diets. Compared to the situation in algae, the effects of UV-B on PUFA concentrations were found to be minimal, despite the fact that our UV-B irradiation conditions were potentially much more damaging than duckweed would experience in a natural setting. Our study indicates that UV-B would not have a major deleterious effect on the supply of essential PUFA from duckweed to freshwater food webs.

Acknowledgments

We acknowledge the financial support of Manitoba Hydro and the University of Winnipeg. We thank Joanna Selby for developing the fatty acid analytical technique, and Samantha Kasloff and Natalka Manastersky for technical assistance with the duckweed cultures. Jeff Babb, Department of Mathematics and Statistics, University of Winnipeg, provided generous assistance with statistical procedures.

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(Manuscript accepted 1 August 2006)