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Short-term response and recovery of *Zostera capricorni* photosynthesis after herbicide exposure

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Abstract

We used photosynthetic activity (measured as chlorophyll *a* fluorescence) and photosynthetic pigment concentrations to assess the effect of pulsed exposure to catastrophic levels of the herbicides Atrazine, Diuron and Irgarol 1051 on the seagrass *Zostera capricorni* Aschers. in laboratory and field experiments. Custom-made in situ chambers were developed so seagrasses could be dosed within the meadow. *Zostera capricorni* was exposed to 10 and 100 $\mu g\,l^{-1}$ herbicide solutions for 10 h. During this time and for the subsequent 4-day recovery period, chlorophyll *a* fluorescence parameters (maximum quantum yield: F_v/F_m and effective quantum yield: $\Delta F/F'_m$) were measured. Laboratory samples exposed to these herbicides were severely impacted during the exposure period and most treatments did not recover fully. $\Delta F/F'_m$ was a more sensitive indicator of herbicide impact than F_v/F_m. In situ samples were also severely impacted by Irgarol and Diuron exposure whereas samples recovered completely after exposure to Atrazine at the same concentrations as the laboratory experiments. Total chlorophyll concentrations showed only limited impact in both laboratory and field situations. This study suggests that laboratory experiments may overestimate the on-going impact of herbicides on seagrass.

Keywords: *Zostera capricorni*; Atrazine; Diuron; Irgarol 1051; Photochemistry; Pollution effects; Chlorophyll *a* fluorescence

1. Introduction

Herbicides, such as Diuron and Atrazine, enter adjacent freshwater ecosystems from agricultural fields, making their way to estuarine areas by spray drift, leaching, run-off or accidental spills (Van den Brink et al., 1997). Antifoulant chemicals such as Irgarol

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1051 and Diuron are used as biocides on boat hulls (Thomas et al., 2000). The impact of these chemicals on seagrass physiology is poorly understood (Leadbitter et al., 1999), furthermore, toxicity testing using estuarine plants is in it's infancy (Lytle and Lytle, 2001).

The *N*-phenylurea herbicide Diuron (DCMU) is predominantly used for general weed control in non-crop and agricultural areas (Voulvoulis et al., 1999). Diuron reversibly inhibits photosynthetic electron flow to the plastoquinone in photosystem II (PSII) by blocking the electron transport chain just after the primary electron acceptor (Q_A) (Miles, 1991; Falkowski and Raven, 1997). This process causes a simultaneous decrease in photochemical and non-photochemical quenching (Brack and Frank, 1998). Diuron contamination is of particular concern in nearshore environments in Queensland, where sediment concentrations can reach as high as $10\,\mu g\,kg^{-1}$ adjacent to sugarcane production areas (Haynes et al., 2000a).

Atrazine is commonly used as a low-cost weed control agent on a variety of crops (Solomon et al., 1996; El Jay et al., 1997) and has been implicated in the decline of *Zostera marina* abundance throughout Chesapeake Bay (Dennison et al., 1993). Atrazine inhibits photosynthesis by blocking the reoxidation of Q_A, similar to urea herbicides (Solomon et al., 1996), hence, the action of these herbicides is not species-specific (Cobb, 1992). Extended exposure to Atrazine results in damage to isolated chloroplasts in the presence of light since it acts as a blocker in the photosynthetic chain. In the absence of light, Atrazine will not harm plants (Solomon et al., 1996).

Atrazine occurs in estuaries dissolved in the water-column or is adsorbed onto suspended sediment and colloidal particles and uptake into seagrass tissue occurs through both the leaves and roots (Schwarzschild et al., 1994; Mersie et al., 2000). Water concentrations have been found as high as $17\,\mu g\,l^{-1}$ in rivers immediately after herbicide treatments (El Jay et al., 1997) and runoff concentrations have been reported as high as $1\,mg\,l^{-1}$ (Schwarzschild et al., 1994). Mersie et al. (2000) reported an average of $36\,\mu g\,l^{-1}$ Atrazine in Recharge Lake, USA but average levels in lakes tend to be between 0.1 and $1\,\mu g\,l^{-1}$ (Schwarzschild et al., 1994). Seagrass growth and productivity have been reduced by concentrations as low as 5–$10\,\mu g\,l^{-1}$ for an exposure period of five or more weeks (Schwarzschild et al., 1994).

The triazine herbicide, Irgarol 1051 has been used increasingly to further the effectiveness of antifouling paints, after the restricted use of tributyltin (Hall et al., 1999; Voulvoulis et al., 1999). There is limited monitoring data available for this chemical (Voulvoulis et al., 1999), but due to the close proximity of marina areas to seagrass beds, this chemical has been accumulated by marine angiosperms such as *Z. marina* (Scarlett et al., 1999a) and *Zostera capricorni* (up to $118\,ng\,g^{-1}$) (Scarlett et al., 1999b). Growth rate of *Z. marina* was reduced by concentrations of Irgarol at and above $10\,\mu g\,l^{-1}$ and *Z. marina* photosynthetic efficiency was significantly reduced (about 10%) at $0.18\,\mu g\,l^{-1}$ and a had 10-day EC50 value of $2.5\,\mu g\,l^{-1}$. Estuaries in SW England, where the assessment was carried out, have $<0.003\,\mu g\,l^{-1}$ Irgarol but concentrations in *Z. marina* leaf tissue were up to 25 000 times this amount which was only 15 times lower than the 10-day EC50 value (Scarlett et al., 1999a).

Due to the ongoing input of these three herbicides into coastal waters, more information on their impact on seagrasses is required. In a laboratory comparison of four herbicides, Ralph (2000) found that *H. ovalis* showed a decreasing sensitivity range: Diuron > Atrazine > Simazine > Glyphosate, the variety of sensitivities being attributed to the variation in

plant tissue impact sites and uptake rates. Graney et al. (1995) stress the importance of validating toxicology data in the field, since the chemicals and biological matter may behave differently in the environment. Correll and Wu (1982) assessed the impact of Atrazine on *Z. marina* oxygen production in mesocosms and found photosynthesis was stimulated by 75 $\mu g\,l^{-1}$ Atrazine but inhibited by 650 $\mu g\,l^{-1}$ Atrazine. Nonetheless, Huber (1993) notes that results from laboratory or model ecosystem studies cannot be directly applied to the situation in the field. To date there has been no noted manipulative investigation of the impact of herbicides on in situ seagrasses. Solomon et al. (1996) highlight the need for research into the response of aquatic organisms to pulsed exposures and recovery periods at concentrations representative of a catastrophic event to model the effect of herbicide release after a rain event. Bowmer (1986) also highlighted the importance of determining the ability of an ecosystem to recover after exposure to temporarily high concentrations of a pollutant.

In this study, seagrasses were subjected to a short-term exposure (10 h) to three herbicides. A significant stress response was defined as a reduction in the photosynthetic efficiency of photosystem II, measured with chlorophyll *a* fluorescence (see Schreiber et al., 1994; Maxwell and Johnson, 2000).

In recent years, there has been considerable advancement in the application and interpretation of chlorophyll *a* fluorescence in plant physiology, ecophysiology and ecology studies (e.g. Schreiber et al., 1994). The pulse amplitude modulated (PAM) measuring system provides a quantitative, non-invasive and rapid technique which is particularly convenient for field studies. Furthermore, with the development of the Diving-PAM fluorometer, in situ monitoring of aquatic plants has become possible (e.g. Ralph et al., 1998; Beer and Björk, 2000).

The specific aims of this study were to: (i) determine the impact of 1-day pulsed exposure to a short-term catastrophic concentration of Atrazine, Diuron and Irgarol (10 and 100 $\mu g\,l^{-1}$ doses) on *Z. capricorni* photosynthesis; (ii) determine whether field and laboratory based experiments provide a similar outcome; (iii) study photosynthetic recovery in *Z. capricorni* after a 1-day exposure to three herbicides, by comparing photosynthetic efficiency and chlorophyll pigments and; (iv) determine the effectiveness of $\Delta F/F'_{\mathrm{m}}$ as an indicator of herbicide impact.

2. Methods

2.1. Culturing samples

Sprigs of *Z. capricorni* (including healthy leaves and about 15 cm of roots and rhizome) were removed from Paradise Beach, NSW, Australia (151°19′E, 33°35′S), transported to the laboratory and washed free of sediment. Four to five sprigs were planted in plastic tubs (175 mm × 120 mm × 55 mm) containing terrestrial sandy loam. The surface was sprinkled with washed sand and tubs were placed in 50 l aquaria under ∼150 μmol photons $m^{-2}\,s^{-1}$. The light cycle was 16 h light, 8 h dark. These aquaria were part of a re-circulating system including a pump (Vortex elite, 2/4000sp, pumping water into the tanks at $1.9 \pm 0.05\,l\,min^{-1}$), activated carbon filter (Eheim, Germany) and a salinity control unit (TPS Minichem, Brisbane). The salinity was maintained at 35 ± 0.4 ppt and the temperature

was 25 ± 1 °C. Samples were cultured in this system for 2 months before being used in the experiments. By this time, the samples were acclimatized and healthy leaf growth was occurring.

2.2. Laboratory experiments

Six 10 l tanks (washed with anionic detergent and 2 M HNO_3 and RO water rinsed twice) were filled with filtered (0.45 μm) seawater and one plastic tub of cultured *Z. capricorni* was placed in each tank under a ~150 μmol photons m^{-2} s^{-1} light at 08:00 h on day 1. The first fluorescence measurements (effective quantum yield, $\Delta F/F'_m$ and maximum quantum yield, F_v/F_m; Van Kooten and Snel, 1990) were then taken for two leaves in each tub with a Diving-PAM (Walz GmbH, Effeltrich, Germany). The calculation of $\Delta F/F'_m$ involves determining the ratio of variable fluorescence in a light-adapted state (ΔF) to maximum fluorescence in a light-adapted state (F'_m), where ΔF is the difference between minimum fluorescence at time t (F_t) and the maximum fluorescence in a light-adapted state (F'_m, measured on application of a saturation pulse which oxidizes the reaction centers). F_v/F_m is dark-adapted equivalent of $\Delta F/F'_m$, where F_v is the difference between F_0 (minimum fluorescence) and F_m (maximum fluorescence) (Van Kooten and Snel, 1990; Schreiber et al., 1994). F_v/F_m was measured in the laboratory using a 20 min dark-adaptation period. This was measured in addition to $\Delta F/F'_m$ to determine which measure was more sensitive to herbicide toxicity. Chlorophyll *a* fluorescence was measured on the second leaf of the terminal bunch, about 5–8 cm from the sediment.

Herbicides were then added (control, 10 and 100 μg l^{-1}, $n = 2$) and fluorescence parameters measured hourly on two leaves per replicate for 10 h. At 18:00 h, the tubs were placed directly into clean aquaria containing fresh seawater and two leaf samples were taken from each tub for chlorophyll pigment analysis. Chlorophyll *a* fluorescence parameters of samples were measured for the next 4 days at 09:00 h to assess recovery and a second sample of leaf material was collected at the end of the experiment for chlorophyll determination. Salinity was monitored over this period to ensure there was no excessive evaporation.

2.3. The in situ chambers

Open-bottom cylindrical chambers described in Macinnis-Ng and Ralph (2002) were used to enclose samples within the seagrass meadow. The chambers were pushed 50 mm into the sediment and pegged with stays. The chambers had four blanking bungs to allow a 2 mm (1 m long) acrylic fibre optic to pass into the chamber while keeping it water tight. These fibres were held in place using custom-made perspex leaf clips.

2.4. Field experiments

The chambers were placed in the *Z. capricorni* meadow at Paradise Beach on the evening before day 1, on the low tide. The leaf clips were put in place and the lids left off overnight. At 08:00 h the following morning, the first chlorophyll *a* fluorescence measurement was taken (0 h), the lids were attached and the chambers dosed with herbicide (control, 10 and 100 μg l^{-1}, $n = 2$). Replication was low due to the time taken to set up the experiments and

collect all the $\Delta F/F'_m$ measurements. After 1 h, the lids were removed from the chambers and fresh seawater was pumped into each. The same concentration of the herbicide was again added to each chamber to decrease the impact of herbicide adsorbing to the chamber surface. Additional to the six chambers, four seagrass leaves outside the chambers were monitored as an external control. $\Delta F/F'_m$ was measured for the next 10 h. The chambers were removed at 18:00 h. The sample leaves were marked and monitored for the next 4 days. Two leaves were taken from each chamber and chilled for pigment analysis at the end of the exposure day. These leaves were frozen (−80 °C) on return to the laboratory. A second set of leaves was collected for pigment analysis at the end of the recovery period.

2.5. Photosynthetic pigment analysis

Pigment extraction was performed in the solvent *N*,*N*-dimethylformamide (DMF) (Moran and Porath, 1980). Leaves were dried with absorbent paper, weighed and cut into thin slivers (ca. 1 mm) to increase the surface area of exposed tissue. Five ml of DMF was placed in a 15 ml brown glass screw-capped bottle with each sample. The bottles were refrigerated (4 °C) for 3 days before spectrophotometric determination (using LKB Ultrospec II UV-Vis model 4050 with spectral resolution of 1.00 nm) in glass cuvettes. Absorbance was measured at 480, 647 and 664 nm and absorbance at 750 nm subtracted to correct for scattering due to turbidity. Pigment concentrations were calculated using the Wellburn (1994) extinction coefficient equations. Only total chlorophyll concentration (chlorophyll *a* + chlorophyll *b*) was considered in the analysis.

2.6. Statistical analysis

After checking for homogeneity of variance (Levene's test) and normal distribution, a one-way ANOVA was used to test for significant differences between treatments at time 2, 10 and 96 h. All chlorophyll *a* fluorescence data were arc-sine transformed before analysis. Total chlorophyll concentration was analyzed using a one-way ANOVA to determine the impact of treatments. Where a significant difference occurred, a Tukey's HSD post hoc comparison was used to determine which treatments were different. All statistical analyses were carried out using Statistica (version 4).

3. Results

3.1. Atrazine

Atrazine caused a dramatic decline in effective quantum yield ($\Delta F/F'_m$) both in the laboratory and in situ experiments within the first hour for the 100 $\mu g\,l^{-1}$ treatment (Fig. 1b and c). The $\Delta F/F'_m$ remained below 0.1 units for the remainder of the exposure period (i.e. until 10 h) in the laboratory (Fig. 1b). In situ, this treatment remained below 0.1 units until 4 h and then recovered to approximately 0.4 units by the end of the exposure period (Fig. 1c). The 10 $\mu g\,l^{-1}$ treatment had more impact in the laboratory than the field, since the $\Delta F/F'_m$ reached as low as 0.1 units in the laboratory (Fig. 1b) but only

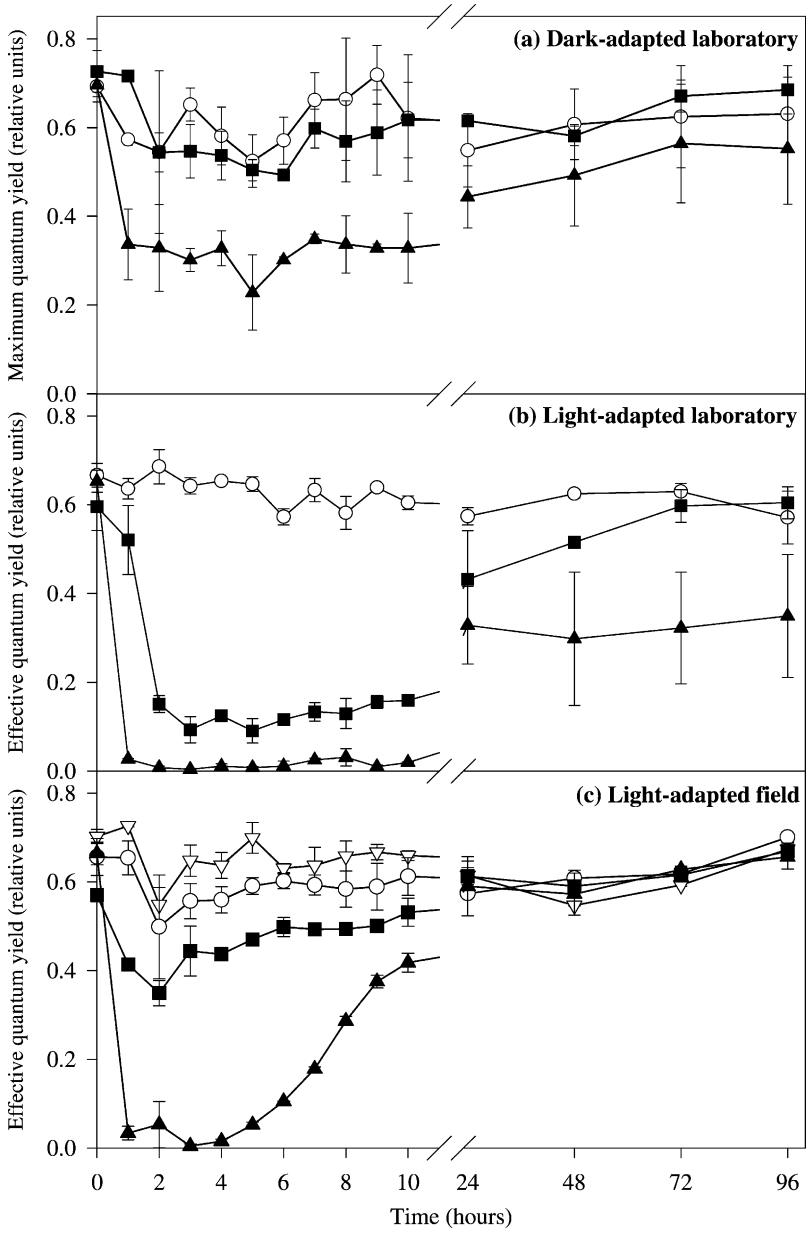

Fig. 1. Maximum quantum yields (F_v/F_m) for (a) laboratory experiment and effective quantum yields ($\Delta F/F'_m$) for (b) laboratory and (c) field experiments over a 10 h exposure period to various concentrations of Atrazine and a subsequent recovery period. Concentrations were: (▽) external control (not present in laboratory experiment); (○) chamber control; (■) 10 μg l^{-1} Atrazine; (▲) 100 μg l^{-1} Atrazine. The break on the abscissa represents the end of the exposure period and the beginning of 'recovery'.

Table 1
P-values from one-way ANOVAs on chlorophyll *a* fluorescence data from controls and two concentrations of herbicides at 2, 10 and 96 h

	2 h					10 h					96 h				
	P	e	c	10	100	*P*	e	c	10	100	*P*	e	c	10	100
Atrazine															
Laboratory F_v/F_m	0.266	ns				0.035	–	a	a	a	0.309	ns			
Laboratory $\Delta F/F'_m$	<0.001	–	a	b	c	<0.001	–	a	b	c	0.034	–	a	a	b
Field $\Delta F/F'_m$	<0.001	a	a	a	b	0.096	ns				0.445	ns			
Diuron															
Laboratory F_v/F_m	<0.001	–	a	b	c	<0.001	–	a	b	b	0.122	ns			
Laboratory $\Delta F/F'_m$	<0.001	–	a	b	b	<0.001	–	a	b	b	0.088	ns			
Field $\Delta F/F'_m$	<0.001	a	a	b	c	<0.001	a	a	a	b	0.044	a	a	a	b
Irgarol															
Laboratory F_v/F_m	<0.001	–	a	b	c	<0.001	–	a	b	c	0.066	ns			
Laboratory $\Delta F/F'_m$	<0.001	–	a	b	c	<0.001	–	a	b	b	0.011	–	a	a	b
Field $\Delta F/F'_m$	<0.001	a	a	b	c	<0.001	a	a	b	c	0.005	a	a	a	b

Significance level: $P < 0.05$, ns: not significant. Where a significant difference occurred, a Tukey's HSD post hoc comparison was used and the differences between treatments were reported as different letters in the table. Treatments were e = external control (not present in laboratory), c = chamber control, 10 = 10 $\mu g\,l^{-1}$ herbicide and 100 = 100 $\mu g\,l^{-1}$ herbicide.

as low as 0.35 units in situ (Fig. 1c). Indeed, the first measurement after the chambers were removed (24 h) showed no difference between the controls and Atrazine treatments in situ (Fig. 1c, Table 1). The 100 $\mu g\,l^{-1}$ treatment remained photosynthetically inhibited in the laboratory (averaging 0.35 units, Fig. 1b) at the end of the experiment (Table 1), implying incomplete recovery, although this treatment was variable during the recovery period.

Maximum quantum yield (F_v/F_m) values measured in the laboratory declined only as low as 0.2 units for the 100 $\mu g\,l^{-1}$ treatment (Fig. 1a) and statistical analysis suggested there were no significant differences between the treatments during the experiment (Table 1). The laboratory F_v/F_m data were more variable than the $\Delta F/F'_m$ data during the exposure period (Fig. 1a), so $\Delta F/F'_m$ appeared to be a more sensitive and accurate indicator of Atrazine stress. Furthermore, there were no significant differences at 2, 10 and 96 h for the F_v/F_m values, whereas the $\Delta F/F'_m$ values were significantly different in all instances (Table 1).

Light-adapted laboratory results reveal that the decline in $\Delta F/F'_m$ during the exposure period was due to an increase in F_t for the Atrazine treatments but this decline may also be due to lower F'_m, particularly during the recovery period (unpublished), at 24 h. The decline in the photosynthetic activity of the in situ samples was initially due to an increase in F_t during the exposure period which then gradually dropped off (Macinnis-Ng, unpublished).

Total chlorophyll concentration revealed no significant difference between the treatments in the field (Table 2). In the laboratory, total chlorophyll concentration was significantly higher in the treatments in comparison to the controls after 10 h, but had recovered to similar levels as the controls after the recovery period (Table 2).

Table 2
Total chlorophyll concentrations (mean values ± standard error, $\mu g\,cm^{-2}$) after 10 and 96 h exposure of *Z. capricorni* to three herbicides in the laboratory and field

	Laboratory		Field	
	10 h	96 h	10 h	96 h
Atrazine				
External control	–	–	18.4 ± 0.7	17.2 ± 0.6
Control	14.3 ± 0.7 a	22.1 ± 4.1	19.7 ± 1.2	17.4 ± 1.0
10 $\mu g\,l^{-1}$ herbicide	32.2 ± 2.2 b	28.9 ± 0.7	19.1 ± 2.3	17.8 ± 1.7
100 $\mu g\,l^{-1}$ herbicide	27.3 ± 5.3 b	24.4 ± 2.8	16.8 ± 0.7	18.8 ± 1.2
ANOVA *P*-value	0.011*	0.286	0.851	0.801
Diuron				
External control	–	–	17.8 ± 1.2 a	16.4 ± 0.6
Control	23.5 ± 3.7 a	32.6 ± 3.3	17.0 ± 0.6 a	16.6 ± 1.2
10 $\mu g\,l^{-1}$ herbicide	23.5 ± 1.7 a	29.9 ± 1.1	15.0 ± 1.0 b	17.5 ± 0.9
100 $\mu g\,l^{-1}$ herbicide	34.0 ± 1.8 b	33.1 ± 3.7	17.9 ± 2.1 a	16.7 ± 1.0
ANOVA *P*-value	0.028*	0.726	0.016*	0.222
Irgarol				
External control	–	–	18.5 ± 1.2	18.6 ± 1.9 a
Control	27.8 ± 2.5	37.5 ± 4.8	16.0 ± 1.1	18.2 ± 0.6 a
10 $\mu g\,l^{-1}$ herbicide	37.5 ± 3.5	39.2 ± 2.7	17.1 ± 1.7	16.3 ± 0.5 b
100 $\mu g\,l^{-1}$ herbicide	38.5 ± 3.1	43.2 ± 1.0	17.1 ± 0.8	17.7 ± 0.9 a,b
ANOVA *P*-value	0.065	0.463	0.508	0.004*

Treatments with the same letter are not significantly different (ANOVA, Tukey's HSD post hoc comparison ($P < 0.05$) test) within each group of treatments.
* $P < 0.05$ significance level.

3.2. Diuron

Photosynthetic activity was severely reduced due to Diuron exposure in the laboratory and field situations within the first hour (Fig. 2a–c). Unlike Atrazine, however, $\Delta F/F'_m$ dropped to less than 0.1 units for both the 10 and 100 $\mu g\,l^{-1}$ treatments in the first 2 h of the experiment. In the laboratory, the 10 $\mu g\,l^{-1}$ treatment continued to have reduced photosynthetic activity (Fig. 2b) where as the in situ samples began to return to normal levels after 2 h (Fig. 2c), reaching levels of nearly 0.6 by 10 h. Despite remaining below 0.1 for the entire 10 h exposure period, the $\Delta F/F'_m$ for the 100 $\mu g\,l^{-1}$ treatment appeared to recover by the first reading during the recovery period (24 h, Fig. 2c). Full recovery did not occur in the laboratory however, as the $\Delta F/F'_m$ remained at around 0.4 units for the 10 $\mu g\,l^{-1}$ treatment and about 0.2 units for the 100 $\mu g\,l^{-1}$ treatment (Fig. 2b). The F_v/F_m for the laboratory experiment was impacted during the exposure period, but showed at least partial recovery (Fig. 2a). Like the Atrazine results, the F_v/F_m was less sensitive to Diuron impacts than $\Delta F/F'_m$ (Fig. 2a and b). The laboratory F_v/F_m and laboratory and field $\Delta F/F'_m$ were all different at 2 and 10 h but the F_v/F_m had recovered by 96 h (Table 1). Like Atrazine, the depressed $\Delta F/F'_m$ in the laboratory and field was due to a combination of the elevation of Ft and depression of F'_m (Macinnis-Ng, unpublished).

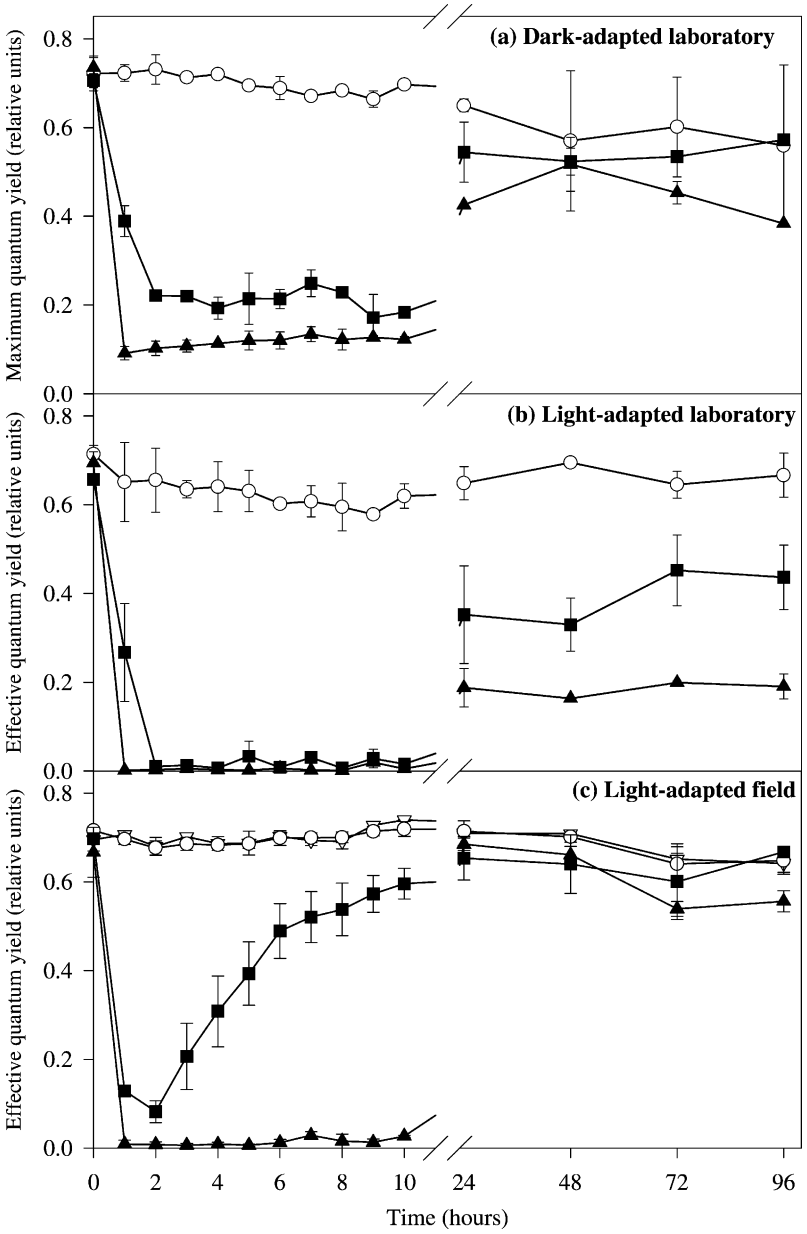

Fig. 2. Maximum quantum yields (F_v/F_m) for (a) laboratory experiment and effective quantum yields ($\Delta F/F'_m$) for (b) laboratory and (c) field experiments over a 10 h exposure period to various concentrations of Diuron and a subsequent recovery period. Concentrations were: (▽) external control (not present in laboratory experiment); (○) chamber control; (■) 10 μg l^{-1} Diuron; (▲) 100 μg l^{-1} Diuron. The break on the abscissa represents the end of the exposure period and the beginning of 'recovery'.

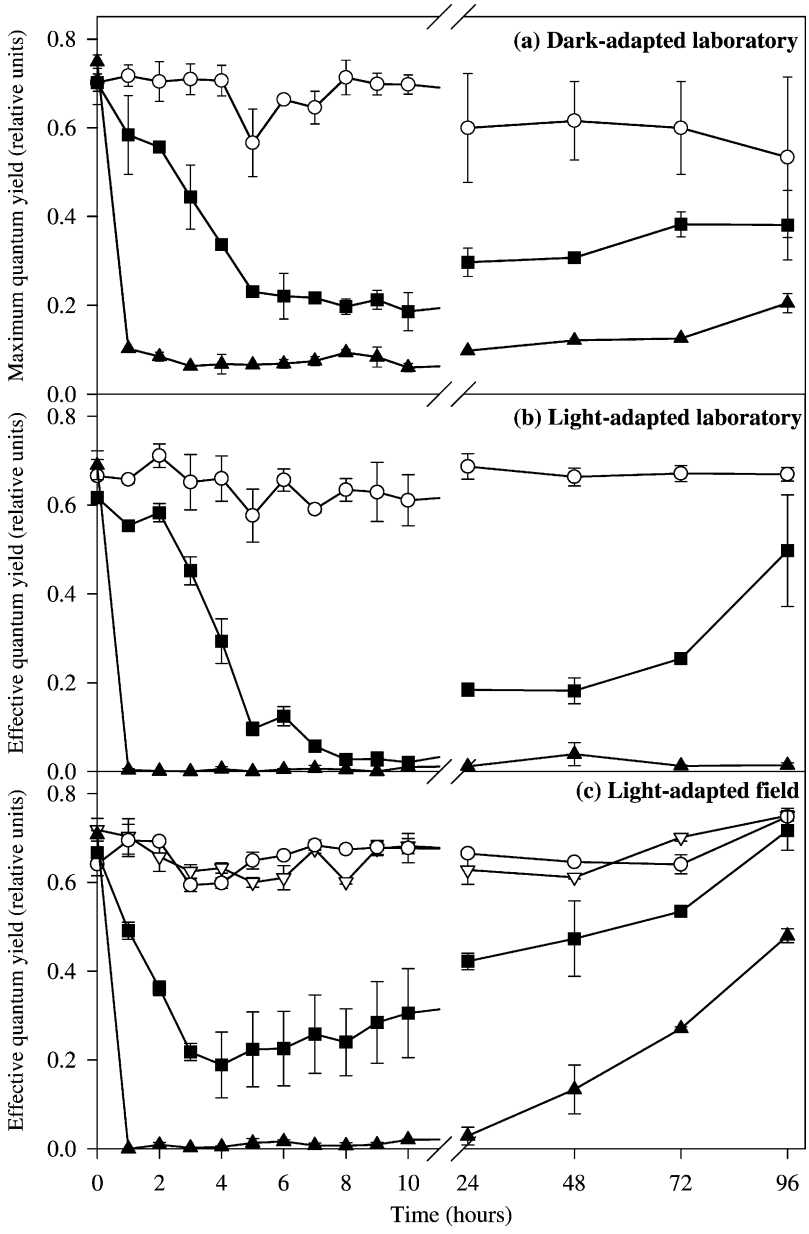

Fig. 3. Maximum quantum yields (F_v/F_m) for (a) laboratory experiment and effective quantum yields ($\Delta F/F'_m$) for (b) laboratory and (c) field experiments over a 10 h exposure period to various concentrations of Irgarol 1051 and a subsequent recovery period. Concentrations were: (▽) external control (not present in laboratory experiment); (○) chamber control; (■) 10 μg l^{-1} Irgarol 1051; (▲) 100 μg l^{-1} Irgarol 1051. The break on the abscissa represents the end of the exposure period and the beginning of 'recovery'.

In the laboratory, the 100 $\mu g\,l^{-1}$ treatment caused elevated levels of total chlorophyll for the 10 h samples but these samples recovered by 96 h (Table 2). In the field, total chlorophyll concentration was significantly lower in the 10 $\mu g\,l^{-1}$ treatment after 10 h but had recovered by 96 h (Table 2).

3.3. Irgarol

Irgarol was the most toxic herbicide to *Z. capricorni* in both the laboratory and field, with incomplete recovery of PSII quantum yield in both situations (Fig. 3a–c), although the action of the 10 $\mu g\,l^{-1}$ treatment was slightly slower than the same concentration for Diuron. $\Delta F/F'_m$ values in the laboratory and field dropped to 0 units after the first hour of exposure for the 100 $\mu g\,l^{-1}$ treatments (Fig. 3b and c), but the impact of the 10 $\mu g\,l^{-1}$ treatment was slower, reaching its lowest photosynthetic activity after 6 h in the laboratory (Fig. 3b) and 5 h in the field (Fig. 3c). Once again, the F_v/F_m was not as sensitive as $\Delta F/F'_m$ (Fig. 3a and b) but there was a fast impact in the 100 $\mu g\,l^{-1}$ treatment and a slower impact in the 10 $\mu g\,l^{-1}$ treatment when F_v/F_m is considered, similar to that of the $\Delta F/F'_m$ results. In the recovery period, partial or full recovery occurred in both Irgarol treatments in the field (Fig. 3c), such that the $\Delta F/F'_m$ returned to pre-exposure levels (above 0.6 units) for the 10 $\mu g\,l^{-1}$ treatment and the 100 $\mu g\,l^{-1}$ treatment returned to over 0.4 units (Fig. 3c). The laboratory samples did not appear to be as robust, with the $\Delta F/F'_m$ for the 100 $\mu g\,l^{-1}$ treatment remaining at just above 0 units and the 10 $\mu g\,l^{-1}$ treatment recovering to about 0.5 units (although this was highly variable). The F_v/F_m values also did not recover in the laboratory (Fig. 3a), with the 100 $\mu g\,l^{-1}$ treatment remaining below 0.2 units and the 10 $\mu g\,l^{-1}$ treatment remaining below 0.4 units at the end of the experiment. Statistically, $\Delta F/F'_m$ values were significantly depressed due to Irgarol at 2, 10 and 96 h in both the laboratory and field, indicating a lack of recovery in the highest treatment, while the 10 $\mu g\,l^{-1}$ treatment had recovered by 10 h in the laboratory and by 96 h in the field (Table 2). The less sensitive F_v/F_m values were significantly different among treatments for 2 and 10 h but not 96 h.

An increase in the minimum fluorescence was the underlying cause of the impact on the photosynthetic activity in the Irgarol experiment, in both the exposure and recovery periods. Despite this trend, the F'_m in the field was clearly depressed during the exposure period (Macinnis-Ng, unpublished).

In the laboratory, the total chlorophyll concentrations were not significantly different (Table 2). In the field, the total chlorophyll concentration was significantly lower in the 10 $\mu g\,l^{-1}$ treatment in comparison to the controls at 96 h (Table 2).

4. Discussion

The use of the in situ chambers in conjunction with the fluorometric determination of photosynthetic activity effectively demonstrated photosynthetic stress in *Z. capricorni* induced by three herbicides. The herbicides tested had a severe initial impact on *Z. capricorni* photosynthetic activity in the laboratory as well as in situ. Toxicities of these three herbicides to *Z. capricorni* ranks as follows: Irgarol > Diuron > Atrazine. Using chlorophyll *a* fluorescence as part of this technique is particularly effective because these herbicides

directly target photosystem II photochemistry (Solomon et al., 1996; Voulvoulis et al., 1999).

All three herbicides have a similar point of action on the photosynthetic apparatus: the reducing side of photosystem II, leading to a decline in photosynthetic activity when the seagrass was exposed to the herbicide. More specifically, Atrazine, Diuron and Irgarol block the reoxidation of Q_A, so absorbed energy cannot be used in photosynthesis (Miles, 1991). The result is an increase in the F_0 signal, as the energy is dissipated non-photochemically, due to the incomplete oxidation of Q_A (Schreiber et al., 1994). This was apparent in the F_0 readings for Diuron and Irgarol and the F_t readings for all three herbicides and implies an increase in non-photochemical quenching, whilst photochemical quenching remained relatively steady. The finding that $\Delta F/F'_m$ is a more sensitive indicator of herbicide stress than F_v/F_m is in accordance with Ralph's (2000) findings. Due to the dark-adaptation period involved in determining F_v/F_m, non-photochemical quenching is reduced and photosynthetic light pressure is removed, whereas $\Delta F/F'_m$ is impacted by both non-photochemical quenching and light pressure (Maxwell and Johnson, 2000). Since non-photochemical quenching has been identified as the major factor reducing PSII photochemical efficiency, it follows that $\Delta F/F'_m$ would be the most sensitive indicator. Furthermore, the removal of light pressure in determining F_v/F_m effectively reduces the pressure on the PSII reaction centers, supported by Solomon et al.'s (1996) finding that Atrazine is less toxic in the dark. This has important implications on the choice of parameter used to determine herbicide toxicity.

Scarlett et al. (1999a) found that F_v/F_m of *Z. marina* was significantly reduced by about 10% by 0.18 $\mu g\,l^{-1}$ Irgarol over a 10-day exposure period. This concentration is lower than the range found in estuaries in Europe (Scarlett et al., 1999a). The concentrations used in the present experiment may be higher than those usually reported for coastal waters (Scarlett et al., 1999b), but they may be indicative of levels found around enclosed marinas or after a rain event (Hall et al., 1999) and thus may represent a catastrophic concentration. The lack of complete recovery after such a pulsed event is a point of concern. It does not necessarily result in permanent cell damage in the short-term however, since recovery of phytoplankton from exposure has been demonstrated after chronic exposure (Solomon et al., 1996). Further study on the long-term implications of a significant decline in photosynthetic activity are required to fully understand chronic impacts of herbicide exposure. In this study, the samples exposed to Atrazine and Diuron showed some recovery of photosynthetic activity but the condition of other biochemical processes in the seagrass are not known. Furthermore, the plants exposed to Irgarol did not fully recover, even in situ. This reduced state of photosynthetic activity may have a longer-term impact on the seagrass and would certainly leave the plants in a poor condition for withstanding additional environmental stresses.

A characteristic of PSII inhibitor herbicide impact on aquatic macrophytes is rapid recovery after the sample is returned to uncontaminated water (Bowmer, 1986). This was confirmed by the recovery of in situ samples in the current study. Faster recovery occurred in the in situ experiments than in laboratory experiments for all herbicides.

Physicochemical factors which could be contributing to the differences include concentrations of dissolved and particulate inorganic carbon (Kester, 1986) which may bind the herbicides, influencing the bioavailability. Estuarine water probably had a greater complexing capacity compared to the filtered seawater used in the laboratory (Graney et al., 1995).

Light levels, temperature and redox potential could all influence the herbicide concentrations in the water. Despite this however, Mitchell (1987) found that variations in light, salinity and cropping did not influence Atrazine toxicity to *Halodule wrightii*. Factors not considered by Mitchell (1987) include complexing, precipitation, absorption and adsorption. Indeed, reduced persistence and bioavailability in field conditions generally makes chemicals less toxic (Graney et al., 1995). Exposure to a pollutant can encourage acclimation (Anderson et al., 1995) yet, in terms of pre-exposure, the laboratory and in situ samples had identical acclimation to herbicides since they were from the same site.

Comparisons between the current studies and the literature show some anomalies. Diuron was more toxic both in situ and in the laboratory in the current study than in the study by Haynes et al. (2000b). Even over a 5-day exposure period, the *Z. capricorni* samples tested by Haynes et al. (2000b) did not show mean $\Delta F/F'_m$ values much below 0.2 units, compared to readings of 0 after only a few hours of exposure. Exposure to 5–10 $\mu g\,l^{-1}$ Atrazine for 5 or more weeks significantly reduced seagrass growth (Schwarzschild et al., 1994) but the current study suggests that just a 10 h exposure period can significantly reduce PSII photochemical efficiency. Scarlett et al. (1999a) found that levels of Irgarol much lower than the current study can reduce photosynthetic efficiency in seagrass, yet they used a longer exposure period and recovery was not considered. These anomalies highlight the need for consistent techniques but also suggest that different populations may show different sensitivities.

The chlorophyll pigment data were difficult to interpret, possibly because these herbicides have not been shown to be inhibitors of pigment biosynthesis. This is in accordance with Schwarzschild et al.'s (1994) finding that Atrazine had no significant effect on total chlorophyll or chlorophyll *a* to chlorophyll *b* ratio for a 40-day period (at concentrations up to 2.46 $mg\,l^{-1}$).

Field validation of toxicology data gives a clearer indication of how chemicals will react in natural conditions (Graney et al., 1995). This study has provided information on the impact of pulses of three herbicides on *Z. capricorni*. The need for experiments to determine seagrass response to short-term pulsed exposure and recovery (Bowmer, 1986; Solomon et al., 1996; Ralph, 2000), as would occur in flowing water has not been previously addressed. Nor has the need to validate laboratory data in the field (Ralph, 2000). Laboratory experiments indicated *Z. capricorni* was more sensitive to herbicides than in situ samples. Dramatic recovery of PSII photochemical efficiency in *Z. capricorni* was demonstrated after in situ exposure to Atrazine and Diuron, while samples exposed to Irgarol remained photosynthetically compromised after the 4-day recovery period. The greater sensitivity of $\Delta F/F'_m$ as a demonstration of herbicide impact highlights that this measure is preferable to the more time consuming F_v/F_m.

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