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Effect of GeO_2 on embryo development and photosynthesis in *Fucus vesiculosus* (Phaeophyceae)

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Germanium dioxide (GeO_2) has been used for many years in the cultivation of red and green algae as a means of controlling the growth of diatoms. Brown algae are sensitive to GeO_2, however, the basis of this sensitivity has not been characterized. Here we use embryos of *Fucus vesiculosus* to investigate morphological and physiological impacts of GeO_2 toxicity. Morphometric features of embryos were measured microscopically, and physiological features were determined using pulse amplitude modulated (PAM) fluorometry. At 5 mg L^{-1} GeO_2, embryos grew slower than controls and developed growth abnormalities. After 24 h, initial zygote divisions were often oblique rather than transverse. Rhizoids had inflated tips in GeO_2 and were less branched, and apical hairs were deformed, with irregularly aligned, spheroidal cells. Minimum fluorescence (F_0) showed minor differences over the 10 days experiment, and pigment levels (chlorophylls *a*, *c* and total carotenoids) showed no difference after 10 days. Optimum quantum yield increased from ca. 0.52 at 24 h to 0.67 at 5 days, and GeO_2-treated embryos had higher mean values (significant at 3 and 5 days). Optimum quantum yield of photosystem II (Φ_{PSII}) was stable in control thalli after 5 days, but declined significantly in GeO_2. Addition of silica (as SiO_2) did not reverse the effects of GeO_2. These results suggest that GeO_2 toxicity in brown algae is associated with negative impacts at the cytological level rather than metabolic impacts associated with photosynthesis.

Key Words: chlorophyll *a* fluorescence; development; *Fucus*; germanium dioxide; photosynthesis; quantum yield

INTRODUCTION

Lewin (1966) reported that germanium (Ge) was toxic to diatoms. Subsequently, this element (as GeO_2) has had an important role in the laboratory culture of marine algae. Accordingly, researchers will typically use 5-10 mg L^{-1} of GeO_2 in their medium to prevent growth of diatoms during initial stages of algal isolation (e.g., McLachlan 1973, Garbary et al. 2004). The basis for this use is that Ge and silica (Si) are chemically very similar, and Ge inhibits deposition of Si into the frustule of the diatoms (Chiappino et al. 1977). This application of GeO_2 has proven effective in the culture of red and green algae (e.g., Garbary and Tam 1989, Scrosati et al. 1994), and only in very high concentrations (20-30 mg L^{-1} GeO_2) is there any inhibition of red algal growth (Tatewaki and Mizuno 1979). It has long been known, however, that brown algae have negative responses to GeO_2. West (unpublished, see Parker 1969) first noticed the inhibition of brown algae in the presence of Ge, and McLachlan et al. (1971) later reported growth

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abnormalities in *Fucus* embryos when grown in the presence of GeO_2. McLachlan (1977), Tatewaki and Mizuno (1979) and Yang (1993) later showed that this was a more general phenomenon for Phaeophyceae, with inhibition in most species occurring at concentrations as low as 1 mg L^{-1}. In addition, Markham and Hagmeier (1982) and Hubbard et al. (2004) reported inhibition of growth of blade discs of *Saccharina* and kelp gametophytes when grown with medium containing GeO_2.

The mechanism of Ge toxicity for brown algae is unknown. While species of Phaeophyceae may contain Si (Parker 1969), this is no more than the concentration in seawater. In addition, the omission of Si from the growth media for brown algae has no apparent deleterious effects (McLachlan 1977). Thus the physiological basis for Ge toxicity in brown algae is unlikely to be similar to that in diatoms, and Shea and Chopin (2007) even reported positive growth responses of low concentrations of GeO_2 on *S. latissima* (L.) Lane et al. (as *Laminaria*). More recently Mizuta and Yasui (2011) showed extensive silicon localization in sporophyte tissue of *S. japonica* (Areschoug) Lane et al. There have been several studies on the direct effects of Ge on algal growth and morphology (McLachlan 1977, Yang 1993, Shea and Chopin 2007). In this paper we examine the effects of GeO_2 on developing embryos of *F. vesiculosus* L. during the first 10 days following fertilization from the perspective of both morphological changes and photosynthetic physiology. Thus, are the inhibitory effects of Ge primarily associated with developmental phenomena, and are these impacts associated with negative effects of GeO_2 on photosynthetic processes?

MATERIALS AND METHODS

Experimental material and culturing

Fucus vesiculosus was collected at Tor Bay Provincial Park (45.19° N, 61.34° W) on the Atlantic coast of Nova Scotia, Canada from mid-June to mid-July 2007. Mature receptacles were collected in the mid-intertidal zone during low tide, washed with seawater, dried with filter paper, and stored in the dark at 4-10°C for up to 10 days. We used the standard technique (Jaffe and Neuscheler 1969, Quatrano 1974) to obtain gametes and to carry out fertilization. Immediately after fertilization (AF) the zygotes were divided into several groups, and each group was placed into 60-mm plastic Petri dishes in Tyndalized seawater at 15°C. Later, the developing zygotes and embryos were kept in Petri dishes with seawater under continuous light provided by cool white fluorescent bulbs at an irradiance of 20-25 μmol photons m^{-2} s^{-1}. Embryos were grown in seawater alone or in seawater supplemented with 5 mg L^{-1} GeO_2 (electronic grade, lot A019218501; Acros Organics, Geel, Belgium). Embryos exposed to GeO_2 are referred to as Ge-treated. Samples for fluorescence measurement and morphometry were taken at 1, 3, 5, 8, and 10 days AF.

Morphometry

Embryos were measured using a calibrated Olympus BH2 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) at 200× or 400× magnification. Ten or 15 embryos from each condition were measured for embryo length (top of embryo, excluding hairs, to tip of rhizoid), embryo diameter, and rhizoid tip diameter. In addition, the number of apical hairs was counted. The apical hairs usually appeared in 6-7 days old embryos and were counted at 7-8 days AF.

FM4-64-staining of membranes

Membrane aggregations were stained in embryos (22-24 h AF) with 5 μM of FM4-64 [N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl) pyridinium dibromide] (Invitrogen, Burlington, ON, Canada) for 30 min (Belanger and Quatrano 2000, Bolte et al. 2004, Hadley et al. 2006). The stain was prepared from the stock solution (10 mM in dimethyl sulfoxide [DMSO]) and diluted in seawater. The embryos were observed by Nikon Microphot FXA microscope (Nikon, Tokyo, Japan) as described by Belanger and Quatrano (2000).

Images from both transmitted light and epifluorescence microscopy were captured using the CCD SPOT2 camera system (Diagnostic Instruments, Sterling Heights, MI, USA) mounted on the Nikon Microphot FXA microscope.

Pulse amplitude modulated (PAM) fluorometry

Fluorescence measurements of *F. vesiculosus* embryos were performed using a microscopy-PAM apparatus (Walz GmbH, Effeltrich, Germany) as described previously (Garbary and Kim 2005, Kim et al. 2006). This system uses a blue (MC-L470) actinic and saturating-pulse light source. The active field diameter was set at 65 μm. The maximum (F_m) and the minimum (F_0) fluorescence, and the intrinsic potential quantum efficiency of PSII (F_v/F_m) were determined for all embryos after being acclimated to darkness for 10 min. Microscopy-PAM was used to as-

sess the effective quantum yield of charge separation in PSII, $\Phi_{PSII} = (F_m' - F)/F_m'$ (Genty et al. 1989); where F'_m is the maximum fluorescence yield for a light-acclimated embryo exposed to the actinic light, and F is the steady-state fluorescence yield of a light-acclimated embryo.

From the rapid light curves (RLC) of photosynthesis we calculated relative electron transport rate ($rETR_{max}$), photosynthetic efficiency (α^{ETR}) and light saturation ($I_k = rETR_{max}/\alpha^{ETR}$). Electron transport rate was calculated as $rETR = 0.84 \times \Phi_{PSII} \times PFD \times 0.5$, where PFD is photon flux density of photosynthetically active radiation (PAR 400-700 nm). RLCs were fitted to a double exponential decay curve (Platt et al. 1980). In cases where photoinhibition was not observed, an asymptotic function was applied (Harrison and Platt 1986).

The photosynthesis experiment was repeated using a different collection from the same site. Based on the similarity of results in the two runs, we only report on the one for which the morphometric data was collected.

Pigment analysis

Ten-days old attached embryos were resuspended and concentrated by centrifugation (500 *g*, 5 min). The supernatant was discarded and the pellet was ground using a manual tissue grinder in 100% acetone with small quantities of Na_2SO_4 and $NaHCO_3$. Several rounds of extraction were made with additional acetone until the extract was colourless. The concentration of acetone was adjusted to 90% (in distilled water), and quantities of chlorophylls *a* and *c* ($c_1 + c_2$) as well as total carotenoids were calculated from data obtained with a Unicam UV2 spectrophotometer (Unicam, Cambridge, UK) using published equations (Jeffrey and Humphrey 1975, Henley and Dunton 1995).

Data analysis

Photosynthetic data were analyzed using a general linear model (GLM) procedure of SYSTAT version 9 (SPSS Inc., Chicago, IL, USA) to determine if significant differences occurred among treatments and times for F_0, F_v/F_m, $rETR_{max}$, α^{ETR}, and I_k. Data were tested for normality and homogeneity of variance using the Kolmogorov-Smirnov and Cochran's tests, respectively. Where a significant difference occurred, a Tukey's HSD *post hoc* comparison was used to determine which times were different. Values are all expressed as mean and standard error (i.e., mean ± SE) or as mean and 95% confidence intervals.

RESULTS

Changes in embryo morphogenesis

GeO_2 at 5 mg L^{-1} altered the growth and morphogenesis of *F. vesiculosus* embryos. Differences between embryos in seawater without (Fig. 1A & B) and with Ge (Fig. 1C-E) were often noticeable after the first zygote division, 22-24 h AF. Within 24 h AF nearly all control embryos became polarized, the rhizoid developed and at least one cell division occurred. The first division of the zygote was perpendicular to the polar axis of the embryo. The cell division pattern of Ge-treated embryos was much less regular, and up to 30-35% of the embryos had irregularities. Some zygotes failed to polarize forming apolar embryos. More commonly, while the rhizoid protuberance developed, the division plane of the first division was not perpendicular to the polarity axis of the embryo but oblique (Fig. 1C-E).

By 3-4 days AF conspicuous morphological differences developed. Ge-treated embryos were smaller, and had wider diameter rhizoid tips (Table 1), which looked inflated (Fig. 1G & H). Differences in rhizoid morphology were particularly apparent, as control embryos had attenuated tips (Fig. 1F). Many of the Ge-treated embryos also had curved rhizoids (Fig. 1H). Whole 5 days AF embryos were significantly longer than those in Ge (Table 1). This size difference was largely a function of rhizoid elongation as there was no difference in embryo 'body' length (i.e.,

Table 1. Characteristics of control and GeO_2 treated embryos of *Fucus vesiculosus*

	Days after fertilization			
	3	5	7	8
Embryo length (μm)				
Control	185 ± 6.9	200 ± 6.3	229 ± 6.9	
Germanium	154 ± 8.3	158 ± 4.5	169 ± 10.3	
Embryo diameter (μm)				
Control	74 ± 2.2	79 ± 2.5	81 ± 1.6	
Germanium	71 ± 2.2	82 ± 2.0	79 ± 3.7	
Rhizoid tip diameter (μm)				
Control	15 ± 0.7	10 ± 0.7	8 ± 0.6	
Germanium	20 ± 1.6	22 ± 1.6	27 ± 3.6	
No. of hairs				
Control	0	0	1.0 ± 0.2	1.8 ± 0.3
Germanium	0	0	0	0.6 ± 0.2

Note: hair numbers for 8 days from different experiment. Values indicate mean ± SE (n = 10).

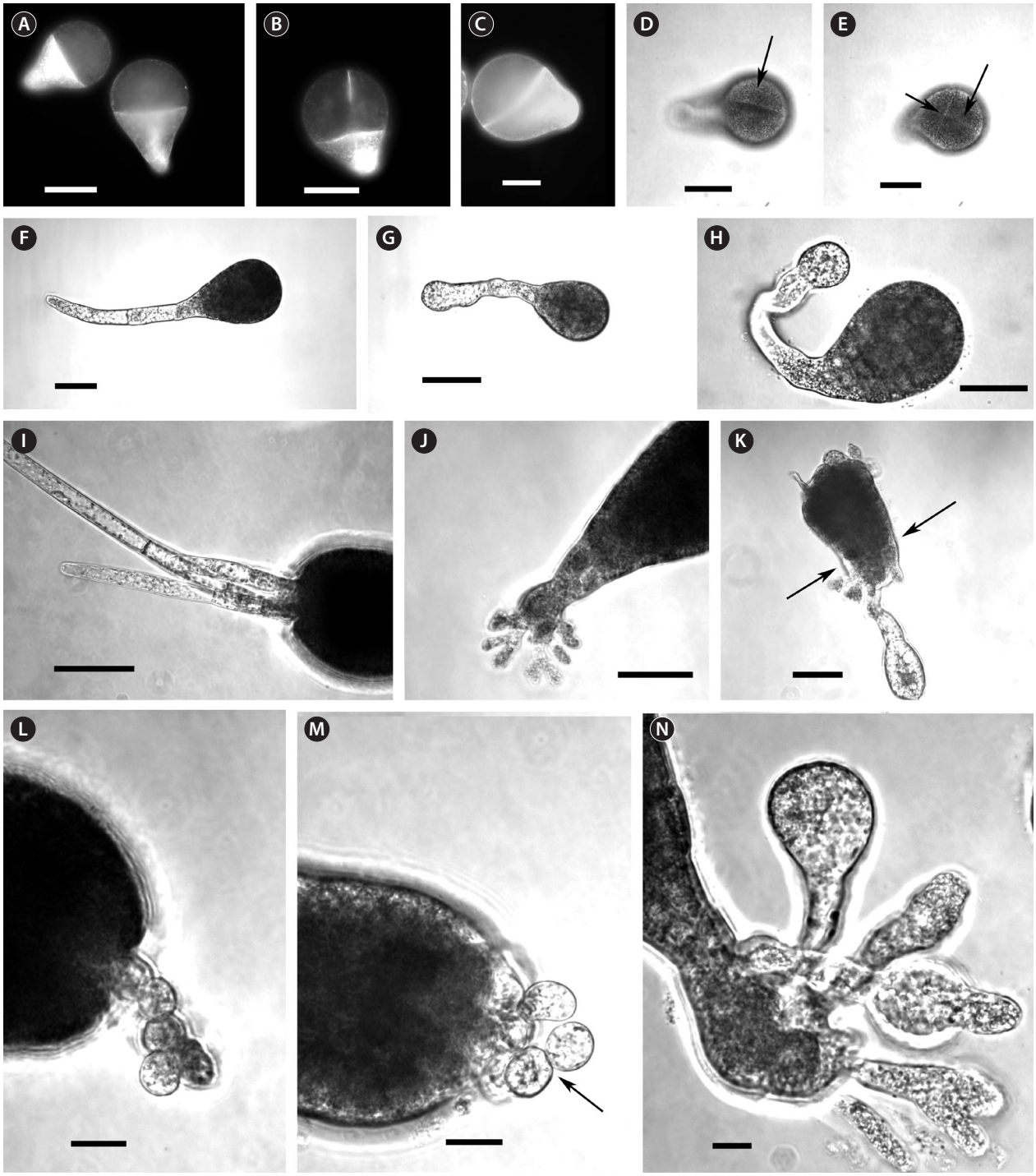

Fig. 1. Developing embryos of *Fucus vesiculosus*. (A & B) 24 h control embryos with one (A) or two (B) whole or partial cell divisions stained with FM4-64 and showing bright membrane concentrations at rhizoid tip and along cell plates. (C) GeO_2 treated 24 h embryo stained with FM4-64 showing oblique division. (D) GeO_2 treated 24 h embryo showing oblique first division. (E) GeO_2 treated 24 h embryo showing oblique first division (short arrow) and subsequent division of thallus cell perpendicular to the first (long arrow). (F) Control embryo at 4 days showing normal development of rhizoid and thallus. (G) GeO_2 treated embryo at 4 days showing rhizoid with inflated tip. (H) GeO_2 treated embryo at 4 days showing hook-shaped rhizoid with inflated tip. (I) Apex of control embryo at 8 days with three normal apical hairs. (J) Base of 8 days control embryo with typical rhizoid development. (K) Whole 8 days GeO_2 treated embryo with irregular shape, inflated rhizoid tip, poor development of apical hairs and secondary rhizoid development from thallus base; arrows indicate common point of breakage of GeO_2 treated embryos. (L & M) Apex of 8 days GeO_2 treated embryo showing the abnormal development of apical hairs. Note the cell disaggregation. (N) Primary and secondary rhizoids of 8 days GeO_2 treated embryo showing the inflated tips. Scale bars represent: A-K, 50 μm; L-N, 20 μm.

the non-rhizoidal portion). All of these metrics were significantly different between control and treatment conditions (Student's t-test, $p < 0.01$).

At 7-8 days AF most control embryos had developed conspicuous up to 3 apical hairs (Fig. 1I). The hairs were up to six cells long, and cells were regularly cylindrical. The rhizoids were often highly branched and often developed a rhizoidal pad (Fig. 1J). The Ge-treated embryos were often misshapen with irregular outlines (Fig. 1K). These embryos were often fragile, and tended to break near the base of the 'body' part of the embryo (Fig. 1K). The apical hairs in Ge-treated embryos were sometimes absent, but always highly deformed. The cells in a given filament were spheroidal, and poorly aligned (Fig. 1L & M), sometimes appearing as a cell cluster at the embryo apex. The rhizoids showed the same inflated structures as previously (Fig. 1K & N). The tips of rhizoids were fragile and sometimes burst following resuspension (by gentle pipetting), which was never observed in control embryos. The number of rhizoid branches was reduced in Ge-treated embryos; however, some embryos did have a more usual number of, albeit deformed, rhizoid tips (Fig. 1N).

Photosynthetic responses

Photosynthetic characteristics in embryos developing in seawater alone and in seawater supplement with 5 mg L^{-1} GeO_2 were measured over the first 10 days AF. The controls differed significantly from Ge-treated embryos in their minimum fluorescence (Table 2) and generally showed higher levels of F_0. There were several reversal over time, and at the end of the experiment, the two conditions were not significantly different (Fig. 2). This corresponds well with the pigments data, that saw no differences in chlorophyll and carotenoid contents between control and Ge-treated embryos after 10 days (Table 3).

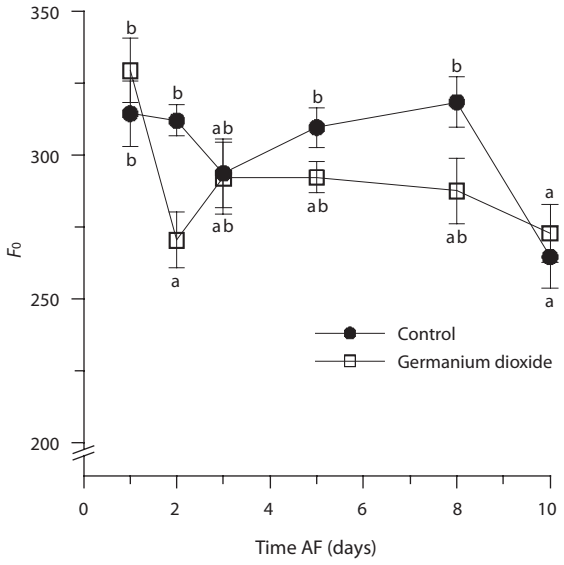

Fig. 2. Dynamics of minimum fluorescence (F_0) in control and GeO_2 treated embryos of *Fucus vesiculosus*. AF, after fertilization.

While the ANOVA suggested no significant overall difference between treatments for maximum quantum yield

Table 2. Summary of ANOVA results for two-way analyses of photosynthetic data shown in Figs 2-6

Photosynthetic parameter	Variable	F-ratio	p
F_0	Time	6.232	<0.001
	Treatment	4.219	0.042
	Treatment × time	2.647	0.026
F_v/F_m	Time	128.889	<0.001
	Treatment	3.781	0.054
	Treatment × time	12.349	<0.001
$rETR_{max}$	Time	51.599	<0.001
	Treatment	6.458	0.012
	Treatment × time	9.236	<0.001
α^{ETR}	Time	7.004	<0.001
	Treatment	2.611	0.109
	Treatment × time	4.183	0.001
I_k	Time	24.094	<0.001
	Treatment	0.509	0.477
	Treatment × time	5.488	<0.001

In all cases treatments refers to control versus GeO_2 conditions (df = 1) and time refers to the six days (df = 5) on which photosynthetic parameters were monitored.

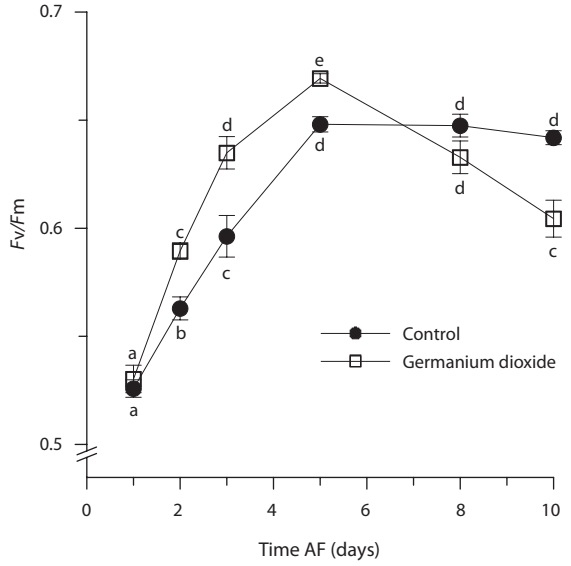

Fig. 3. Dynamics of maximum quantum yield (F_v/F_m) in control and GeO_2 treated embryos of *Fucus vesiculosus*. AF, after fertilization.

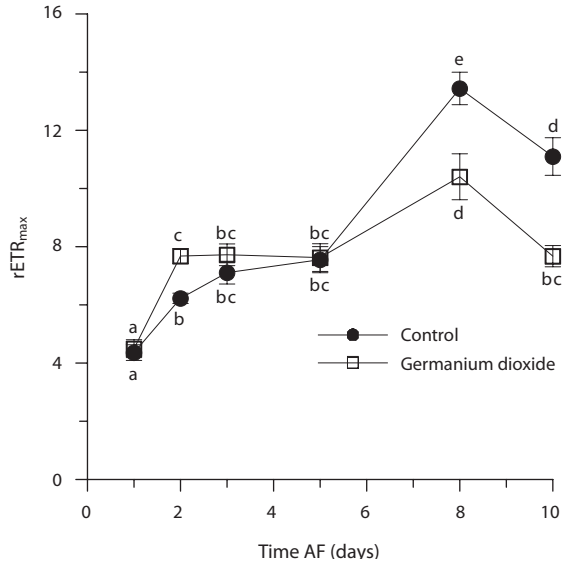

Fig. 4. Dynamics of maximum electron transport rate ($rETR_{max}$) in control and GeO_2 treated embryos of *Fucus vesiculosus*. AF, after fertilization.

Table 3. Photosynthetic pigment content in 10 days after fertilization and GeO_2 treated embryos of *Fucus vesiculosus*

	Pigment content (μg mg^{-1} FW)	
	Control	**GeO_2**
Chlorophyll *a*	0.64 (0.06)	0.66 (0.06)
Chlorophyll $c_1 + c_2$	0.13 (0.02)	0.13 (0.02)
Total carotenoids	0.32 (0.06)	0.37 (0.04)

Means given (± conf. int., α = 0.05; n = 3); none of the differences are significant (Student's t-test).

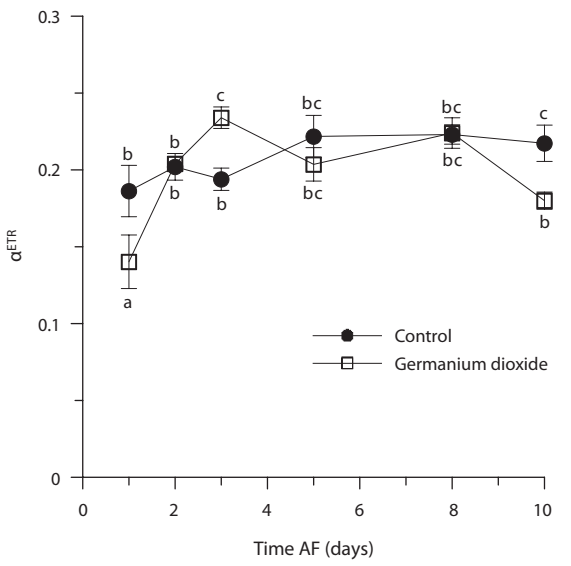

Fig. 5. Dynamics of photosynthetic efficiency (α^{ETR}) in control and GeO_2 treated embryos of *Fucus vesiculosus*. AF, after fertilization.

(Table 2), this is confounded by the differences attributable to time and the highly significant time × treatment interation. The F_v/F_m in 1 day *Fucus* embryos was about 0.53 (Fig. 3). During the first 5 days AF it gradually increased, and then stabilized at about 0.65. In Ge-treated embryos, quantum yield did not differ from controls after 24 h AF (Fig. 3). F_v/F_m then increased sharply, and over the next 4 days of embryogenesis it remained significantly higher than in control embryos. The maximum difference between control and experiment conditions was about 6% at 4 days AF. After 5 days when F_v/F_m in control embryos reached a steady-state level, it gradually decreased in Ge-treated embryos. Thus at the end of the experiments (10 days AF) maximum quantum yield of photosystem II was significantly lower in *Fucus* embryos growing in the presence of Ge. Despite the decline, the mean of about 0.6 still indicates normal functioning (Garbary and Kim 2005, Kim et al. 2006).

Time, treatment and the time × treatment interaction were all significant for $rETR_{max}$ (Table 2, Fig. 4). The maximum value of $rETR_{max}$ was about 13.5 in 8 days control embryos. Higher values for $rETR_{max}$ were evident at the end of the experiment when controls were over 30% higher than Ge-treated embryos. Photosynthetic efficiency (α^{ETR}) in both control and experimental embryos remained relatively stable during the whole experiment, fluctuating slightly between 0.18-0.22 (Fig. 5). While time and treatment × time interaction were significant factors, there was no overall difference between Ge and control embryos (Table 2).

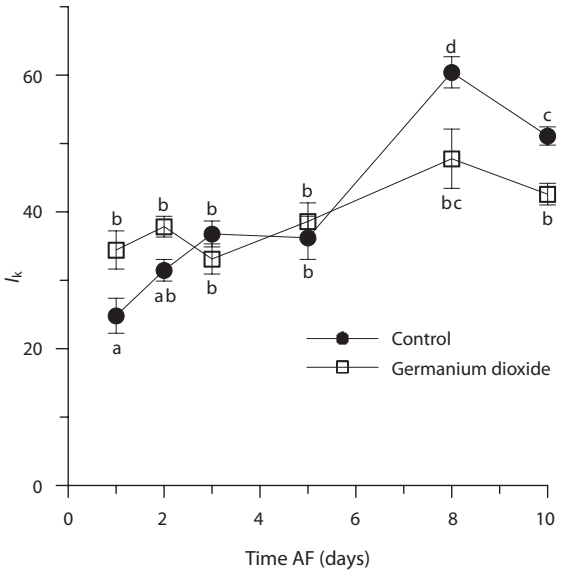

Fig. 6. Dynamics of optimum light intensity (I_k) in control and GeO_2 treated embryos of *Fucus vesiculosus*. AF, after fertilization.

After 24 h, control and Ge-treated embryos had different optimal light intensities (I_k). Control embryos saturated at an irradiance of about 25 μmol photons m^{-2} s^{-1} while the I_k of Ge-treated embryos was ~28% higher (Fig. 6). This value of optimum light intensity in Ge-treated embryos remained stable throughout the experiment, while the I_k of control embryos increased gradually in the first five days AF, and then dramatically at eight days AF up to ~60 μmol photons m^{-2} s^{-1}. Thus at the end of the experiments (10 days AF) the I_k in control embryos was significantly higher than in Ge-treated ones.

The rhizoid cells of control and Ge-treated two-celled embryos were significantly differed in minimum fluorescence: F_0 of rhizoid cells in Ge-treated embryos was almost 30% higher than in controls (Table 4). At the same time, there were no differences in other photosynthetic parameters between these cells.

Table 4. Comparison of photosynthetic parameters in rhizoid cells of control and GeO_2 treated 2-celled embryos of *Fucus vesiculosus*

	Control	GeO_2
F_0	140.20 (26.51)*	191.20 (18.16)*
F_v/F_m	0.54 (0.02)	0.56 (0.02)
$rETR_{max}$	4.47 (0.49)	5.08 (0.92)
α^{ETR}	0.17 (0.03)	0.18 (0.03)
I_k	27.94 (7.97)	29.91 (10.21)

Means given (± conf. int., α = 0.05), significant differences marked with an asterisk (Student's t-test, $p < 0.01$).

DISCUSSION

Morphological responses

Many aspects of germanium toxicity were manifest as abnormalities of *Fucus* embryo morphogenesis, including zygote polarization, the pattern of rhizoid growth and apical hair formation. The induction of axis polarity in fucoid zygotes has been extensively investigated (e.g., Quatrano 1974, Kropf 1997, Homblé and Léonetti 2007). Fucoid eggs have spherical shape and are initially apolar. Polarity can be observed within 14-16 h AF when the rhizoid protuberance appears on the zygote surface. The rhizoid appearance is preceded by a series of processes, including asymmetric ion fluxes (Homblé and Léonetti 2007), redistribution of cytoskeletal elements (e.g., Bisgrove and Kropf 2001*a*, Bisgrove et al. 2003) and targeted vesicular secretion (Kropf 1997). Subsequently, the zygote has a single axis of symmetry that defines the embryo growth axis, and normally the first division of the zygote is perpendicular to this axis and positioned by telophase nuclei (Bisgrove and Kropf 2004). In the presence of Ge, many zygotes failed to polarize before the first division, and many 2-celled embryos had a cell plate at an oblique angle to the polarity axis. The relatively high level of minimum fluorescence (F_0) in rhizoid cells of Ge-treated embryos (Table 3) also may be the consequence of disturbed polarization. Generally, high values of this parameter reflect either a stressed state, or relatively high chlorophyll *a* content. The former is less likely, since there was no decrease in F_v/F_m or ETR (Table 3). Thus, the high chlorophyll content (i.e., numerous plastids) in rhizoid cells could result from incorrect positioning of cell plates during the first division. This division is normally highly asymmetric, with most mitochondria in the rhizoid cell and most chloroplasts in the non-rhizoidal one (Quatrano 1974). In brown algae, cytokinesis includes participation of microfilaments in cell plate formation (Bisgrove and Kropf 2004). The delay of polarization and abnormal position of cell plates in Ge-treated zygotes suggests that Ge affects (possibly indirectly) the localization of microfilaments in *Fucus* cells.

Yang (1993) observed morphological changes in Ge-treated cells of brown algae. He showed that Ge altered cell wall structure in fucoid embryos, particularly in the rhizoid region; this is consistent with our data. The wall looseness and disorganized arrangement of fibrils in the rhizoid tips might be a direct effect of Ge on the cell wall (Yang 1993), but these symptoms also point to cytoskeletal malfunction. In fucoid algae the cell wall organization

and function is regulated by F-actin (Bisgrove and Kropf 2001*b*).

Another hypothesis for how GeO_2 affects cell walls of brown algae is the substitution for other elements with similar chemical properties to Ge. In diatoms, Ge toxicity can be reduced by the addition of Si to the growth medium (Lewin 1966), although McLachlan et al. (1971) pointed out that this was not the case with fucoids. In our case, Ge was also not less toxic even when Si (as SiO_2) was added to the medium in double the concentration of added Ge (data not shown). Although this does not rule out the possibility that Si-dependent processes have no role in Ge toxicity, inhibitory effects of GeO_2 may not always be based on Si substitution.

A possible mechanism for Ge toxicity comes from higher plants where Ge leads to increased root branching and leaf necrosis (Skok 1957, McIlrath and Skok 1966). Ge may partially substitute for boron (B), where it affects cell permeability, structural integrity of cell membranes and cell wall formation (McIlrath and Skok 1966, Cakmak et al. 1995). Boron resembles Si in physical and chemical properties, although unlike Si, which is not essential for most plants, B is almost universally required. Boron is normally absorbed by plants as undissociated boric acid, and it accumulates in both the cytoplasm and cell walls (Dembitsky et al. 2002, Bassil et al. 2004). Free and complex forms of accumulated borate occur in different seaweeds, including green, red and brown algae. Unlike other algae, brown algae contain considerable B in diverse B-containing compounds, including complexes with mannitol, laminarin, and alginic acid (Chuda et al. 1997, Dembitsky et al. 2002). If Ge can also substitute for B in brown algae, that might explain their increased sensitivity to GeO_2 compared with green or red algae (Tatewaki and Mizuno 1979). Both B and Ge can form complex compounds with other membrane and cell wall components such as phenols (Cakmak et al. 1995), and brown algae have high contents of phenolic compounds (Schoenwaelder 2002, 2008). Phenolics play a critical role in early development of fucoids participating in cell wall formation, zygote adhesion to the substratum and polar axis formation (Schoenwaelder and Wiencke 2000, Bisgrove and Kropf 2001*b*). Most Ge-treated embryos had abnormal rhizoid growth. Algal rhizoid elongation is mediated via tip growth. The requirements for this process were extensively investigated in pollen tubes and root hairs (e.g., Holdaway-Clarke and Hepler 2003, Galway 2006). Elimination of boric acid from culture media often leads to pollen tubes bursting at their tips (Cheng and McComb 1992, Holdaway-Clarke and Hepler 2003); we observed this symptom in rhizoids of Ge-treated *Fucus* embryos.

Photosynthetic responses

Our observations on the development of the photosynthetic apparatus in fucoid embryos are consistent with the previous investigations of this process carried out with similar (Lamote et al. 2003, Kim et al. 2006, Kim and Garbary 2009) or other methods (McLachlan and Bidwell 1978, Major and Davison 1998, Tarakhovskaya and Maslov 2005). Kim et al. (2006) had similar results with another fucoid, *Ascophyllum nodosum*. The major difference in photosynthetic development during embryogenesis between *Fucus* and *Ascophyllum* is that the latter did not show a significant increase in maximum quantum yield, whereas in *Fucus* we observed a 20% increase during the first 5 days AF.

There were no conspicuous changes in F_0 in Ge-treated embryos. Since F_0 provides a relative measure of chlorophyll *a* content, we conclude that Ge apparently had no effect on synthesis or degradation of this pigment. The direct measurements of photosynthetic pigment content in *Fucus* embryos support this conclusion, for even after 10 days there was no difference between control and experimental conditions in chlorophylls *a* and *c* and total carotenoids.

While maximum quantum yield and $rETR_{max}$ significantly increased in Ge-treated embryos during the first 5 days AF, at the same time, total growth was reduced and highly abnormal. This seems counterintuitive; however, Lewin (1966) showed that GeO_2, though extremely deleterious for diatoms, had no negative influence on energy metabolism of the cells, and even at 400 mg L^{-1} GeO_2, cell respiration did not decrease. Shea and Chopin (2007) also reported that low concentrations of GeO_2 (up to 0.5 mg L^{-1}) might sometimes have a slightly positive effect on growth of brown algae. After 5 days, F_v/F_m and $rETR_{max}$ significantly decreased in Ge-treated embryos, relative to control. This effect may reflect the morphological observations of embryos. Normally, apical hairs form at this stage of embryo development. We showed that Ge-treated embryos failed to form normal hairs, though they previously formed primary and secondary rhizoids. Thus *Fucus* embryos can develop (though highly abnormally) in the presence of Ge, up to about the 5-6 days AF. Subsequently, development nearly ceases, embryos never form normal apical hairs, and embryo disintegration occurs. McLachlan et al. (1971) observed apical necrosis in older *Fucus* embryos treated with Ge. The decline of photosynthetic processes may also reflect this general

collapse of embryo development. At 5 days AF, F_v/F_m in control embryos reached a steady-state, suggesting that electron transport processes and coupled biochemical reactions in the Calvin cycle were equilibrated (Roháček 2002). This was not the case in Ge-treated embryos where it was not the structural components of the photochemical part of the assimilation apparatus (e.g., pigments) that were affected, but the processes maintaining the balance between electron transport and carbon reduction.

The developmental abnormalities of *Fucus* that result from exposure to GeO_2 support the notion that Ge is interfering with essential cell processes including growth rate, morphogenesis, and to a lesser extent, photosynthesis. The affects of Ge in Phaeophyceae as shown here are more complex than previously considered and warrant further study.

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