JOURNAL OF PLANKTON RESEARCH | VOLUME 25 | NUMBER 8 | PAGES 000–000 | 2003

SHORT COMMUNICATION

Long-term exposure of dinoflagellates to ^{14}carbon: effects on growth rate and measurements of carbon content

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Exposure of photosynthetic dinoflagellates to low specific activities of ^{14}C for several days led to a significant reduction in growth rates and overestimation of ^{14}C-based measurements of cellular C content. No effect was observed in other taxonomic groups examined.

Photosynthetic plankton incorporate radiolabelled carbon (^{14}C) through photosynthesis when grown in a culture medium containing inorganic ^{14}C. This process was first exploited by Steemann Nielsen to quantify photosynthesis (Steemann Nielsen, 1952). Since then the method has become one of the most commonly used techniques for measurements of aquatic primary production. Radiolabelling has also been applied to measure growth rates and ratios of carbon to chlorophyll *a* (Chl *a*) (Welschmeyer and Lorenzen, 1984), cellular carbon (C) content (Putt and Stoecker, 1989; Crawford and Stoecker, 1996) and respiration rates of planktonic micro-organisms (Manahan, 1983; Stoecker and Michaels, 1991). In addition, ^{14}C has been used to label algal prey cells in order to quantify grazing by heterotrophic protists (Montagnes and Lessard, 1999). Despite the extensive use of ^{14}C-labelling methods, the effect of radioactivity on plankton physiology is unknown. It has been assumed that the short incubation times of up to 24 h, combined with typically low specific activities of ^{14}C, have no physiological consequences. In this paper we report observations made whilst using the ^{14}C method to determine the cellular C content of dinoflagellates. Our results show that long-term exposure (i.e. several days) to ^{14}C had an adverse effect on the physiology of phototrophic dinoflagellates, but no effect was observed for heterotrophic dinoflagellates or nondinoflagellate phytoplankton.

Twelve plankton species (Table I) were cultivated without agitation in medium prepared from filtered sea water (30‰ salinity) on a 16 : 8 light : dark cycle. Photosynthetic phytoplankton was maintained either in f/2-medium (Guillard, 1975) at 18°C and an irradiance of 50 µmol photons m^{-2} s^{-1}, or in B-medium (Hansen, 1989) at 15°C and 75 µmol photons m^{-2} s^{-1}. Initial dissolved inorganic carbon concentrations of the growth medium were measured either with a type 225-Mk3 infrared gas analyser (Analytic Development Co. Ltd, Hoddesden, UK) or manometrically using a purge and trap procedure (Kroopnick, 1985). Radiolabelled growth medium was prepared by adding 25–50 µl ^{14}C stock in aqueous solution of $NaH^{14}CO_3$, with a specific activity of 100 µCi ml^{-1} and a pH of 10.1, to 62 ml of growth medium. The labelled growth medium had a final specific activity of 0.04–0.08 µCi ml^{-1}. ^{14}C stocks were obtained both from Carbon 14 Centralen, Denmark, and from ICN Biochemicals Inc., Costa Mesa, CA, USA. Photosynthetic phytoplankton was uniformly ^{14}C-labelled by culturing cells for at least five divisions in air-sealed culture bottles. Heterotrophic dinoflagellates were fed ^{14}C-labelled prey (*Ditylum brightwellii*) and separated from the prey by use of

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Table I: Plankton species used in this study

Species	Source
Phototrophic dinoflagellates	
Akashiwo sanguinea (Hirasaka) Hansen & Moestrup (= *Gymnodinium sanguineum* Hirasaka)[a]	CCMP[c] 1740
Amphidinium carterae Hulburt[a]	NEPCC[d] 629
Fragilidium subglobosum (v. Stosch) Loeblich[b]	MBL[e]
Heterocapsa triquetra (Ehrenberg) Stein[b]	MBL
Prorocentrum minimum (Pavillard) Schiller[b]	MBL
Scrippsiella trochoidea (Stein) Loeblich[a]	NEPCC 15
Other phototrophs	
Isochrysis galbana Parke[b]	MBL
Phaeodactylum tricornutum Bohlin[b]	MBL
Rhodomonas salina (Wislouch) Hill & Wetherbee[b]	MBL
Heterotrophic dinoflagellates	
Noctiluca scintillans (Macartney) Ehrenberg[a]	Admiralty Inlet, WA, USA[f]
Protoperidinium oceanicum (Vanhöffen) Balech[a]	Copalis Beach, WA, USA[f]
P. pellucidum (Berg) Schütt[a]	Admiralty Inlet, WA, USA[f]

[a] f/2-medium (Guillard, 1975) at 18°C and an irradiance of 50 μmol photons m^{-2} s^{-1}.
[b] B-medium (Hansen, 1989) at 15°C and an irradiance of 75 μmol photons m^{-2} s^{-1}.
[c] Provasoli-Guillard National Center for Culture of Marine Phytoplankton, USA.
[d] North East Pacific Culture Collection, Canada.
[e] Culture collection of the Marine Biological Laboratory, Helsingør, Denmark.
[f] Wild-type isolate.

a micropipette. Uniformity of the ^{14}C-labelling was ensured by monitoring the accumulation of ^{14}C in cells over time (i.e. decays min^{-1} $cell^{-1}$).

To determine if specific growth rate was affected by the addition of ^{14}C, cells were grown in medium labelled with ^{14}C as well as in controls without added ^{14}C. Growth rates were determined for three photosynthetic dinoflagellates (*Fragilidium subglobosum*, *Heterocapsa triquetra* and *Prorocentrum minimum*), one haptophyte (*Isochrysis galbana*), one diatom (*Phaeodactylum tricornutum*) and one cryptophyte (*Rhodomonas salina*). Since repeated samplings from a single culture bottle lead to exchange of CO_2 between the culture medium and ambient air, 36 replicate 62-ml culture bottles were prepared for each species. Cultures of initial cell densities were prepared in larger batches and distributed amongst the culture bottles. Half of the bottles contained growth medium with ^{14}C at a final activity of 0.04–0.08 μCi ml^{-1}. At intervals of approximately one division, three ^{14}C-labelled cultures and three controls were fixed with Lugol's solution, and cell concentrations were determined microscopically. Specific growth rates, μ (day^{-1}), were calculated by determining the slope of the linear regression of log-transformed cell concentration. Only exponential parts of curves were used in calculations of growth rate. Prior to the experiments and at the end of incubations, the pH of all culture media was measured using a Sentron pH-meter model 2001.

To determine the ^{14}C content of large cells (>15 μm diameter), 10–50 cells were isolated individually with a micropipette, rinsed three times in growth medium, and transferred to scintillation vials. Small cells (<15 μm diameter) were retained on GF/C filters which were rinsed and transferred to scintillation vials. Three to five filters were prepared per species. Vials were then acidified to remove inorganic ^{14}C and radioactivity was measured with a liquid scintillation analyser (Packard). Cellular C content of ^{14}C-labelled cells was calculated as described in Welschmeyer and Lorenzen (Welschmeyer and Lorenzen, 1984). The ^{14}C-based cellular C contents were compared with previously published C contents measured with a CHN analyser (Menden-Deuer and Lessard, 2000; Skovgaard, 2000; Skovgaard *et al.*, 2000). The CHN-based C measurements and the herein reported ^{14}C-based C measurements were made on the same cultures.

Tests of statistically significant differences between mean growth rates were made using Student's *t*-test for differences between two slopes (Zar, 1999). Student's *t*-test was also applied to testing for differences between mean C values. When data were not normally distributed or

had unequal variances, the Mann–Whitney rank sum test was used.

Growth rates of the three photosynthetic dinoflagellates (*F. subglobosum*, *H. triquetra* and *P. minimum*) grown in ^{14}C-labelled medium were significantly lower (13–28%, $P < 0.05$) than growth rates from non-labelled culture medium, whereas the growth rates of the other phytoplankton organisms were unaffected by ^{14}C in the growth medium (Figure 1; Table II). To test if the reduction in growth rate could be attributed to a contaminant in the ^{14}C stock solution rather than the radioactivity, a growth experiment was performed, in which the ^{14}C stock was acidified with HCl 2 days prior to use to drive off ^{14}C as $^{14}CO_2$. There was no difference between the growth rate of *F. subglobosum* incubated in acidified ^{14}C-labelled medium or in non-labelled culture medium (Figure 2; Table II).

The radiolabelling method also resulted in an apparent increase in the cellular C content of photosynthetic dinoflagellates compared with data obtained from CHN analysis. Cellular C content estimates of five phototrophic dinoflagellate species, based on the specific activity of uniformly ^{14}C-labelled cells, were significantly higher (49–233%, $P < 0.05$) than C contents measured with a CHN analyser on the same cultures (Table III). Therefore, measurements of biomass of photosynthetic dinoflagellates would lead to significant overestimates when based on the radiolabelling method. In contrast, measurements of cellular C content for two species of heterotrophic dinoflagellates showed no significant difference between the two C measurement methods (Table III).

Consequently, it appears that exposure to ^{14}C results in reduced growth rates and an overestimation of C content of the photosynthetic dinoflagellates investigated. These results were particularly surprising given the low specific activity the cells were exposed to. The amount of radioactivity used in the present study was up to one order of magnitude lower than what has been successfully applied to other planktonic protists (Welschmeyer and Lorenzen, 1984; Stoecker and Michaels, 1991; Crawford and Stoecker, 1996). The reduction in growth rate cannot be attributed to a rise in pH and subsequent bicarbonate depletion in the air-sealed culture bottles, as is known to be harmful to phytoplankton (Goldman *et al.*, 1982; Schmidt and Hansen, 2001), since the pH in all growth media used (labelled, non-labelled, and labelled and acidified) was similar, and pH in labelled cultures never exceeded 8.5 at the end of incubations (data not shown). It is unlikely that an acid-volatile, toxic contaminant could have caused the observed effects, because such a contaminant would have to have been extremely potent, since the ^{14}C stock solution was diluted by a factor of 1.2×10^3–2.5×10^3 in the final ^{14}C-labelled culture medium.

Based on our results, we propose that ^{14}C itself resulted in the reduction in growth rate and the apparent increase in cellular C content. At this point we have no definite explanation of how exposure to ^{14}C causes the observed effects. However, it is intriguing to note that phototrophic dinoflagellates seem to be particularly sensitive to long-term exposure to ^{14}C. It is possible that damage to the cell's DNA replication and repair mechanism induced by beta-radiation is the mechanism involved. Radioactivity has been identified as a key factor in DNA damage leading to mammalian cell mortality (Wheeler *et al.*, 1992) and even low amounts of beta-radiation (few µCi ml^{-1}) can cause DNA damage when radioactive compounds are incorporated into the cell's DNA

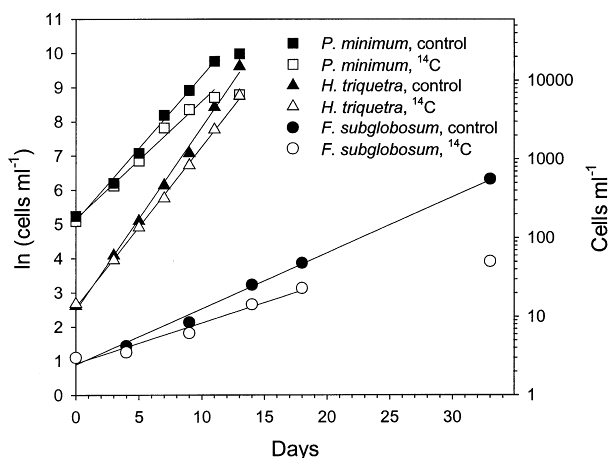

Fig. 1. Growth of three phototrophic dinoflagellate species (*Fragilidium subglobosum*, *Heterocapsa triquetra* and *Prorocentrum minimum*) in culture medium containing ^{14}C and in nonradioactive control medium. Symbols represent means of triplicates. Lines are linear regressions.

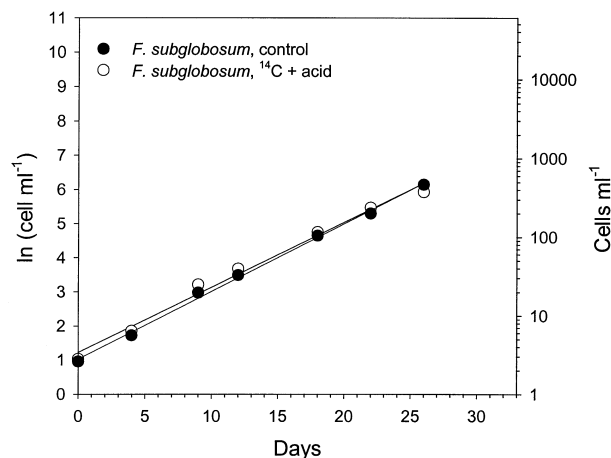

Fig. 2. Growth of *Fragilidium subglobosum* in culture medium containing a ^{14}C stock solution that had been treated with acid prior to addition and in control medium with no ^{14}C stock added. Symbols represent means of triplicates. Lines are linear regressions.

Table II: Growth rates of phototrophic phytoplankton species in ^{14}C-labelled culture medium (μ) and in control medium without ^{14}C added ($\mu_{control}$). SE of slope from linear regression (Figures 1 and 2) shown in parentheses. Difference between growth rate in ^{14}C-labelled culture and control is listed only when statistically significant ($P < 0.05$)

Species	^{14}C-labelled cultures μ(day^{-1})	Control cultures $\mu_{control}$ (day^{-1})	Difference $\mu < \mu_{control}$ (%)
Dinoflagellates			
Fragilidium subglobosum	0.12 (0.01)	0.17 (0.01)	27.9
Fragilidium subglobosum + acid[a]	0.19 (0.01)	0.20 (0.01)	–
Heterocapsa triquetra	0.47 (0.01)	0.53 (0.01)	12.5
Prorocentrum minimum	0.34 (0.02)	0.42 (0.02)	18.7
Others			
Isochrysis galbana	0.55 (0.03)	0.57 (0.02)	–
Phaeodactylum tricornutum	0.71 (0.04)	0.72 (0.04)	–
Rhodomonas salina	0.61 (0.02)	0.62 (0.02)	–

[a] The ^{14}C stock was treated with acid prior to addition to cultures.

Table III: Cellular C content of dinoflagellates measured by the ^{14}C-labelling method and by CHN analysis

Species	^{14}C method C content (pg cell^{-1})	CHN method C content (pg cell^{-1})	Difference ^{14}C > CHN (%)
Phototrophs			
Akashiwo sanguinea	7443 (724)	4647[a] (320)	60
Amphidinium carterae	663 (18)	259[a] (2)	155
Fragilidium subglobosum	11 338 (307)	7609[b] (519)	49
Prorocentrum minimum	238 (12)	71[c] (3)	233
Scrippsiella trochoidea	3358 (107)	2011[a] (75)	67
Heterotrophs			
Noctiluca scintillans	38 723 (1,459)	35 340[a] (4,142)	–
Protoperidinium spp.	8141 (769)	9147[a] (727)	–

SE in parentheses. Difference between results of the two methods is listed only when statistically significant ($P < 0.05$).
[a] Previously published (Menden-Deuer and Lessard, 2000).
[b] Previously published (Skovgaard *et al.*, 2000).
[c] Measured with an infrared gas analyser (Skovgaard, 2000).

(Bedford *et al.*, 1975; Sundell-Bergman and Johanson, 1980). The dinoflagellate nucleus is unique in several respects, including permanently condensed chromosomes and a high DNA content (Spector, 1984; Rizzo, 1987). These characteristics may make the dinoflagellate nucleus particularly susceptible even to low amounts of radioactivity. Reduction in growth due to DNA damage has previously been observed for phytoplankton exposed to increased levels of ultraviolet radiation (Jokiel and York, 1984). There might, however, be other explanations for the observed negative effect of ^{14}C on phototropic dinoflagellates. For example, phytoplankton are known to discriminate against heavier C isotopes during C assimilation (Beardall *et al.*, 1982). It is possible

that intracellular C isotope fractionation can lead to impediment of metabolic processes, and thereby cause the observed effects.

For the application of ^{14}C-labelling it is important to consider whether short-term exposure to ^{14}C could have the same effect as observed here. The ^{14}C method is typically used for incubation times of a few hours [e.g. (Parsons *et al.*, 1984)], which is considerably shorter than the long-term incubations needed to obtain uniform labelling of cells. If ^{14}C has the same consequences during short-term incubations, it would lead to systematic methodological errors for the measurements of primary production in dinoflagellate-dominated phytoplankton populations. Considering the wide use of ^{14}C methods in aquatic ecology, it is essential to study this effect and its causes further. Specifically, it is important to quantify the reduction in growth rate at a higher temporal resolution and extend the comparison of cellular C content to non-dinoflagellate species. Those results will provide insights into the particular physiology of phototrophic dinoflagellates and could have important ramifications for the use of radioactive labelling in aquatic ecology.

ACKNOWLEDGEMENTS

We thank Prof. D. K. Stoecker for inspiration and discussion and Prof. E. J. Lessard for discussion and support through NSF grant OCE-9403426. A.S. was supported by the European Commission (Research Directorate General, Environment Programme, Marine Ecosystems) through the project BIOHAB (Biological Control of Harmful Algal Blooms in European Coastal Waters: Role of Eutrophication), contract EVK3-CT99-00015. BIOHAB is part of the EC EUROHAB cluster. Deutscher Akademischer Austausch Dienst (DAAD) provided support for S.M.D.

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Received on August 19, 2002; accepted on April 15, 2003