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Comparative study of selenium requirements of three phytoplankton species: *Gymnodinium catenatum*, *Alexandrium minutum* (Dinophyta) and *Chaetoceros* cf. *tenuissimus* (Bacillariophyta)

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Abstract. This study investigated the selenium (Se) requirements of three phytoplankton species which commonly bloom in southern Australian estuaries. The present study showed that the toxic dinoflagellate *Gymnodinium catenatum* Graham had an obligate requirement for Se (IV) in culture. After two transfers (~4 weeks ≈ 7 generations) in Se-deficient seawater medium, this phytoplankton species exhibited a decline in growth rate (25%) and biomass yield (90%), while complete cessation of cell division occurred under prolonged (8 weeks ≈ 12 generations) Se starvation. Addition of 10^{-9}–10^{-7} M H_2SeO_3 to nutrient-enriched seawater medium resulted in increased *G.catenatum* growth and biomass yields in direct proportion to the Se concentrations offered. In contrast to *G.catenatum*, Se limitation was observed in the dinoflagellate *Alexandrium minutum* Halim after four transfers (5 weeks ≈ 20 generations) in Se-deficient medium. Exponential growth rates of *A.minutum* decreased slightly (5–10%) when Se was not supplied, but biomass yields decreased as much as 80–90%. The diatom *Chaetoceros* cf. *tenuissimus* Meunier showed no evidence of Se limitation even after eight transfers (8 weeks; >60 generations) in Se-deficient medium. Variations in growth rates and biomass yields between transfers provide valuable information about the relative potential for Se limitation in the three species studied. In addition, differences in Se requirement between these bloom-forming phytoplankton species suggest that this micronutrient may play a role in structuring phytoplankton communities in southern Australian waters.

Introduction

Selenium (Se) is now widely recognized as an important nutritional element for microalgal growth. Pintner and Provasoli (1968) first demonstrated the stimulatory effects of Se on the growth of three axenic marine *Chrysochromulina* spp. (prymnesiophytes), but since then there have been numerous studies which have shown that some phytoplankton species [e.g. the dinoflagellate *Peridinium cinctum* fa. *westii* (Lindström and Rodhe, 1978), the prymnesiophyte *Chrysochromulina breviturrita* (Wehr and Brown, 1985) and the diatom *Thalassiosira pseudonana* (Price *et al*., 1987)] have an absolute requirement for Se or that its addition to culture medium stimulates growth (Wheeler *et al*., 1982; Keller *et al*., 1984; Harrison *et al.*, 1988).

Selenium is present in sea water in three different chemical forms: selenite (SeO_3^{2-}, Se IV), selenate (SeO_4^{2-}, Se VI) and organic selenides (e.g. selenomethionine; Se -II) (Cutter and Bruland, 1984). Particulate Se can be found in

any oxidation state (-II, 0, IV, VI), either as adsorbed/co-precipitated selenite and selenate, organic selenides or elemental Se (Se 0; Cutter, 1985). Dissolved selenite appears to be the most bioavailable form of inorganic Se (Wrench and Measures, 1982), but selenate and organic selenides are generally the most abundant species in estuarine waters (e.g. Cooke and Bruland, 1987; Cutter, 1989a,b). Selenium sources in estuaries include riverine, atmospheric, sediment and point source (pollutant) inputs (e.g. oil refineries) (Cutter, 1989b).

While Se is essential for some phytoplankton at low concentrations (0.1–100 nM), higher concentrations can be toxic (Wheeler *et al*., 1982; Foe and Knight, 1986; Price *et al*., 1987; Boisson *et al*., 1995) and this depends on the chemical form of the element. Some studies have shown that selenate is more toxic than selenite [e.g. the diatom *T.pseudonana* (Price *et al*., 1987)], while others have shown that for different species [e.g. the prymnesiophyte *Cricosphaera elongata* (Boisson *et al*., 1995)] the reverse is true.

Studies on the uptake of Se into algal cells show that the element is incorporated into various biochemical components: amino acids and proteins, soluble carbohydrates, lipids and polysaccharides (Bottino *et al*., 1984; Vandermeulen and Foda, 1988), but the role of Se in cell function is not clear. Some evidence suggests that Se is important in cell division processes and maintaining internal membrane integrity (Doucette *et al*., 1987), while it is also an essential part of the enzyme glutathione peroxidase, which protects cells against the destructive effects of hydrogen peroxide (Overbaugh and Fall, 1982; Price and Harrison, 1988).

In Australia, blooms of toxic dinoflagellates that produce paralytic shellfish poisons (PSP) are frequent events in south-east Tasmanian waters (*Gymnodinium catenatum* Graham), and the Port River estuary, South Australia (*Alexandrium minutum* Halim). *Gymnodinium catenatum* blooms occur within a seasonal temperature window (January–June) following a rainfall 'trigger' and extended periods of calm weather (Hallegraeff *et al*., 1995). *Alexandrium minutum* blooms are also linked to heavy rainfall (and low-salinity sewage effluent), causing resting cyst germination and bloom development in the upper Port River estuary (Cannon, 1993). In addition, blooms of small diatoms such as *Chaetoceros* cf. *tenuissimus* Meunier are common in these estuarine and coastal waters, but are generally not coincident with dinoflagellate blooms (G.M.Hallegraeff, unpublished data). The incidence of toxic dinoflagellate blooms after rainfall and the potential riverine and municipal effluent input of dissolved Se (e.g. Cutter, 1989b) suggests that Se could play a role in phytoplankton species dominance and succession in southern Australian estuaries.

To gain a better understanding of the role of this trace element in phytoplankton bloom dynamics, we examined the Se requirement of these bloom-forming species in culture. The present study compares growth and biomass yields of *G.catenatum*, *A.minutum* and *C.* cf. *tenuissimus* in the absence of Se and with 10^{-9} M added selenite (H_2SeO_3). This is followed by a discussion of the importance of Se in coastal waters and the implications of species-specific Se requirements in bloom development.

Method

Strain history

Cultures were obtained from the CSIRO Collection of Living Microalgae, Hobart, Tasmania, where they are maintained in enriched seawater medium (salinity 28) with GPM nutrients (Loeblich, 1975), soil extract and 10^{-8} M Se (referred to as GSe medium in this laboratory). One xenic clonal strain of each species was used to determine the Se requirement. Strain codes, isolation details and toxicity information are provided in Table I. The diatom *C.* cf. *tenuissimus* Meunier sensu Rhines and Hargraves (1988) was identified by G.M.Hallegraeff using TEM (see Doblin, 1998, p. 32) and is synonymous with *Chaetoceros galvestonensis*, *Chaetoceros simplex* var. *calcitrans*, *Chaetoceros calcitrans* and *Chaetoceros calcitrans* forma *pumilus* (see Rhines and Hargraves, 1988).

Culture conditions

Experimental cultures were grown in sea water (collected at an offshore station near Maria Island, south-east Tasmania), which was filtered through a series of glass-fibre, activated charcoal and 0.22 µm filters, and autoclaved in Teflon bottles. Salinity was adjusted to 28 using sterile Milli-Q (MQ) water. Nutrients were added to yield GPM medium (Loeblich, 1975) at a concentration of 1/10 of the original recipe with the addition of Se as selenite (10^{-9} M H_2SeO_3; Aldrich Chemical Co., lot no. 1124KH) and no added soil extract (GSe/10; Table II). Cultures were grown in acid-washed, 50 ml Pyrex or KIMAX tubes (loosely capped Teflon-lined lids) at a temperature of 18°C with bottom illumination of 180 µmol photons photosynthetically active radiation (PAR) m^{-2} s^{-1} (measured at the base of tubes using a Biospherical Instruments QSL-100 light sensor with integrating sphere) from Philips Deluxe cool white fluorescent lights on a 12:12 h light:dark cycle. Cultures were not axenic, but precautions were taken to minimize bacterial contamination by carefully timed transfers (late exponential phase) and minimal carry-over volumes. In addition, light microscope (Zeiss Axioplan) inspections were made on randomly selected exponential phase cultures to check for bacterial colonization.

Table I. Characteristics of algal strains used in experiments

Species	Strain code	Source	Date of isolation	Toxic
Gymnodinium catenatum (GCDE08)	CS-301	Derwent Estuary, Tasmania	15 June 1987	+ (PSP)
Alexandrium minutum (AMAD06)	CS-323	Port River, South Australia	27 October 1987	+ (PSP)
Chaetoceros cf. *tenuissimus*	CS-365/1	St Helens, Tasmania	1989	–

Table II. Composition of GSe/10 growth medium (based on GPM medium; Loeblich 1975)

Additive	Nutrient	Concentration (μM)
Nitrate	KNO_3	200
Phosphate	K_2HPO_4	20
Metals (pH adjusted to 7.5)	$EDTA(Na,H_2O)_2$	10
	H_3BO_3	55
	$FeCl_3$·6H2O	0.5
	$MnCl_2·4H_2O$	2.0
	$CoCl_2·6H_2O$	0.053
	$ZnCl_2$	0.23
Selenium	H_2SeO_3	0.001
Vitamins:	Vitamin B_{12}	0.74
	Thiamin·HCl	3.0
	Biotin	0.0004

Growth measurements

Growth rates of cultures were calculated from changes in *in vivo* fluorescence (Brand *et al*., 1981), measured at the same time each day (3 h after the start of the light period):

$$\mu\ (\text{day}^{-1}) = \ln (F_1/F_0)/(t_1 - t_0) \qquad \text{(Guillard, 1973)}$$

Each treatment had a minimum of three replicates plus at least one blank (medium containing no algae); fluorescence readings (F_1, F_0) at each time period (t_1, t_0) were corrected for fluorescence of blank tubes at the same time.

Yield estimates

Cultures were sampled at the end of exponential phase for cell density and chlorophyll *a* (Chl *a*) biomass. Duplicate 1 ml samples were taken for cell counts and immediately fixed with acid Lugol's solution (0.2%; Lovegrove, 1960). Depending on cell concentration, samples were diluted with sterile culture medium and counted with a Sedgwick–Rafter counting chamber (Rigosha, Japan) under bright field using a Zeiss Axioplan microscope. When possible, estimates were based on counting a minimum of 400 cells (~10% error; Guillard, 1973). Water mounts of unpreserved cultures were also made for observations of living cells.

Subsamples were also filtered through 25 mm GF/F filters under dim light and filters stored at –20°C for later Chl *a* analysis. Pigments on filters were extracted with 100% acetone and the mixture sonicated for 45 s (0°C). Extracts were left on ice in the dark for ≥2 h, centrifuged (2000 *g* for 5 min) and Chl *a* in the supernatant determined spectrophotometrically, using the appropriate equations of Jeffrey and Humphrey (1975).

Growth of G.catenatum *in the presence and absence of Se (IV)*

Experimental *G.catenatum* cultures were inoculated with GSe exponential phase cells into GSe/10 containing no added Se (–Se) or GSe/10 with 10^{-9} M Se (IV)

added as H_2SeO_3 (+Se). The initial cell concentration in each culture was ~1 × 10^5 cells l^{-1}. Cultures were transferred approximately every 18 days to maintain cells in the exponential phase of growth, with the inoculum (1 ml) being diluted 1:30 (the minimum carry over for sustained growth of *G.catenatum* in culture; M.A.Doblin, unpublished data). Growth was monitored by fluorescence, and after exponential phase during the second transfer, cultures were sampled for cell density and Chl *a*.

To test the recovery rate of cells after long-term Se limitation, three replicates in each treatment (+Se, –Se) were transferred for a third time into Se-replete medium and another three replicates transferred to Se-deplete medium (yielding ++, +–, –+ and –Se treatments). Subsequent growth was monitored by fluorescence and cultures were sampled at the end of the third exponential phase for cell density and Chl *a*.

Growth of G.catenatum *in different Se (IV) concentrations*

To test the lower limit of requirement and level of tolerance of Se in *G.catenatum*, cells were grown for two transfers (~5 weeks) in nutrient-enriched seawater medium (GSe/10), with added H_2SeO_3 concentrations of 10^{-11}, 10^{-9} and 10^{-7} M. GSe/10 with no added Se formed the control. Growth was monitored by *in vivo* fluorescence and during the exponential phase of the second transfer, estimates of bacterial abundance were made by aseptically subsampling cultures and plating out onto seawater agar (with added vitamins; Lewis *et al*., 1986). Cultures were sampled for cell counts and Chl *a* biomass at the end of the second exponential phase.

Se requirement of other phytoplankton species

Two other phytoplankton species were tested for their Se requirement: another toxic (PSP-producing) dinoflagellate, *A.minutum*, and a south-east Tasmanian, bloom-forming diatom, *C.* cf. *tenuissimus*. Unialgal cultures of both species were maintained over 4–8 transfers (~8 weeks) in GSe/10 containing no added Se (–Se) or GSe/10 with 10^{-9} M H_2SeO_3 (+Se). Dilution ratios between transfers were 1:100 for *A.minutum* and 1:300 for *C.* cf. *tenuissimus*. Differential growth rates between species resulted in an unequal number of transfers (*G.catenatum* = 3, *A.minutum* = 5, *C.* cf. *tenuissimus* = 8), but after a period of ~8 weeks, cultures were sampled for cell density and Chl *a* at the end of exponential phase. Eight weeks was equivalent to ~12 generations for *G.catenatum*, >30 generations for *A.minutum* and >60 generations for *C.* cf. *tenuissimus*.

Statistical analyses

Growth rates were determined by least squares linear regression using a minimum of four successive fluorescence values. These points were selected from early exponential phase and yielded the greatest R^2 value. All slopes were significantly greater than zero, except those from *G.catenatum* cultures which were in Se-deficient medium for three successive transfers. Differences in growth rates,

cell yields and Chl *a* content between transfers or Se treatments were tested using ANOVA and, when appropriate, planned comparisons were used to determine which transfer(s)/treatment(s) were different. The analyses were calculated using Systat 5.1 at a significance level of $\alpha = 0.05$.

Results

Growth of G.catenatum *in the presence and absence of Se (IV)*

Exponential growth rates of *G.catenatum* were identical in the absence of Se and with 10^{-9} M added Se during the first transfer (~2.5 weeks). However, during the second transfer, cells in Se-deficient medium grew 35% slower than in Se-replete medium ($\mu = 0.17$ day^{-1} compared to $\mu = 0.27$ day^{-1}; Figure 1a and b; $n = 12$, $F = 168$, $P < 0.0001$). In addition, cell yields in Se-deficient cultures were 90% lower compared to Se-replete cultures (Figure 1c; $n = 6$, $F = 44$, $P < 0.0001$).

Cells supplied with Se (10^{-9} M) for two transfers and which were then, on the third transfer, transferred into either Se-replete (++Se) or Se-deficient medium (+–Se) had the same growth rate ($\mu = 0.32$ day^{-1}; Figure 2a and b; $n = 3$, $F = 0.13$, $P = 0.73$), but cell yields were considerably reduced in the +–Se treatments ($n = 3$, $F = 14$, $P = 0.01$). Cells which were not supplied with Se for two transfers and which were then transferred for a third time into Se-replete medium (–+Se) grew 25% more slowly than cells which were cultured in the presence of Se for all transfers ($\mu = 0.23$ day^{-1}; Figure 2b; $n = 3$, $F = 73$, $P = 0.001$). Cells cultured in medium containing no Se for three transfers ceased to grow (Figure 2a and b).

No obvious morphological differences between *G.catenatum* cells grown in the presence or absence of Se were observed at the light microscope level; however, there was a higher proportion of single cells and 2- to 4-celled chains in Se-deficient cultures compared with normal 8-celled chains in Se-replete medium (85% single cells compared to 57%; $n = 3$, $F = 4.72$, $P = 0.10$).

Growth of G.catenatum *in different Se (IV) concentrations*

During the second transfer into medium with no added Se and with 10^{-11} M Se, *G.catenatum* cultures had the same exponential growth rate and biomass yields (Figure 3), but at Se concentrations $>10^{-11}$ M, growth and biomass yields of *G.catenatum* were stimulated. Cells supplied with 10^{-9} and 10^{-7} M Se grew 10–20% faster ($\mu = 0.24$ day^{-1}) than those supplied with 0 or 10^{-11} M added Se ($\mu = 0.20$ day^{-1}; Figure 4a; $n = 4$; $F = 28$, $P = 0.0003$). Addition of 10^{-9} and 10^{-7} M Se caused a substantial increase in cell yields compared to cultures with no added Se (Figure 3b; $n = 4$, $F = 137$, $P < 0.0001$). Chlorophyll *a* biomass in cultures with 10^{-9} and 10^{-7} M Se was also elevated compared to cultures with 0 and 10^{-11} M Se (Figure 3c; $n = 4$, $F = 244$, $P < 0.0001$).

Bacterial abundance in cultures did not change as a result of Se addition ($n = 3$, $F = 0.37$, $P = 0.71$), and with the added precautions of inoculating and transferring cultures with exponential phase cells using minimal culture volumes, we are confident that addition of Se caused a direct effect on algal growth and that it was not due to a bacterial interaction.

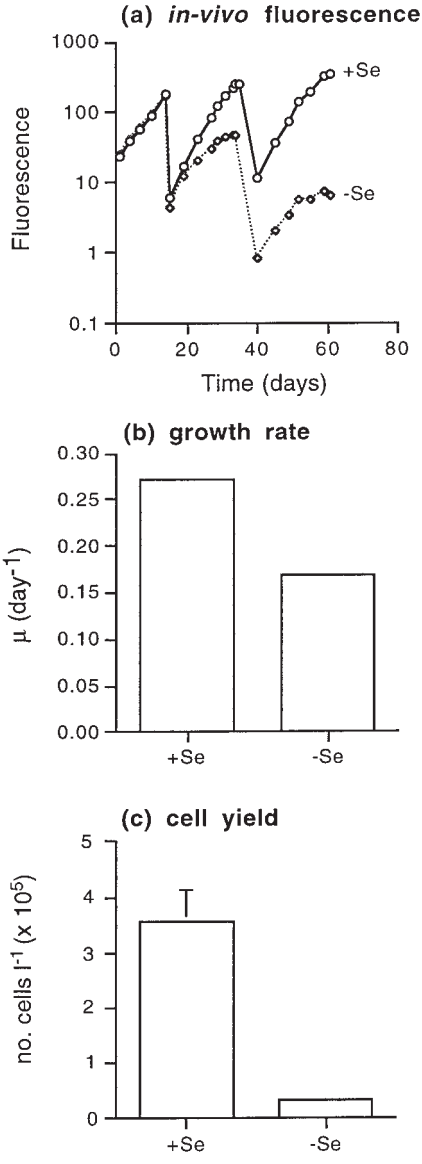

Fig. 1. (**a**) Growth of the dinoflagellate *G.catenatum* determined by *in vivo* fluorescence over three transfers in enriched seawater medium (GSe/10), (**b**) exponential growth rates and (**c**) cell yields of *G.catenatum* during the second transfer in enriched seawater medium (GSe/10). –Se, no added selenium. +Se, with 10^{-9} M H_2SeO_3; n = 12; error bars = SE.

Se requirement of other phytoplankton species

During the first, second and third transfers into media containing no or 10^{-9} M Se, exponential growth rates of *A.minutum* were the same (μ = 0.65 day^{-1}; Figure 4a and b; n = 4, F = 0.20, P = 0.66). During the fourth and fifth transfers, however,

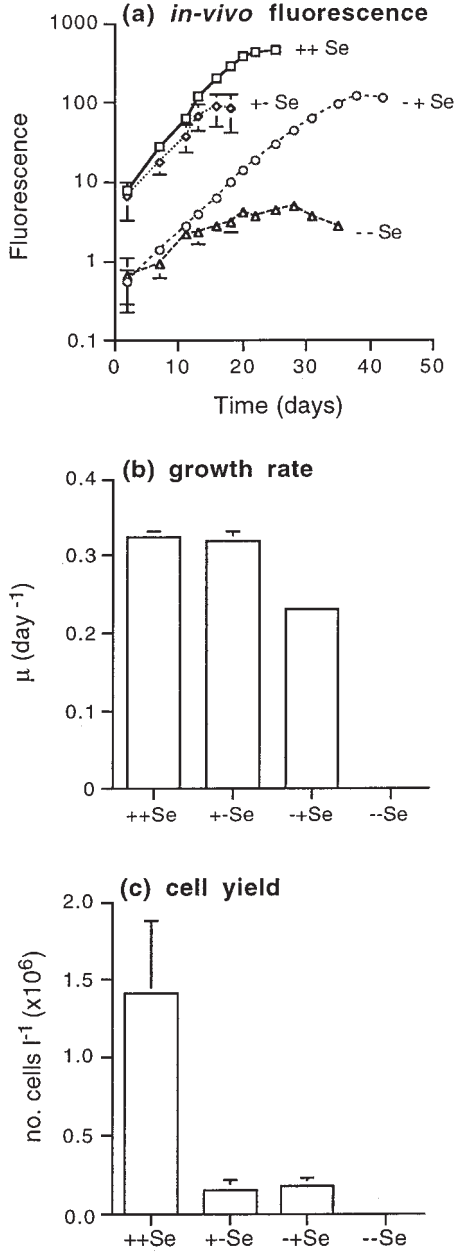

Fig. 2. (**a**) *Gymnodinium catenatum* growth curves determined by *in vivo* fluorescence, (**b**) exponential growth rates and (**c**) cell yields after two growth cycles in the presence or absence of Se and transfer of cells in late exponential phase into enriched seawater medium with no added selenium (–Se) or with 10^{-9} M H_2SeO_3 (+Se). ++Se/– Se, cells grown for three consecutive transfers (equivalent to 12 generations; ~8 weeks) in +Se/–Se medium (respectively). +–Se, cells grown for two transfers in +Se medium and then transferred to –Se medium; –+Se, cells grown for two transfers in –Se medium and then transferred to +Se medium. n = 3; error bars, SE.

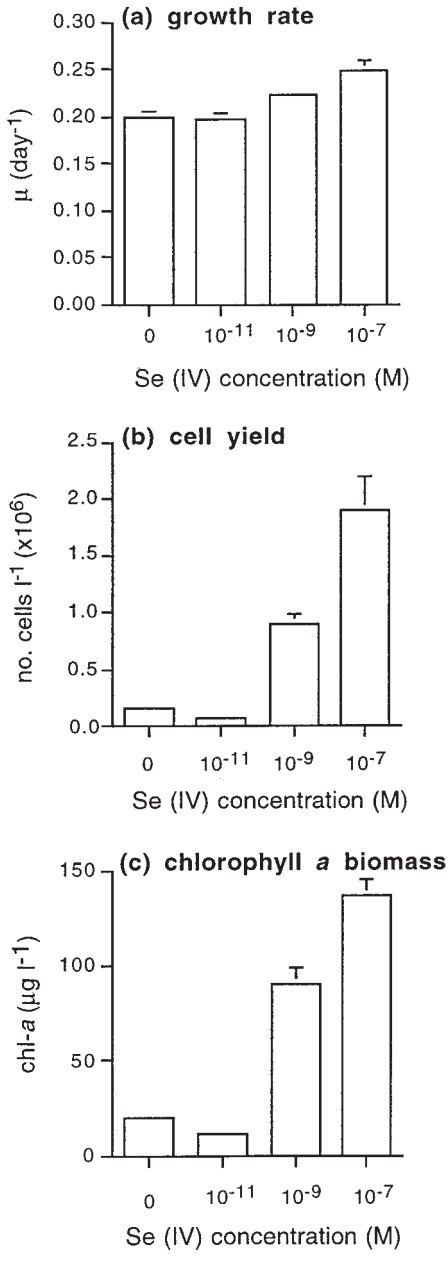

Fig. 3. (**a**) Exponential growth rates, (**b**) cell yields and (**c**) chlorophyll *a* biomass of *G.catenatum* after two transfers (~5 weeks) in enriched seawater medium (GSe/10) with 0, 10^{-11}, 10^{-9} and 10^{-7} M added H_2SeO_3. $n = 4$, error bars = SE.

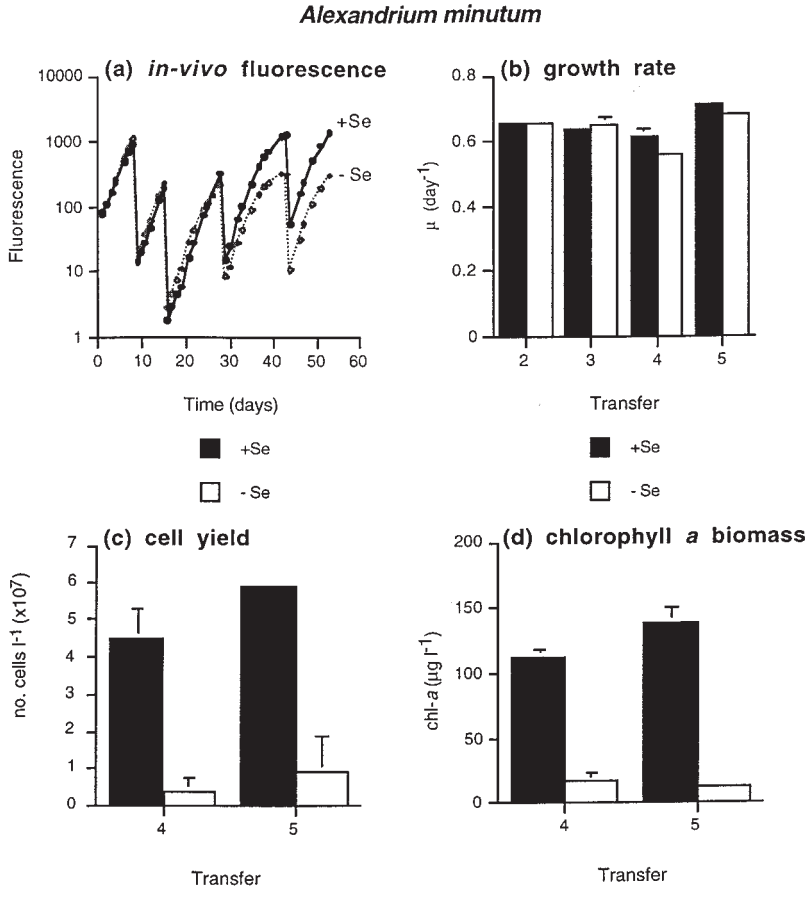

Fig. 4. (**a**) *In vivo* fluorescence, (**b**) exponential growth rates, (**c**) cell yields and (**d**) chlorophyll *a* biomass of the dinoflagellate *A.minutum* during five transfers (>30 generations; ~8 weeks) in enriched seawater medium (GSe/10) with no added selenium (–Se) and with 10^{-9} M H_2SeO_3 (+Se). Data for transfers 4 and 5 only are shown in (c) and (d). $n = 4$; error bars = SE.

growth was 5–10% slower in cultures not supplied with Se ($\mu = 0.61$ day^{-1} compared to 0.66 day^{-1}; $n = 4$, $F = 8.0$, $P = 0.015$). Cell yields were also lower in –Se cultures during the fourth and fifth transfer (Figure 4c; $n = 4$, $F = 122$, $P < 0.0001$), as were Chl *a* levels (Figure 4d; $n = 4$, $F = 53$, $P < 0.0001$).

In contrast to both the dinoflagellates *A.minutum* and *G.catenatum*, growth rates and cell yields of the diatom *C.* cf. *tenuissimus* were the same over all eight transfers (Figure 5a–d). Furthermore, growth rates and biomass yields did not decline when cells were not supplied with Se ($\mu = 1.4$–1.6 day^{-1}; $n = 4$, $F = 0.76$, $P = 0.40$; cell density: $n = 4$, $F = 0.03$, $P = 0.87$; Chl *a*: $n = 4$, $F = 0.01$, $P = 0.92$).

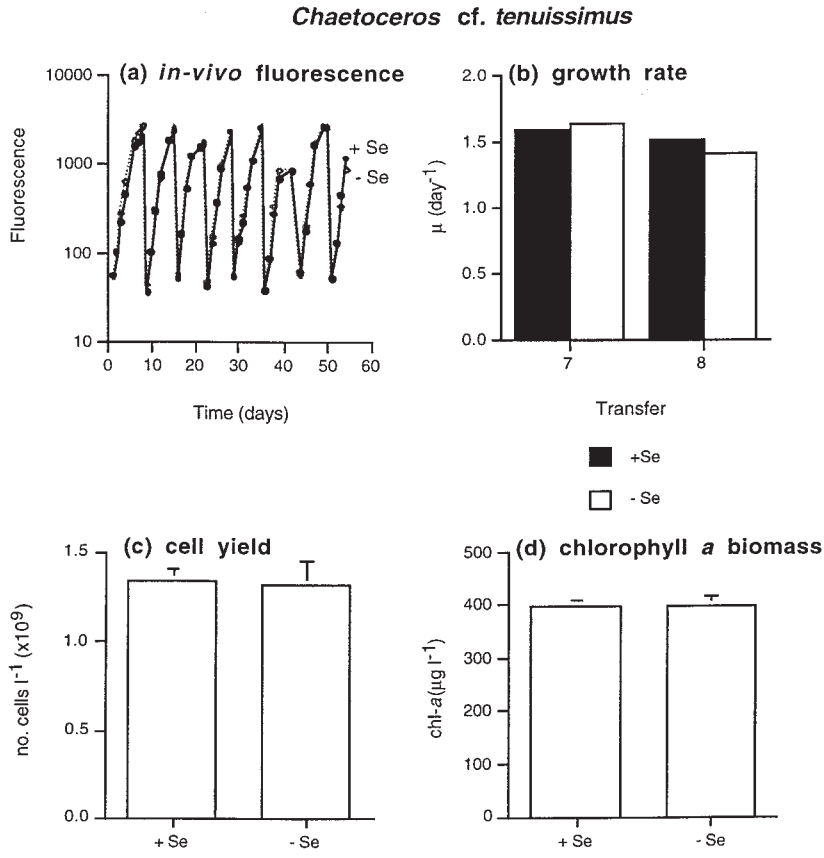

Fig. 5. (**a**) *In vivo* fluorescence, (**b**) exponential growth rates, (**c**) cell yields and (**d**) chlorophyll *a* biomass of the diatom *C.* cf. *tenuissimus* during eight transfers (>60 generations; ~8 weeks) in enriched seawater medium (GSe/10) with no added selenium (–Se) and with 10^{-9} M H_2SeO_3 (+Se). Data for transfers 7 and 8 are shown in (b), while data from transfer 8 only are shown in (c) and (d). $n = 4$; error bars = SE.

Discussion

Se requirement of G.catenatum *and recovery from Se limitation*

The obligate requirement for Se by *G.catenatum* was conclusively demonstrated by the reduction of exponential growth rates and biomass yields during the second transfer into enriched seawater medium (GSe/10) with no added Se. Cessation of cell division in cultures which had not been supplied with Se for ~10 generations (5 weeks) and the resumption of growth upon addition of 10^{-9} M H_2SeO_3 to Se-deplete stationary phase cultures provide further evidence of a Se requirement.

Cells were able to recover from Se starvation, depending on the length of time

they were exposed to Se-deficient conditions. Recovery from short-term Se limitation at the end of exponential phase is relatively rapid, in the order of a few days (Doblin *et al*., 1999). When cells which had been cultured for two transfers in Se-deficient medium were resupplied with Se (–+Se; Figure 2a), growth increased, but it was still slower than growth of cells which had received a continuous supply of Se. Cells cultured without Se for ≥10 generations (≥5 weeks) ceased to grow, indicating that this is the number of cell divisions required to reach the minimum Se cell quota.

No obvious change in *G.catenatum* cell morphology could be observed at the light microscope level as a result of Se limitation, unlike other studies which have shown that Se deficiency causes morphological and ultrastructural changes to cells. For example, Price *et al*. (1987) found that Se-limited cells of *T.pseudonana* had larger cell volumes, were elongated in the pervalvar axis, and had a different mitochondrial, chloroplast and reticular membrane system configuration (Doucette *et al*., 1987). However, changes occurred in the growth habit of *G.catenatum* when grown under Se-deficient conditions, with an increasing (but not significantly different) proportion of single cells and shorter chains. The break-up of chains is typical of *G.catenatum* at the end of exponential phase (Blackburn *et al*., 1989) and under suboptimal growth conditions (e.g. nitrogen limitation) (S.I.Blackburn, unpublished data); however, in this study, shorter chain lengths were observed even during early exponential phase in Se-deficient medium.

Growth of G.catenatum *in different Se (IV) concentrations*

Growth and biomass yields of cultures with 10^{-11} M added Se were the same as those which had no added Se, indicating that background Se levels in the enriched seawater medium are of this order of magnitude. Price *et al*. (1987) estimate the background Se in ESAW medium (with nutrient solutions which have passed through a Chelex 100 column to eliminate cationic trace metal contaminants) to be $<10^{-12}$ M. In comparison, concentrations of Se from 10^{-9} to 10^{-7} M stimulated *G.catenatum* growth and biomass production (Figure 3), with no indication of toxicity. Higher Se concentrations were not tested, mainly because they exceed levels typically found in estuarine and coastal waters (Takayanagi and Wong, 1984; Cutter, 1989b). While our experiments did not determine the upper limit of Se tolerance, we clearly showed that in sea water, low (nM) levels of Se are limiting to *G.catenatum* growth and biomass production.

Se requirement of other phytoplankton species

Monitoring of cultures over consecutive transfers in Se-replete and Se-deficient medium showed that like *G.catenatum*, the dinoflagellate *A.minutum* also has a Se requirement. It took a greater number of generations (~30), but the same amount of time (~5 weeks), for *A.minutum* cells to show a reduction in exponential growth rates and biomass yields. In contrast, there was no change in growth or biomass yields in *C.* cf. *tenuissimus* cultures after ~60 generations, indicating that this diatom species has no Se requirement, or that its Se requirement

was met by the background Se levels in the culture medium. This adds to the findings of Harrison *et al.* (1988) who demonstrated the variable Se requirement within the *Chaetoceros* genus, with *C.debilis*, *C.pelagicus* and *C.vixvisibilis* having a Se requirement, and *C.gracilis* and *C.simplex* not having one. Doblin *et al*. (submitted) showed that intraspecific differences in Se requirements also exist, with some *G.catenatum* strains having a greater requirement for Se than others.

Addition of Se to cultures has been demonstrated to stimulate other toxic or harmful bloom-forming species of phytoplankton. For example, Usup and Azanza (1998) have shown that Se (added in the form of selenite and organic selenide) increases cell yields of the dinoflagellate *Pyrodinium bahamense*. Similarly, growth and cell yields of the marine chrysophyte *Aureococcus anophagefferens* are stimulated in the presence of selenite (Cosper *et al*., 1993). In addition, growth of the red tide raphidophyte flagellate, *Chattonella verruculosa*, is increased by addition of selenite at concentrations of 1.0–10 nM (Imai *et al*., 1996). There appears to be no consistency in the Se requirements of toxic versus non-toxic phytoplankton or those belonging to certain algal classes, but a review of the literature indicates that the number of species with demonstrated Se requirements is increasing (Table III).

Role of Se in structuring phytoplankton populations

Several field investigations have demonstrated that the distribution of Se is directly related to phytoplankton abundance and productivity. Wrench and Measures (1982) found that temporal changes in Se levels (relative proportion of Se VI and Se IV) were correlated with pulses of primary productivity in Bedford Basin, Nova Scotia, Canada, a fjord ecosystem. A similar pattern was observed by Lindström (1983) in Lake Erken, Sweden, where the vertical distribution of Se IV was correlated with the abundance of the dinoflagellate *Woloszynskia ordinata*, showing a decline between 1 and 5 m coincident with the subsurface bloom maximum. Ishimaru *et al*. (1989), in their investigations of the toxic dinoflagellate *Gymnodinium nagasakiense*, found that Se IV levels during the month preceding the 1988 bloom in Wakayama waters, Japan, were low (<0.1 nM) and greatest concentrations (2.8 nM) coincided with maximum cell density.

Inorganic Se (Se IV and VI) concentrations in the Huon Estuary, south-east Tasmania, are <0.01 nM (below detection; G.Cutter, personal communication) in spring during low flow periods and are thus potentially limiting for the toxic dinoflagellates *G.catenatum* and *A.minutum* (but not for the diatom *C.* cf. *tenuissimus*). The absence of any significant anthropogenic sources of Se (e.g. municipal and industrial effluents) in the Huon Estuary suggests that riverine input of dissolved inorganic Se during early summer run-off events (when water temperatures are >15°C) may be a critical trigger for dinoflagellate blooms in this estuary. Additionally, factors which co-vary with Se in river run-off, such as input of other nutrients (e.g. iron) and associated changes in salinity, temperature, pH, dissolved oxygen and optical properties, would also affect phytoplankton growth and may play a role in regulating bloom dynamics.

Table III. Phytoplankton species with demonstrated selenium requirement

Species	No. of transfers	Reference
Diatoms		
Amphiprora hyalina	2	Harrison *et al*., 1988
Chaetoceros debilis	2	Harrison *et al*., 1988
Chaetoceros pelagicus	1	Harrison *et al*., 1988
Chaetoceros vixvisibilis	1	Harrison *et al*., 1988
Coscinodiscus asteromphalus	2	Harrison *et al*., 1988
Corethron criophilum	2	Harrison *et al*., 1988
Ditylum brightwellii	1	Harrison *et al*., 1988
Skeletonema costatum (strain 18c NEPCC)	2	Harrison *et al*., 1988
Skeletonema costatum (strain 611 NEPCC)	2	Harrison *et al*., 1988
Skeletonema costatum (strain 616 NEPCC)	1	Harrison *et al*., 1988
Stephanodiscus hantzschii var. *pusillus*?	1	Lindström, 1983
Stephanopyxis palmeriana	3	Harrison *et al*., 1988
Thalassiosira pseudonana	2	Price *et al*., 1987; Harrison *et al*., 1988
Thalassiosira oceanica	2	Harrison *et al*., 1988
Thalassiosira rotula	1	Harrison *et al*., 1988
Thalassiosira aestivalis	5	Harrison *et al*., 1988
Dinoflagellates		
Alexandrium minutum[a]	4	Present work
Gymnodinium catenatum[a]	2	Present work
Gymnodinium nagasakiense[a]	1	Ishimaru *et al*., 1989
Katodinium rotundatum?	3	Harrison *et al*., 1988
Peridinium cinctum fa. *westii*	1	Lindström and Rodhe, 1978
Pyrodinium bahamense[a]	1	Usup and Azanza, 1998
Prymnesiophytes		
Chrysochromulina breviturrita	2	Wehr and Brown, 1985
Chrysochromulina kappa	?	Pintner and Provasoli, 1968
Chrysochromulina brevefilum	?	Pintner and Provasoli, 1968
Chrysochromulina strobilus	?	Pintner and Provasoli, 1968
Chrysochromulina polylepis[a]	1	Dahl *et al*., 1989; Edvardsen *et al*., 1990
Raphidophytes		
Chattonella verruculosa[a]	2	Imai *et al*., 1996
Chlorophytes		
Platymonas subcordiformis	1	Wheeler *et al*., 1982
Chrysophytes		
Aureococcus anophagefferens[a]	4	Cosper *et al*., 1993

?Uncertainty about requirement or number of transfers.
[a]Harmful algae.

Preliminary data indicate that the major Se fraction in the Huon Estuary is 'organic selenide' (Se II + 0; operationally defined as the difference between total Se and Se VI + VI; Cutter and Bruland, 1984) and that organic selenide concentrations are positively correlated with phytoplankton biomass and salinity (G.Cutter, personal communication). The low concentration of inorganic Se and relatively high levels of organic selenide are typical of unpolluted estuaries and indicate rapid biological utilization of inorganic Se, resulting in minimal accumulation of Se IV and VI (Lindström, 1983). Such nutrient-like behaviour is also found in oceanic waters such as the Atlantic Ocean, where Se IV and VI show surface-water depletion and deep-water enrichment, and organic Se

concentrations are highest at the surface and rapidly attenuate with depth (Cutter and Cutter, 1995).

Although accumulation of organic Se in surface waters suggests that it is relatively refractory, uptake of organo-selenium compounds may occur if inorganic Se concentrations are limiting. Direct uptake of organic Se (-II, 0) has been demonstrated in the diatom *Stephanodiscus hantzschii* var. *pusillus* (seleno-methionine and seleno-cystine; Lindström, 1983) and in the prymnesiophyte *Chrysochromulina breviturrita* (DL-seleno-methionine only; Wehr and Brown, 1985). More recently, Gobler *et al*. (1997) demonstrated that organic Se released by viral lysis of the chrysophyte *A.anophagefferens* was slightly more bioavailable to the diatom *T.pseudonana* than inorganic Se IV after exposure to sunlight (one photoperiod). While riverine Se input during early summer run-off events may provide a pulse of relatively bioavailable inorganic Se for phytoplankton uptake, recycling of organic Se in the surface layer may provide an adequate supply of Se for an overwintering phytoplankton population.

This study demonstrates that the toxic dinoflagellates *G.catenatum* and *A.minutum* have a requirement for Se, while the small diatom *C.* cf. *tenuissimus* does not. Very low (<0.01 nM) concentrations of Se found in the Huon Estuary suggest that this trace element could be limiting for growth of some phytoplankton (e.g. *G.catenatum* and *A.minutum*) and that riverine input of Se after rainfall may be a critical trigger for *G.catenatum* blooms in this region. In comparison, blooms of the diatom *C.* cf. *tenuissimus*, with no Se requirement, have no such pre-requisite for river run-off (Se input). The interspecific differences in Se requirement demonstrated in this study provide valuable information about the factors determining phytoplankton species succession and dominance in southern Australian estuaries and may help to unravel the complexity of harmful algal bloom ecology.

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