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Iron and zinc content of *Hormosira banksii* in New Zealand

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Abstract In the Northern Hemisphere, brown seaweeds in the Order Fucales have been used extensively as bio-monitors of heavy metal contamination in sea water, but in New Zealand there has been no assessment of the suitability of the Fucales in this role. We measured iron (Fe) and zinc (Zn) concentrations in the intertidal fucalean seaweed *Hormosira banksii* at three sites in Otago, south-eastern New Zealand and two sites at Leigh, north-eastern New Zealand. There was no evidence of Zn or Fe contamination at any site studied. Zn levels for *H. banksii* from Otago Harbour followed a trend similar to an earlier study of *Ulva* sp. and the Fe content reflected patterns previously observed in sea water. Thus, as for Northern Hemisphere fucalean seaweeds, *H. banksii* is potentially useful as a bio-monitor for heavy metals in sea water. Levels of Fe and Zn were among the lowest of any brown seaweed worldwide, and the Fe content of *H. banksii* from Waterfall Reef, Goat Island Marine Reserve, Leigh, was 50% lower than samples from Otago. These low trace metal levels led us to examine if the growth of *H. banksii* in New Zealand is limited by Fe or Zn. The physiological requirements of Fe and Zn for growth were calculated and compared with measured values and we suggest that despite low Zn and Fe levels, these trace elements **do not** limit the growth of *H. banksii*.

Keywords bio-monitor; *Hormosira banksii*; Fe; Zn; New Zealand; seaweed; trace metal

INTRODUCTION

Seaweeds fulfil the major requirements for suitable bio-monitors of metal pollution of the surrounding aquatic environment because they are sedentary, large, robust enough to be handled in laboratory experiments, widespread geographically, and for many species (Order Fucales in particular) the uptake and accumulation kinetics of many metals and compounds has been well researched (e.g., Munda 1979; Munda & Hundik 1986; Stengel & Dring 2000; Vasconcelos & Leal 2001; Rainbow et al. 2002). It is well known that trace metal concentrations in seaweeds are many times greater than their concentrations in the sea (e.g., Phillips 1990; Vasconcelos et al. 2001) and are not subject to short-term fluctuations thus providing a time-integrated measure of the supply of a bio-available metal (Rainbow 1995).

The seaweeds most commonly used as bio-monitors of trace metals in sea water are brown

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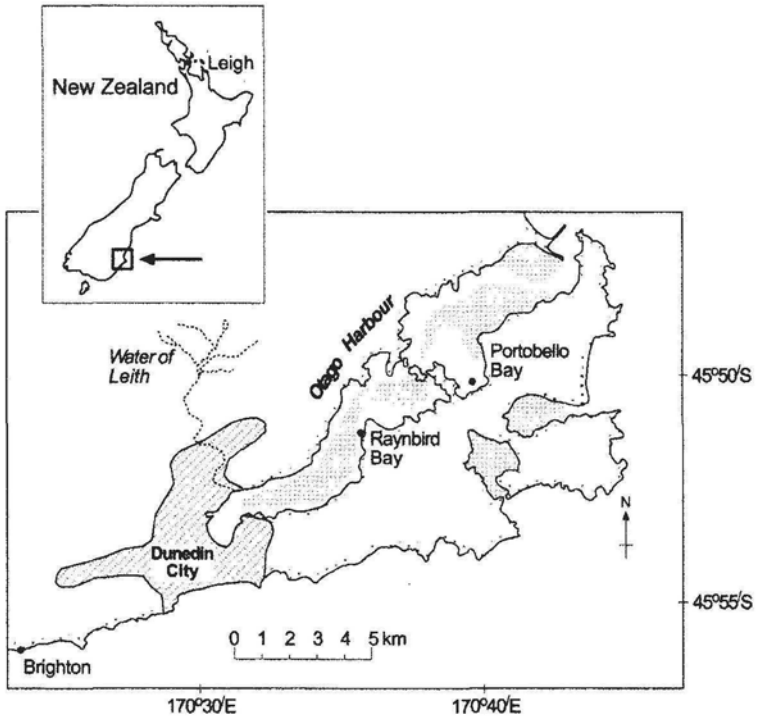

Fig. 1 Map of New Zealand showing locations of Leigh and Otago, and close-up map of the Otago coastline showing location of Otago sampling sites.

seaweeds of the Order Fucales (such as *Fucus* sp. and *Ascophyllum nodosum*) or green seaweeds (such as *Ulva* or *Enteromorpha*). These seaweeds are often chosen as bio-monitors because they are widely distributed and so any studies conducted in different regions can be directly compared (e.g., Say et al. 1990). To date, most bio-monitor studies using seaweeds have been conducted on Northern Hemisphere species (e.g., Fuge & James 1973, 1974; Forsberg et al. 1988; Say et al. 1990; Riget et al. 1995; Leal et al. 1999; Vasconcelos et al. 2001; Rainbow 2002), with only a few Southern Hemisphere studies (Higgins & Mackey 1987a,b; Vásquez et al. 1999) and only one published study on New Zealand seaweeds (Brown et al. 1999). *Hormosira banksii* (Turner) Descanaisne is an intertidal seaweed of the Order Fucales found throughout New Zealand and southern Australia. Our first objective was to examine the spatial and temporal variations of the iron (Fe) and zinc (Zn) content of *H. banksii* from an open coastal site and within a more sheltered estuarine environment (Otago Harbour) to compare its suitability as a bio-monitor with the green seaweeds used previously for this purpose by Brown et al. (1999) and with Northern Hemisphere species. We also measured Fe and Zn in samples from two sites at Leigh, north-eastern New Zealand, including Waterfall Reef at the relatively pristine Goat Island marine reserve (established in 1965 as the first marine reserve in the world).

Iron and Zn are not just indicators of trace-metal contamination but they are also essential nutrients which can be limiting to macro-algal growth and production. Fe has well-characterised roles in photosynthesis, respiration, nitrogen, and sulfur metabolism, and the metabolism of reactive oxygen species (Raven et al. 1999). Zn has important roles as an essential component of carbonic anhydrase (CA) (Raven et al. 1999). Fe is known to limit growth rates of phytoplankton in High Nitrogen Low Chlorophyll (HNLC) regions of the Pacific ocean (e.g., Coale et al. 1996) and may also limit seaweed growth in some coastal waters (Suzuki et al. 1995). In New Zealand coastal waters nitrate concentrations are rarely below the limits of detection and typically above 0.5 μM (Hay 1990; Phillips & Hurd 2004; Rees unpubl. data). The apparent year-round availability of nitrate to seaweeds in New Zealand led us to question if the Fe content of *H. banksii* might be limiting growth of this species. Further evidence that a brown seaweed has, in its recent

evolutionary history, suffered from Fe deficiency comes from the observation that embryos of *Fucus edentatus* develop colourless nutrient-absorbing hairs when cultured in Fe-deficient media (MacLachlan 1977). There is less information on the role and potential of Zn as a limiting factor for seaweed growth (Raven et al. 1999).

Our second objective was to examine if the growth of *H. banksii* might be limited by its Fe and Zn content. This was achieved by modelling the Fe and Zn requirements and comparing them to levels measured from *H. banksii* collected from both the northern and southern marine area in New Zealand. Fe concentrations in sea water at the northern-most site at Waterfall Reef are 10-fold lower (0.018 nmol g^{-1}) than those in Otago Harbour (Croot & Hunter 2000), and we predicted a lower Fe content in *H. banksii* at the northern site.

MATERIALS AND METHODS

Site descriptions

Samples of *H. banksii* were collected from three sites in Otago, South Island, New Zealand (Fig. 1): Brighton Beach, Portobello Bay, and Raynbird Bay. Brighton Beach (45°56′75"S, 170°20′E) is an open coastal site that can be regarded as a clean site in terms of pollution (Ryder et al. 1997). Portobello Bay (45°50′25"S, 170°39′50"E) and Raynbird Bay (45°51′20"S, 170°35′75"E) are located within Otago Harbour. Portobello Bay was selected as a site within the harbour that is closest to the entrance of Otago Harbour at which *H. banksii* was found and hence would be less influenced by industrial sources than a site further towards the head of Otago Harbour. Raynbird Bay was selected as a site that is nearest to Dunedin City (population 120 000) at which *H. banksii* was found and was expected to be influenced more strongly by the industrial sources from Dunedin City than the other sites. This site was also near a domestic sewage outfall at Company Bay which was closed in 1995.

To compare if the Zn and Fe contents of *H. banksii* differ between seaweeds growing at their northern and southern distributional limits in New Zealand, samples were also collected from two sites near Leigh Marine Laboratory, North Island, in midsummer (February) 1999. The two sites were Waterfall Reef (36°16′S, 174°47′E) and Matheson Bay (36°18′S, 174°48′E). Waterfall Reef is within the Goat Island Marine Reserve whereas the nearby Matheson Bay is subject to rural run-off.

Experimental design

Otago samples were collected in March 1998 (late summer), June 1998 (midwinter), and January 1999 (midsummer). All collections were made at low tide. Within each site, three areas were selected in which *H. banksii* beds were found. A 10 m transect line was placed in the area and 10 points for sample collection were selected using a random numbers table. If there was no seaweed within 30 cm of the selected point, the point was excluded. A total of 90 seaweed samples were collected in each season with the same areas used for each season. The seaweed samples collected were 10–25 cm in height and 90–150 g (wet weight). No distinction of the age of the seaweed was made. Co-polymer film gloves (trace metal clean) were used in all sample collections and preparation. Because of time constraints, five of the original 10 samples from each area in Otago, each season, were randomly chosen (using a random numbers table) and analysed for Zn and Fe.

The experimental design used to collect samples during February 1999 in the vicinity of the Leigh Marine Laboratory was the same as that for the Otago sites, with six samples being collected from each of three areas within two sites, Matheson Bay and Waterfall Reef.

Sample preparation

After collection, each seaweed sample was transported back to the laboratory in a sealed, labelled plastic bag. All epibionts were then immediately removed by hand (using co-polymer film gloves to avoid any contamination) or removed after storage at 4°C overnight. The samples were then carefully cleaned in sea water and rinsed in Milli-Q® to minimise the risk of contamination by surface particles. Each cleaned sample was put into a clean plastic bag and frozen to c. –20°C. The frozen samples were then freeze-dried until a constant weight was achieved (c. 4 days) and again stored frozen at c. –20°C until further analysis.

Sample digestion

All glassware was acid washed in 10% HCl (AnalaR grade) for at least 48 h and then rinsed in Milli-Q®. A 2% HNO_3 solution was prepared by dilution with Milli-Q® of a concentrated HNO_3 prepared by quartz distillation in a Class 100 Clean laboratory. The method of digestion was adapted from McEntyre (1996). Each whole freeze-dried seaweed sample was weighed to obtain the dry weight (DW), placed in a conical flask (50 ml or 100 ml depending on the size of the sample) and slow-ashed (450°C). During

this ashing process, the temperature was slowly raised to 200°C where it was held for 2 h and then subjected to the following temperature regime: raised to 240°C and held for 2 h, raised to 280°C and held for an hour, and then finally raised to 450°C. The samples were kept at this temperature until they exhibited a uniform white colour (c. 72 h). This temperature programming was found to be necessary to avoid unpredictable violent bumping of the solid material which could lead to loss of material. We ascribe this phenomenon to the high natural salt content of the seaweed material which interferes with the temperature-induced oxidation of the organic matrix.

The ash residue for each sample was then ground up using an acid-washed glass pestle in the same flask to minimise contamination, until homogenous in colour and texture. If necessary, excess sample was removed to leave 1–2 g of solid sample. This was accurately weighed and acid-digested by adding two 2 ml portions of a 9:1 mixture of HNO_3:HCl (AnalaR grade) and then heated to dryness on a hotplate (c. 80°C) to break down the remaining inorganic material and release any trace metals. The digested samples were then further ashed at 450°C for 24 h to oxidise any remaining organic material which sometimes remained even after this initial acid digestion. Fifteen ml of 2% HNO_3 was added to the solid residue remaining after this ashing and the resulting solution transferred into a 25.0 ml volumetric flask and accurately made up to volume with 2% HNO_3.

A primary reference standard was used consisting of a sample of the certified reference material DOLT-2 (dogfish liver, National Research Council Canada). This was processed in exactly the same way as the seaweed samples, and one reference sample was processed along with every 10 seaweed samples to verify that the digestion method used was giving rise to accurate metal concentrations. A blank sample (an empty conical flask) was also processed using the above method with every batch of 10 unknown samples to verify that no contamination was occurring during the entire sample preparation procedure. The mean level of Zn detected in the digested DOLT-2 reference material was 1293 ± 3 nmol g^{-1} which is well within the certified value of 1313 ± 38 nmol g^{-1}. The mean Fe level detected in the digested DOLT-2 was 17164 ± 18 nmol g^{-1} which was consistently some 14 % lower than the certified value of 19845 ± 846 nmol g^{-1}. We therefore multiplied all of the raw Fe values measured for the seaweed samples by FAAS by a factor of 1.16 to allow for this effect.

Analysis for Zn and Fe

Samples were analysed for Fe and Zn using flame atomic absorption spectrometry (FAAS, Perkin Elmer Analyst 100) operating in concentration mode. Samples were accurately diluted 5 times (i.e., 4.0 ml 2% HNO_3 was added to 1.0 ml sample), for the concentrations of the samples to be within the range of the instrument (up to 15 nmol Zn g^{-1}, up to 180 nmol Fe g^{-1}) and analysed for Zn and Fe.

The mean value was calculated from five replicate measurements of each sample solution. Sea water standards of 0.00 (blank) and 15.3 nmol Zn g^{-1} in 2% HNO_3 were measured using FAAS to determine if there was a matrix effect from the sea water that could interfere with the observed signal. Since the water content of seaweed can vary considerably, all results in the present study are expressed on a DW basis.

Statistical analysis

For the Otago data, a mixed-model ANOVA with plot nested within site and time was used to test for differences in Zn and Fe content of *H. banksii* between sites and season. Data were log transformed before analysis to meet the assumptions of ANOVA. Significant differences that were detected at $P = 0.05$, were examined using a Scheffe comparison of means. For the Leigh data, differences between sites for the Zn and Fe content of *H. banksii* were tested using a Student's *t*-test. To determine if the Zn and Fe content of *H. banksii* from Otago and Leigh differed, data collected in summer 1999 from each geographic region were pooled (Leigh $n = 6$; Otago $n = 9$) and compared using a Student's *t*-test.

RESULTS

Iron levels in *H. banksii* from the three sites in Otago ranged from 716 to 1768 nmol g^{-1} DW but there was no statistically significant variation with site or season, although there was a significant interaction between these variables ($P < 0.01$, Fig. 2). The Zn content of *H. banksii* from Raynbird Bay was higher than that from Brighton or Portobello, which exhibited similar Zn levels ($P < 0.0001$, Fig. 3). Zn content also varied seasonally, with samples collected in June having on average a higher content than samples in January and March ($P < 0.01$, Fig. 3) although this trend was not apparent for the Raynbird Bay site.

For *H. banksii* samples collected from Leigh, there were no differences between the Waterfall Reef and

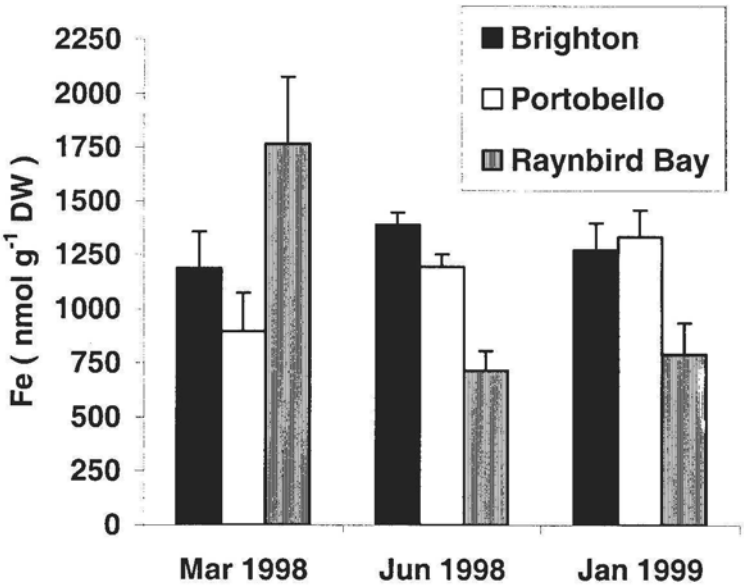

Fig. 2 Iron (Fe) content of *Hormosira banksii* collected from three sites in Otago, New Zealand, during 1998/99. Bars represent means of three replicate plots at each site (± 1 SE). (DW, dry weight.)

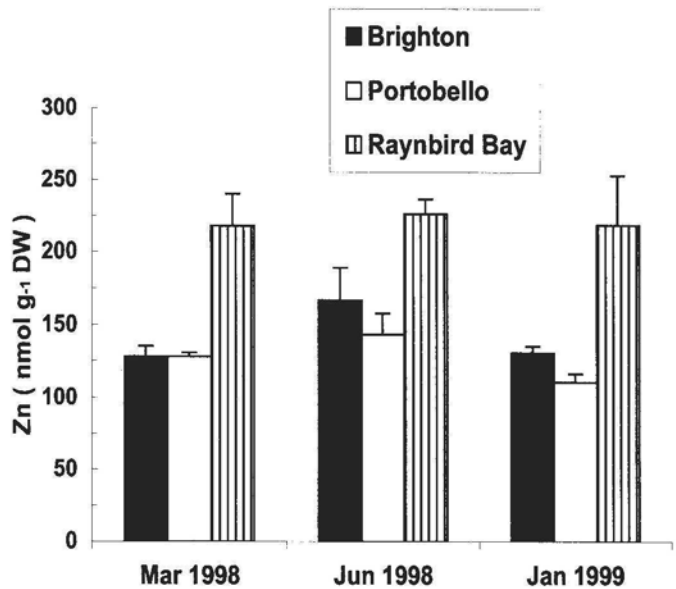

Fig. 3 Zinc (Zn) content of *Hormosira banksii* collected from three sites in Otago, New Zealand, during 1998/99. Bars represent means of three replicate plots at each site (± 1 SE). (DW, dry weight.)

Matheson Bay sites in terms of either the Zn or Fe content ($P > 0.05$, Fig. 4). In January 1999, the average Fe content of *H. banksii* (pooled from the three sites) from Otago was 1167 nmol g^{-1} DW which was twice that of Leigh (492 nmol g^{-1} DW $P < 0.005$). The average Zn content of *H. banksii* from Otago was 155 nmol g^{-1} DW which was similar to that for specimens from Leigh (148 nmol g^{-1} DW $P > 0.05$).

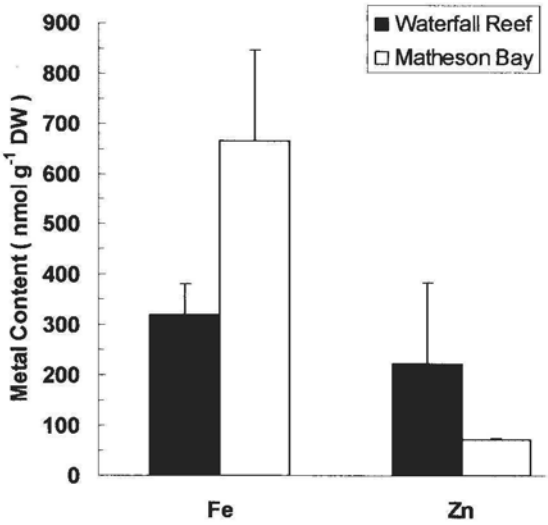

Fig. 4. Iron (Fe) and zinc (Zn) content of *Hormosira banksii* collected from two sites at Leigh, New Zealand, during February 1999. Bars represent the means of three replicate plots ±1 SE. (DW, dry weight.)

DISCUSSION

Levels of Zn in *H. banksii* were within the range of those previously reported by Brown et al. (1999) for *Ulva* (107 nmol g^{-1} DW) and *Enteromorpha* (306 nmol g^{-1} DW) in the middle region of Otago Harbour (zone 1 in Brown et al. 1999). Zn levels at Raynbird Bay, the site closest to Dunedin City at which *Hormosira* is found, were higher than those at Portobello and Brighton, but were still relatively low compared with levels recorded in *Ulva* and *Enteromorpha* growing at the inner harbour basin close to Dunedin (zone 2 in Brown et al. 1999). These spatial patterns for Zn in *Ulva*, *Enteromorpha*, and *Hormosira* reflect patterns in Otago Harbour sea water which give rise to lower Zn concentrations (0.12–0.18 pmol g^{-1}) at the harbour entrance than in the Otago Harbour Basin (1.53 pmol g^{-1}, Hunter & Tyler 1987). Our data for *H. banksii* support the conclusion reached by Brown et al. (1999) that the middle region of Otago Harbour, and the outer coast site at Brighton, are not contaminated by Zn. As for Northern Hemisphere Fucalean seaweeds, *H. banksii* is thus a potential indicator of Zn levels in sea water. In Otago Harbour, however, *Ulva* and *Enteromorpha* are more useful bio-monitors because of their wider distribution; Brown et al. (1999) found the inner basin of Otago Harbour to be moderately contaminated by Zn.

Our measurements of Fe content in *H. banksii* are the first to be published on any New Zealand seaweed. The lack of a seasonal pattern in Fe content of *H. banksii* reflects Fe concentrations in Otago Harbour sea water which also showed no consistent seasonal pattern (Croot & Hunter 2000). However, using *H. banksii* we did not detect differences between the harbour sites (Raynbird Bay and Portobello) and the outer coast site at Brighton, which contrasts with the spatial patterns in Fe sea water concentration (Croot & Hunter 2000): levels of total Fe in sea water at Otago Harbour mouth (site 7 in Croot & Hunter 2000) which is closest to the present Brighton site were typically lower (0.18–1.7 nmol g^{-1}) than sites in the main shipping channel nearest to Portobello and Raynbird Bay which were similar and ranged from 0.36 to 2.97 nmol g^{-1}. The low levels of Fe in *H. banksii* both within and outside Otago Harbour compared with other seaweeds (see below and Table 1) indicate that the sites studied were not contaminated by Fe. However, the potential usefulness of *H. banksii* as a bio-monitor for Fe requires further investigation using *in situ* Fe-enrichment experiments and/or laboratory culture experiments.

The Fe content of *H. banksii* from Waterfall Reef, Leigh, was 2–5-fold lower than the Otago samples and this may be explained in part by the lower Fe concentration in sea water near the Waterfall Reef site (0.018 nmol g^{-1}, T. A. V. Rees unpubl. data). The Fe and Zn content of *H. banksii* from Leigh and Otago will also be affected by their growth rates and hence metabolic requirements and future culture studies should examine the effect of supply and demand of trace elements on metal content.

The Fe and Zn contents of *H. banksii* from New Zealand are within the lower range of values recorded for brown seaweeds (Table 1). Furthermore, the average concentrations of Fe (318 ± 62 nmol g^{-1} DW) and Zn (72 ± 4 nmol g^{-1} DW) for *H. banksii* from Leigh are the lowest reported for any brown seaweed except for Fe in *Lessonia trabeculata* and Zn in *Macrocystis pyrifera* (Table 1; table 7 in Rainbow et al. 2002). The low levels of Fe and Zn lend support to the idea that the growth of *H. banksii* may be trace-element limited, and this idea is further explored below by modelling Fe and Zn requirements and relating these to our measured values.

Roles of, and quantitative requirement for, Fe in the Phaeophyceae

To calculate the requirements of *H. banksii* for Fe and Zn, we first describe the role of these elements

in key metabolic pathways and quantify the number of atoms required for each biochemical process. The Fe and Zn contents of these processes are then summed to give a total requirement, and these estimates are then compared to our measured values of Fe and Zn content for *H. banksii* from Otago and Leigh. We know little about the Fe requirements of seaweeds so, for much of what follows, the Fe per catalytic complex values are derived from data on other organisms, and the data for brown algae come from algae other than *H. banksii*.

Table 2 summarises the Fe content of the catalysts involved in the photosynthetic, respiratory, and photorespiratory pathways and of nitrate, nitrite, and sulfite reduction in brown algae.

The values in Table 2 can be used, with other data, to estimate the Fe content of various catalysts in *H. banksii* on a DW basis. The estimated total of Fe in catalysts is some 76 nmol Fe g^{-1} DW (Table 3). The lowest measured content of Fe in *H. banksii* reported in the present paper is 318 nmol Fe g^{-1} DW for specimens from Leigh. Hence we estimate that 24%

Table 1 Iron (Fe) and Zinc (Zn) contents of members of the Phaeophyceae (nmol metal g^{-1} dry weight (DW)) from a range of sites with or without major anthropogenic inputs. Where necessary a fresh matter to dry matter ratio of 6 has been assumed.

Species	Fe	Zn	References
Ascophyllum nodosum	772–20600	920–1770	Black & Mitchell (1952) Stengel & Dring (2000)
Colpomenia sinuosa	–	830	Munda & Hudnik (1991)
Cystoseira compressa	–	765	Munda & Hudnik (1991)
Cystoseira spicata	–	550–650	Munda & Hudnik (1991)
Dictyota dichotoma	–	440	Munda & Hudnik (1991)
Dictyopteris membranacea	–	690–1300	Munda & Hudnik (1991)
Ecklonia radiata	630–4100	60–240	Higgins & Mackey (1987a,b)
Ectocarpus siliculosus	–	430	Munda & Hudnik (1991)
Fucus serratus	3393–12800	960–1200	Black & Mitchell (1952) O'Leary & Breen (1997)
Fucus spiralis	11400–60500	950	Black & Mitchell (1952)
Fucus vesiculosus	1201–48400	230–12500	Black & Mitchell (1952) Bryan & Hummerstone (1973) Forsberg et al. (1988) Morris & Bale (1975) O'Leary & Breen (1997, 1998) Riget et al. (1995) Rainbow et al. (2002)
Fucus virsoides	–	510–1130	Munda & Hudnik (1991)
Halopteris scoparia	–	1300	Munda & Hudnik (1991)
Hormosira banksii	318–1768	73–226	This study
Laminaria cloustonii (= *L. hyperborea*)	2850–5070	1160–2080	Black & Mitchell (1952)
Laminaria digitata	398–28100	367–1510	Black & Mitchell (1952) Bryan (1969)
Lessonia trabeculata	270–2860	–	Vásquez et al. (1999)
Macrocystis integrifolia	2300	360	Rossell & Srivastava (1984)
Macrocystis pyrifera	1400–1500	55–180	Manley (1981, 1984) North (1980)
Nereocystis luetkeana	1800	130	Rossell & Srivastava (1984)
Padina gymnospora	–	384	Amada Filho et al. (1997)
Padina pavonica	–	550–1610	Munda & Hudnik (1991)
Padina tetrastromatia	42000	230	Burdon-Jones et al. (1982)
Pelvetia canaliculata	3490–10120	610–6461	Black & Mitchell(1952) O'Leary & Breen (1997)
Sargassum filipendula	–	308	Amada Filho et al. (1997)
Sargassum pallidum	540	250	Khristoforova et al. (1983)
Scytosiphon lomentaria	–	440–1560	Munda & Hudnik (1991)

of the total measured Fe content of *H. banksii* is catalytically active which is similar to the value of 28% estimated for a C_3 land plant leaf by Hewitt (1983). Even the lowest Fe content in Table 1 (for *L. trabeculata*) is 4 times the computed catalytic Fe requirement for *H. banksii*. For coastal marine phytoplankton the measured Fe content is in general agreement with the predictions of catalytic Fe content by Raven (1988, 1990; Sunda et al. 1991; Sunda & Huntsman 1997), but the estimates are higher than the measured values for the oceanic diatom *T. oceanica* (Sunda et al. 1991; Sunda & Huntsman 1995; Maldonado & Price 1996). Agreement between modelled and measured Fe content in the oceanic diatom has been achieved by dynamic modelling and estimates of the content of Fe-containing catalysts in the *Thalassiosira oceanica* cells (Flynn & Hipkin 1999; Strzepek & Harrison 2002; Strzepek 2003). For the micro-algae many of the data relate to Fe-limited, or marginally Fe-limited, organisms. For the land plant leaves and for *H. banksii* there is no external evidence of Fe limitation, although there is evidence of Fe limitation of primary productivity in estuarine and coastal environments, and especially those with anthropogenic input of available nitrogen and phosphorus and of Fe-sequestering material (Suzuki et al. 1995; Kawaguchi et al. 1997; Zhang et al. 1999; see also Wells 1999) or on calcareous substrata (Duarte et al. 1995).

Is there any further evidence that the 24% of total Fe which can be attributed to catalytic Fe in *H. banksii* indicates Fe limitation of growth? Certainly the lowest Fe level in *H. banksii* is lower than any of the other values for brown algae except *L. trabeculata* (Table 1 of this paper; Wheeler & Naldi 1999) collected from natural or anthropogenically influenced environments. How much of the Fe in *H. banksii* is in the cell walls, and hence catalytically inactive? *Ecklonia radiata*, which has more Fe per unit DW (630 nmol Fe g^{-1} DW) than do the Leigh specimens of *H. banksii* (318 nmol Fe g^{-1} DW), only loses 7% of this Fe in a 15-min exposure to 1 mol m^{-3} EDTA in sea water (Higgins & Mackey 1987a). Although this treatment might not extract all cell wall Fe, these data suggest that the estimated non-catalytic proportion of 65% of total Fe in *H. banksii* is not likely to all be in the cell walls. Large fractions of thallus Fe were found to be associated with the extracellular space in other studies. Thus, Rossell & Srivastava (1984) found that 47% of thallus Fe was removed from *Macrocystis integrifolia* by treatment with 100 m*M* HCl whereas Manley (1981, 1984) found that a similar treatment removed 40% of thallus Fe from *M. pyrifera*. However, before concluding that up to half of the Fe in *Macrocystis* thalli is extracellular, further work on the integrity of the cells after the HCl treatment would be helpful.

There is also the likelihood that the Fe content in catalysts has been over or underestimated. The amount of chlorophyll per unit DW and per photosystem II, and the ratio of photosystem II to cytochrome b_6-f and to photosystem I could all vary by ±30% relative to our assumptions (Raven 1990;

Table 2 Iron (Fe) content of major catalytic systems in photosynthetic eukaryotes with special reference to brown algae and other heterokonts.

Catalytic system	Fe content	References
Thylakoid and stromal redox system II, cytochrome b_6-f complex, cytochrome c_6, photosystem I, ferredoxin, NAD(P)H-PQ oxidoreductase, Fe superoxide dismutase and ascorbate peroxidase	≤23 Fe per photosystem II	Raven (1988, 1990) Asada (1999) Raven et al. (1999) Strzepek & Harrison (2002)
Mitochondrial redox systems, including NADH-UQ oxidoreductase, cytochrome b-c_1 complex, cytochrome c, cytochrome oxidase, aconitase	≤28 Fe per cytochrome c	Hewitt (1983) Raven (1988, 1990) Nicholls & Ferguson (1992)
Peroxisomal catalase consuming H_2O_2 produced by glycolate oxidase	1 Fe per catalase	Iwamato & Ikawa (1997) Raven et al. (1999, 2000)
Nitrite and nitrite reductase	≤7 Fe per nitrite reductase	Hewitt (1983) Raven (1988, 1990) Flynn & Hipkin (1999)
Sulfite reductase	5 Fe per sulfite reductase	Hewitt (1983)

Table 3 Computed iron (Fe) content of major Fe-containing catalysts in *Hormosira banksii* and comparison with the total Fe measured in this study. Minor Fe-containing components, e.g., cytochrome P_{450} in the endoplasmic reticulum and *b*-type cytochrome(s) in the plasmalemma, are not considered. (DW, dry weight.)

Computed Fe content of catalysis and measured Fe content	Fe (nmol g^{-1} DW)	Computation of Fe content in catalysts on a dry matter basis from values in Table 2
Computed Fe content of thylakoid catalysis and associated superoxide	66	600 chlorophylls $a + c$ per photosystem II in *Macrocystis pyrifera* (Raven 1990) and 2.87 dismutase and ascorbate peroxidase nmol chlorophylls $a + c$ per g DW in *Hormosira banksii* (Raven et al. 1995)
Computed Fe content of mitochondrial redox catalysts	8.8	One-fifth as much cytochrome *c* in mitochondria as cytochrome c_6 in plastids, i.e., 0.11 cytochrome *c* per photosystem II (Chapter 6 of Raven 1984; Raven 1988, 1990)
Computed Fe content of catalase in peroxisomes	≤0.7	Brown algae have a H_2O_2-generating photorespiratory carbon oxidation cycle (Iwamoto & Ikawa 1997). However, the flux through the cycle is a smaller fraction of the carbon fixed than in C_3 land plants because of a higher CO_2/O_2 selectivity of ribulose bisphosphate carboxylase-oxygenase (Raven et al. 2000) and the occurrence of a carbon concentrating mechanism (Raven et al. 1995), so that the Fe content in catalase is less than the 0.1 Fe in catalase per cytochrome b_6-*f* (Hewitt 1983) or ≤0.2 Fe per photosystem II.
Computed Fe content of nitrate, nitrite, and sulfite reductases	≤0.4	<0.36 nmol Fe in nitrate and nitrite reductases per g dry DW based on the specific reaction rates of the reductases the C/N ratio (28) of *H. banksii*, and photosynthetic rates on a DW basis (Raven & Osmond 1992; Loughnan 1997; Kawamitsu & Boyer 1999 and references therein). <0.036 nmol Fe in sulfite reductase per g DW based on the specific reaction rate of the reductase and the ratio of reduced sulfur to reduced nitrogen in brown algae of <0.1 (Raven 1984, 1988).
Sum of computed Fe content of catalysts	≤75.8	
Measured Fe content of *H. banksii*	≥318	

Table 4 Computed Zinc (Zn) content of the major Zn-containing catalysts in *Hormosira banksii* and comparison with the total Zn measured in this study. Minor Zn-containing catalysts, such as ethanol dehydrogenase and phosphatases (Hewitt 1983; Marschner 1995) are not considered. Note that brown algae have Fe and Mn, but not Cu-Zn, superoxide dismutases (Raven et al. 1999). (DW, dry weight.)

Computed Zn content of catalysis and measured Zn content	Zn (nmol g^{-1} DW)	Computation of Zn content in catalysts on a DW basis from values in Hewitt (1983) and Marschner (1995)
Computed Zn content of carbonic anhydrase	≤17	Less than the 17 nmol Zn per g dry matter in carbonic anhydrase calculated from the content of the 6 Zn per enzyme molecule in C_3 higher plants, since the *H. banksii* has a carbon concentrating mechanism (Raven et al. 1999, 2000). Not clear what family/ies of carbonic anhydrase (α, β, γ, Cadmium-containing) occurs in brown algae (Giordano & Maberly 1989; Surif & Raven 1989, 1990; Sültemeyer 1998).
Computed Zn content of RNA polymerase and zinc fingers	≤2.5	
Sum of computed Zn content of catalysts	≤19.5	
Measured Zn content of *H. banksii*	≥72.5	

Raven et al. 1999, 2000). This could alter the predicted Fe content of the alga from half to twice the quoted values for the dominant, photosynthesis-related Fe-containing catalysts. Thus the available data could be consistent with a catalytic Fe content which is 70% or more of the total Fe content as well as catalytic Fe only accounting for c. 15% of the total Fe.

Roles of, and quantitative requirements for, Zn in the Phaeophyceae

The computed Zn content in catalysts of *H. banksii* (Table 4) is not more than 19.5 nmol Zn per g^{-1} DW. The lowest value of Zn, from the Matheson Bay site at Leigh, is 72.5 nmol Zn g^{-1} DW (as low as any of the values in Table 1) so that the predicted Zn in catalysts (CA) is 27% of the total Zn. However, Higgins & Mackey (1987a) found that 88% of tissue Zn in *Ecklonia radiata* (a total of 240 nmol Zn g^{-1} DW) could be removed by a 15-min treatment with 1 m*M* EDTA, and is presumably a minimum estimate of the cell wall content. Thus, the predicted catalytic Zn content could be more than that of the intracellular Zn in *H. banksii*. However, the estimated catalytic Zn content could exceed the actual requirement even in C_3 land plants, based on problems with inducing phenotypic effects attributable to lowered CA activity as a result of Zn deficiency and of down-regulation of CA by genetic manipulation in C_3 land plants (Ohki 1976; Majeau et al. 1994; Price et al. 1994).

CONCLUSIONS

Although the Fe and Zn content of *H. banksii* are among the lowest recorded for any brown seaweed to date, there is no evidence that these elements limit the growth of this seaweed. Laboratory culture studies and/or *in situ* enrichment would be required to confirm this suggestion that the growth of *H. banksii* in New Zealand is not limited by Fe or Zn. We suggest that *H. banksii* would make a suitable bio-monitor of heavy metal contamination in sea water and data presented here provide a useful baseline of Fe and Zn levels to which future measurements of the content of these metals in *H. banksii* could be compared.

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REFERENCES

Amado Filho, G. M.; Karez, C. S.; Androde, L. R.; Yoneshigue-Valertin, Y.; Pfeiffer, W. C. 1997: Effects on growth and accumulation of Zn in six seaweed species. *Ecotoxicology and Environmental Safety 37*: 223–228.

Asada, K. 1999: The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology 50*: 601–639.

Black, W. A. P.; Mitchell, R. L. 1952: Trace elements in the common brown algae and sea water. *Journal of the Marine Biological Association U.K. 30*: 575–584.

Brown, M. T.; Hodgkinson, W. M.; Hurd, C. L. 1999: Spatial and temporal variations in the copper and Zn concentrations of two green seaweeds from Otago Harbour, New Zealand. *Marine Environmental Research 47*: 175–184.

Bryan, G. W. 1969: The absorption of Zn and other metals by the brown seaweed *Laminaria digitata*. *Journal of the Marine Biological Association U.K. 49*: 225–243.

Bryan, G. W.; Hummerstone, L. G. 1973: Brown seaweed as an indication of heavy metals in estuaries of south-east England. *Journal of the Marine Biological Association UK 53*: 705–720.

Burdon-Jones, C.; Denton, G. R. W.; Jones, G. B.; McPhie, K. A. 1982: Regional and seasonal variations of trace metals in tropical Phaeophyceae from north Queensland. *Marine Environmental Research 7*: 13–20.

Coale, K. H.; Johnson, K. S.; Fitzwater, S. E.; Gordon, R. M.; Tanner, S.; Chavez, F. P.; Ferioli, L.; Sakamoto, C.; Rodgers, P.; Millero, F.; Steinberg, P.; Nightingale, P.; Cooper, D.; Cachlan, W. P.; Landry, M .R.; Constantinou, J.; Rollwagen, G.; Trasvina, A.; Kudela, R. 1996: A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature 383*: 495–501.

Croot, P. L; Hunter, K. A. 2000: Labile forms of Fe in coastal sea water: Otago Harbour, New Zealand. *Marine and Freshwater Research 51*: 193–203.

Duarte, C. M.; Merino, M.; Gallegos, M. 1995: Evidence of Fe-deficiency in seagrasses growing above carbonate sediments. *Limnology and Oceanography 40*: 1153–1158.

Flynn, K. J.; Hipkin, C. R. 1999: Interactions between Fe, light, ammonium, and nitrate: insights from the construction of a dynamic model of algal physiology. *Journal of Phycology 35*: 1171–1190.

Forsberg, Å.; Söderlund, S.; Frank, A.; Petersson, L. R.; Pedersén, M. 1988: Studies on metal content in the brown seaweed, *Fucus vesiculosus*, from the archipelago of Stockholm. *Environmental Pollution 49*: 245–263.

Fuge, R.; James, K. H. 1973: Trace metal concentrations in brown seaweeds, Cardigan Bay, Wales. *Marine Chemistry 1*: 281–293.

Fuge, R.; James, K. H. 1974: Trace metal concentrations in *Fucus* from the Bristol Channel. *Marine Pollution Bulletin 5*: 9–12.

Giordano, M.; Maberly, S. C. 1989: Distribution of oceanic carbonic anhydrase in British marine algae. *Oecologia 81*: 534–539.

Hay, C. H. 1990: The distribution of *Macrocystis* (Phaeophyta: Laminariales) as a biological indicator of cool sea surface temperature, with special reference to New Zealand. *Journal of the Royal Society of New Zealand 20*: 313–336.

Hewitt, E. J. 1983: A perspective of mineral nutrition: essential and functional metals in plants. *In*: Robb, D. A.; Pierpoint W. S. *ed.* Metals and micronutrients: uptake and utilization by plants. London, Academic Press. Pp. 277–323.

Higgins, H. W.; Mackey, D. J. 1987a: Role of *Ecklonia radiata* (C.Ag.) J. Agardh in determining trace metal availability in coastal waters. I: Total trace metals. *Australian Journal of Marine and Freshwater Research 38*: 307–315.

Higgins, H. W.; Mackey D. J. 1987b: Role of *Ecklonia radiata* (C.Ag.) J. Agardh in determining trace metal availability in coastal waters. II: Trace metal speciation. *Australian Journal of Marine and Freshwater Research 38*: 317–328.

Hunter, K.; Tyler, S. A. 1987: The distribution of Zn and reactive silicate in Otago Harbour, New Zealand. *Marine Chemistry 20*: 377–387.

Iwamato, K.; Ikawa, T. 1997: Glycolate metabolism and subcellular distribution of glycolate oxidase in *Spatoglossum pacificum* (Phaeophyceae, Chromophyta). *Phycological Research 45*: 77–83.

Kawaguchi, T.; Lewitus, A. J.; Aelion, C. M.; McKellar, H. N. 1997: Can urbanization limit Fe availability to estuarine algae? *Journal of Experimental Marine Biology and Ecology 213*: 53–69.

Kawamitsu, Y.; Boyer, J. S. 1999: Photosynthesis and carbon storage between tides in a brown alga, *Fucus vesiculosus*. *Marine Biology 133*: 361–369.

Khristoforova, N. K., Bogdanova, N. N.; Tolstova, L. M. 1983: Metals present in *Sargassum* (brown) algae of the Pacific Ocean as related to the problem of water pollution monitoring. *Oceanology 23*: 200–204.

Leal, M. F. C.; Vasconcelos, M. T. S. D.; Sousa-Pinto, I.; Cabial, J. P. S. 1999: Biomonitoring with benthic macroalgae and direct assay of toxic metals in sea water of the Oporto Coast (Portugal). *Marine Pollution Bulletin 34*: 1006–1015.

Loughnan, A. 1997: Polyphenolic levels in five intertidal brown seaweeds, and the ecological role of polyphenolics in *Hormosira banksii*. Unpublished BSc (Hons) thesis, University of Otago, Dunedin, New Zealand.

McEntyre, C. 1996: Trace metal characteristics of New Zealand dredge oysters (*Tiostrea chilenis*) from Foveaux Strait, and the Nelson-Marlborough region. Unpublished research report for MARI 480, Department of Marine Science, University of Otago, Dunedin, New Zealand.

MacLachlan, J. 1977: Effects of nutrients on growth and development of embryos of *Fucus edentatus* Pyl. (Phaeophyceae, Fucales). *Phycologia 16*: 329–338.

Majeau, N.; Arnoldo, M.; Coleman, J. R. 1994: Modification of carbonic anhydrase activity by antisense and overexpression constructs in transgenic tobacco. *Plant Molecular Biology 25*: 377–385.

Maldonado, M. T.; Price, N. M. 1996: Influence of N substrate on Fe requirements of marine centric diatoms. *Marine Ecology Progress Series 141*: 161–172.

Manley, S. L. 1981: Fe uptake and translocation by *Macrocystis pyrifera*. *Plant Physiology 63*: 914–918.

Manley, S. L. 1984: Micronutrient uptake and translocation by *Macrocystis pyrifera* (Phaeophyta). *Journal of Phycology 20*: 192–201.

Marschner, H. 1995: Mineral nutrition of higher plants (2nd ed.). London, Academic Press.

Morris, A. W.; Bale, A. J. 1975: The accumulation of cadmium, copper, manganese and Zn by *Fucus vesiculosus* in the Bristol Channel. *Estuarine and Coastal Marine Science 3*: 153–163.

Munda, I. M. 1979: Temperature dependence of Zn uptake in *Fucus virsoides* (Don.) J. Ag. and *Enteromorpha prolifera* (O.F. Müll.) J. Ag. from the Adriatic Sea. *Botanica Marina 22*: 149–152.

Munda, I. M.; Hudnik, V. 1986: Growth responses of *Fucus vesiculosus* to heavy metals, singly and in dual combinations, as related to accumulation. *Botanica Marina 29*: 401–412.

Munda, I. M.; Hudnik, V. 1991: Trace metal content in some seaweeds from the Northern Adriatic. *Botanica Marina 34*: 241–249.

Nicholls, D. G.; Ferguson, S. J. 1992: Bioenergetics 2. Academic Press, London.

North, W. J. 1980: Trace metals in giant kelp, *Macrocystis*. *American Journal of Botany 67*: 1097–1101.

Ohki, K. 1976: Effect of Zn nutrition on photosynthesis and carbonic anhydrase activity in cotton. *Physiologia Plantarum 38*: 300–304.

O'Leary, C.; Breen, J. 1997: Metal levels in seven species of mollusc and in seaweeds from the Shannon Estuary. *Biology and Environment: Proceedings of the Royal Irish Academy 97B*: 121–132.

O'Leary, C.; Breen, J. 1998: Seasonal variation of heavy metals in *Mytilus edulis*, *Fucus vesiculosus* and sediment from the Shannon Estuary. *Biology and Environment: Proceedings of the Royal Irish Academy 97B*: 153–169.

Phillips, D. J. H. 1990: Use of macroalgae and invertebrates as monitors of metal levels in estuaries and coastal waters. *In*: Furness, R. W.; Rainbow, P. S. *ed.* Heavy metals in the marine environment. Boca Raton, FL, United States. Pp. 81–89.

Phillips, J. C.; Hurd, C. L. 2004: The nitrogen ecophysiology of intertidal seaweeds from New Zealand: patterns of uptake, storage and utilisation in relation to shore position and season. *Marine Ecology Progress Series*: in press.

Price, G. D.; von Caemmerer, S.; Evans, J. R.; Yu, J-W.; Oja, V.; Kell, P.; Harrison, K.; Gallagher, A.; Badger, M. R. 1994: Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA activity in transgenic tobacco plants for minor effects on photosynthetic CO_2 assimilation. *Planta 193*: 331–340.

Rainbow, P. S. 1995: Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin 31*: 183–192.

Rainbow, P. S.; Smith, B. D.; Lau, S. S. S. 2002: Biomonitoring of trace metal availabilities in the Thames estuary using a suite of littoral biomonitors. *Journal of the Marine Biological Association U.K. 82*: 739–799.

Raven, J. A. 1984: Energetics and transport in aquatic plants. New York, A. R. Liss.

Raven, J. A. 1988: The Fe and molybdenum use efficiencies of plant growth with different energy carbon and nitrogen sources. *New Phytologist 109*: 279–287.

Raven, J. A. 1990: Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway. *New Phytologist 116*: 1–18.

Raven, J. A.; Osmond, C. B. 1992: Inorganic C assimilation processes and their ecological implications in inter- and sub-tidal macroalgae of North Carolina. *Functional Ecology 6*: 41–47.

Raven, J. A.; Beardall, J.; Johnston, A. M.; Kübler, J. E.; Geoghegan, I. 1995: Inorganic carbon acquisition by *Hormosira banksii* (Phaeophyta : Fucales) and its epiphyte *Notheia anomala* (Phaeophyta : Fucales). *Phycologia 34*: 267–277.

Raven, J. A.; Evans, M. C. W.; Korb, R. E. 1999: The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. *Photosynthesis Research 60*: 111–149.

Raven, J. A.; Kübler, J. E.; Beardall, J. 2000: Put out the light, then put out the light. *Journal of the Marine Biological Association U.K. 80*: 1–25.

Riget, F.; Johansen, P.; Asmund, G. 1995: Natural seasonal variation of cadmium, lead and Zn in brown seaweed (*Fucus vesiculosus*). *Marine Pollution Bulletin 30*: 409–413.

Rossell, K. G.; Srivastava, L. M. 1984: Seasonal variations in the chemical constituents of the brown algae *Macrocystis integrifolia* and *Nereocystis luetkeana*. *Canadian Journal of Botany 62*: 2229–2236.

Ryder, R.; Steven, B; Watson, M. 1997: Green Island wastewater treatment upgrade: assessment of environmental effects. Appendices A and D. Dunedin, New Zealand, Dunedin City Council.

Say, P. J.; Burrows, I. G.; Whitton, B. A. 1990: *Enteromorpha* as a monitor of heavy metals in estuaries. *Hydrobiologia 195*: 119–126.

Stengel, D. B.; Dring, M. J. 2000: Copper and Fe concentrations in *Ascophyllum nodosum* (Fucales, Phaeophyta) from different sites in Ireland and after culture experiments in relation to age and epiphytism. *Journal of Experimental Marine Biology and Ecology 246*: 145–161.

Strzepek, R. F. 2003: Photosynthetic Fe requirements of marine diatoms. Unpublished PhD thesis, University of British Columbia, Canada.

Strzepek, R. F.; Harrison, P. J. 2002: The photosynthetic Fe requirements of coastal and oceanic diatoms. *EOS Transactions, American Geophysical Union 83(4)*. Ocean Sciences Meeting Abstract OS12N-10.

Sültemeyer, D. 1998: Carbonic anhydrase in eukaryotic algae: characterization, regulation and possible functions during photosynthesis. *Canadian Journal of Botany 76*: 962–972.

Sunda, W. G.; Huntsman, S. A. 1995: Fe uptake and growth limitation in oceanic and coastal phytoplankton. *Marine Chemistry 50*: 189–206.

Sunda, W. G.; Huntsman, S. A. 1997: Interrelated influence of light, Fe and cell size on marine phytoplankton growth. *Nature 390*: 389–392.

Sunda, W. G.; Swift, D. G.; Huntsman, S. A. 1991: Low Fe requirement for growth in oceanic phytoplankton. *Nature 351*: 55–57.

Surif, M. B.; Raven, J. A. 1989: Exogenous inorganic carbon sources for photosynthesis in sea water by member of the Fucales and Laminariales (Phaeophyta): ecological and taxonomic implications. *Oecologia 78*: 97–105.

Surif, M. B.; Raven, J. A. 1990: Photosynthetic gas exchange under emersed conditions and eulittoral and normally submersed members of the Fucales and Laminariales: interpretation in relation to C isotope ratio and N and water use efficiency. *Oecologia 82*: 68–80.

Suzuki, Y.; Kuma, K.; Kudo, I.; Matsunaga, K. 1995: Fe requirement of the brown macroalgae *Laminaria japonica*, *Undaria pinnatifida* (Phaeophyta) and the crustose coralline alga *Lithophyllum yessoense* (Rhodophyta), and their competition in the northern Japan Sea. *Phycologia 34*: 201–205.

Vasconcelos, M. T. S. D.; Leal, M. F. C. 2001: Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Marine Chemistry 74*: 65–85.

Vásquez, J. A.; Vega, J. M. A.; Matsuhiro, B.; Urzúa, C. 1999: The ecological effects of mining discharges on subtidal habitats dominated by macroalgae in northern Chile: population and community level studies. *Hydrobiologia 398/399*: 217–229.

Wells, M. L. 1999: Manipulating Fe availability in nearshore waters. *Limnology and Oceanography 44*: 1002–1008.

Wheeler, P. A.; Naldi, M. 1999: Sequestration of Fe in marine and estuarine ecosystems by marine macroalgae. Abstracts of the XVI International Botanical Congress, St. Louis, USA, August 1–7, 1999 P.618 (Abstract 1954).

Zhang, J.; Yu, J. G.; Wang, J. T.; Ren, J. L.; Chen, H. T.; Xiong, H.; Dong, L. X.; Xu, W. Y. 1999: The subtropical Zhujiang (Pearl River) Estuary; nutrient, trace species and their relationship to photosynthesis. *Estuarine, Coastal and Shelf Science 49*: 385–400.