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Evaluation of iron as a triggering factor for red tide blooms

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ABSTRACT: We have examined the relationship between Fe and blooms of the toxic dinoflagellate *Alexandrium tamarense* (Balech) (formerly *Gonyaulax tamarensis* var. *excavata* (Lebour)) using a chemical method that estimates the biological availability of Fe in seawater. The Fe requirement for optimal growth of *A. tamarense* in sequential batch culture (ca 3 nM 'available' Fe) was compared with Fe concentrations in waters of the Gulf of Maine, USA. Results indicated that Fe did not limit growth of the organism in nearshore coastal waters or over Georges Bank, but that the organism may have been Fe-limited in Gulf of Maine basin waters. The distribution of *A. tamarense* in the Gulf of Maine is consistent with these Fe data. Red tide outbreaks in the nearshore environment did not correlate with changes in total Fe or the estimated Fe availability. Although Fe did not appear to trigger outbreaks of *A. tamarense* in Maine coastal waters, the findings are consistent with suggestions that pulsed inputs of Fe may be important for the development of toxic dinoflagellate blooms in regions (e.g. Florida) where outbreaks are initiated offshore.

INTRODUCTION

Toxic dinoflagellates flourish in many different regions of the world, generating conditions known as 'red tides', and circumstantial evidence suggests that these occurrences are becoming more frequent (Anderson 1979, Mihnea 1979, Maclean & White 1985). In the Gulf of Maine, USA, blooms of the saxitoxin-producing dinoflagellate *Alexandrium tamarense* (Balech), formerly *Gonyaulax tamarensis* var. *excavata* (Lebour), develop repeatedly in coastal waters between May and October. These outbreaks are sporadic and often localized, hence difficult to monitor in situ. Effective management of shellfish and aquaculture industries depends on monitoring the saxitoxin concentrations in bivalve tissues, an expensive, labor-intensive process. In consequence, there have been increased efforts to understand the mechanisms triggering red tide outbreaks in order to develop a predictive index to ameliorate health and fisheries management, and to determine the extent to which human activities may increase the frequency and exacerbate the severity of these outbreaks. However, there remains considerable uncertainty about the specific conditions necessary for initiating red tide development.

Red tide dinoflagellates, like most dinoflagellates, appear to be well adapted to the early stages of decaying turbulence where nutrient concentrations are relatively high (Margalef et al. 1979, Bowman et al. 1981). For example, blooms often appear along frontal boundaries between water masses (Pingree et al. 1975) or in regions where high tidal energies are dissipated as turbulence (Yentsch et al. 1986). Hydrographic forces may likewise serve to concentrate toxic dinoflagellate populations, contributing to patchiness within a bloom area. Red tides are also often preceded by periods of heavy rainfall (Slobodkin 1953, Collier 1954, Aldrich & Wilson 1960, Collier et al. 1969, Glover 1978, Smayda & Packard 1979, Robinson & Brown 1983). In Swedish coastal waters, blooms of toxic dinoflagellates are most intense in Laholm Bay, which receives 3 times more freshwater input per kilometer of coastline than any other part of the Swedish coast (Graneli et al. 1986). However, because decaying turbulent regimes and runoff do not always result in red tides (Kamykowski 1979, Evans & Taylor 1980, Martin & White 1988), these conditions alone are not sufficient to initiate bloom development.

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Efforts to understand the mechanisms of red tide development have focused on the physiochemical conditions associated with increased turbulence and runoff. Because red tides do not correlate consistently with particular temperature, salinity or light regimes, a nutritional 'trigger' has been suspected; that is, a specific nutritional factor, or factors, that stimulate the selective growth of toxic dinoflagellates over that of other phytoplankton when conditions are otherwise (supra)optimal for growth.

Of the possible nutritional triggers, N or P does not appear to be involved; Rounsefell & Dragovich (1966) found no correlation between red tide outbreaks in Florida and organic phosphate, total phosphate, nitrate-nitrite nitrogen, ammonia nitrogen, or total organic nitrogen. Graneli et al. (1986) observed a slight enhancement of toxic dinoflagellate growth in waters from Laholm Bay (Sweden) upon addition of nitrogen- and phosphorus-rich agricultural drainage waters. However, even greater growth stimulation occurred with the addition of river water from regions affected by acid rain, and hence rich in trace metals.

Trace metal regimes are known to be an important determinant of phytoplankton species composition, with Fe having perhaps a central role (e.g. Brand et al. 1983, Murphy et al. 1984). Additions of Fe to natural population cultures stimulated the growth of 2 red tide dinoflagellates (Wilson 1966, Martin & Martin 1973, Graneli et al. 1986) and red tide outbreaks in Florida (USA) correlated strongly with fluvial Fe inputs to coastal waters over a 25 yr period (Ingle & Martin 1971, Kim & Martin 1974). The Fe requirement of red tide organisms may be greater than that of other phytoplankton species, as suggested by the almost complete removal of Fe from a red tide-dominated estuary when only moderate Fe removal occurred in a nearby estuary containing a diatom bloom (Matsunaga et al. 1984). Laboratory studies also indicate that red tide species may be more susceptible to iron stress than many other coastal phytoplankton species (Doucette & Harrison 1990). However, the relationship between Fe and red tide outbreaks has remained ambiguous, primarily because the proportion of total Fe in seawater that is available to the phytoplankton is unknown. Phytoplankters utilize directly only free (or reactive) soluble Fe species (Anderson & Morel 1982) which, because of the insolubility of Fe, constitute a small portion of the total Fe in oxic seawater. The bulk of Fe occurs in particulate and probably colloidal forms, which must dissolve to produce the soluble species assimilated by phytoplankton.

We have developed a method for measuring a fraction of the total Fe in seawater which we define as 'labile' iron (i.e. accessible to the measuring technique), and found that the growth of several phytoplankton species was a direct function of Fe lability (Wells et al. in press). Moreover, we have shown that the labile fraction of total Fe in seawater is not constant, but can differ substantially across small spatial and temporal scales in nature (Wells & Mayer in press). Therefore, the total Fe content of seawater is not a reliable index to the relationship, if any, between iron requirements of the bloom-forming organism and the development of large populations.

In this study, the relationship between Fe and red tides was re-examined using measurements of labile Fe in seawater. The timing and severity of blooms of the toxic dinoflagellate *Alexandrium tamarense* in Maine coastal waters were compared with concurrent measurements of total Fe and Fe lability. Fe concentrations were also compared with the Fe requirement of *A. tamarense* determined in (sequential) batch cultures. The data were used to evaluate the role of Fe as a triggering factor for red tide development in Maine coastal waters.

MATERIALS AND METHODS

Culture experiments. Growth experiments with *Alexandrium tamarense* (clone GT429, axenic from the Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, Maine) were conducted to determine its minimum Fe requirement for growth and to calibrate this requirement with measurements of labile Fe. Surface seawater was collected by hand off Pemaquid Neck, Maine, (Fig. 1a) in an acid-cleaned, 20 l low density polyethylene carboy, filtered through acid-washed 0.2 µm membrane filters (Nuclepore® polycarbonate) to reduce particulate and colloidal Fe concentrations, and stored in an acid-cleaned, low density polyethylene carboy.

The seawater was enriched with a modification of f/2 nutrients (Guillard & Ryther 1962); f/2 amounts of nitrate (883 µM), phosphate (36.3 µM), vitamins (5 to 100 µg l^{-1}) and f/20 levels of trace metals Mn (90 nM), Co (5 nM), Zn (8 nM), and Mo (3 nM) were added to give the basal growth medium. No artificial chelators were added. Cu was not added because *Alexandrium tamarense* is known to be particularly sensitive to cupric ion activity (Anderson & Morel 1978, Schenck 1984). The basal medium was sterilized by microwaving for 8 min l^{-1} in a 1 l teflon bottle (Keller et al. 1988). Culture tubes (60 ml Nalgene® polycarbonate centrifuge tubes with polypropylene caps) were acid-washed, filled with deionized water (Barnstead Nanopure®) and sterilized by microwave (5 min l^{-1}). The tube contents were emptied and 50 ml of the cooled basal medium was pipetted aseptically into each tube using acid-washed, sterile, disposable polypropylene

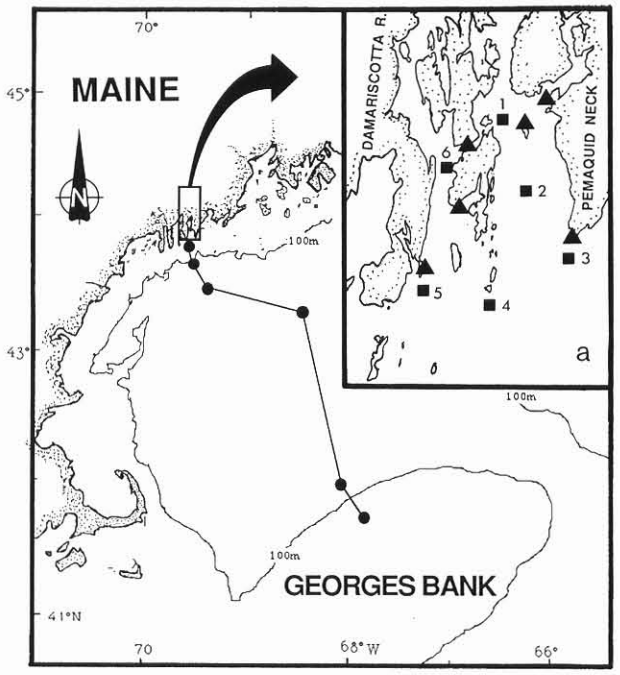

Fig. 1. Sampling stations in the Gulf of Maine, USA. (a) Coastal water sampling stations (■) and blue mussel shore collection sites (▲)

tips. (This procedure avoided clouding of the polycarbonate from contact with hot seawater.)

To minimize Fe contamination, the vessels and media were handled as described under 'Fe analysis'. Fe contamination in the N and P nutrient stocks was minimized by extraction with 8-hydroxyquinoline, also described below. Vitamin and trace metal stocks were prepared from reagent grade chemicals and used without further modification; Fe contamination from these stocks was not significant (see 'Discussion').

Fe was added to culture tubes from a 4×10^{-4} M ferrihydrite stock prepared by adding $FeCl_3 \cdot 6H_2O$ to filtered (0.2 µm), room temperature deionized water. Fe in this stock exists as a colloidal precipitate (Wells et al. 1983). Because this Fe stock is altered chemically by either filtration or heating, it was not sterilized before addition to the culture tubes. While conditions in the final growth experiments were therefore not axenic, bacterial populations estimated from microscopic examinations were small, if evident at all.

The Fe requirement of *Alexandrium tamarense* was determined in 3 consecutive long-term growth experiments, in which the second and third series were inoculated with Fe-deficient cells from the previous series (as in Brand et al. 1983). The initial *A. tamarense* stock, obtained in standard f/2 (Cu and EDTA added), was maintained through 3 transfers in the modified f/2 medium described above (plus 100 nM Fe) to reduce EDTA concentrations. The stock was then transferred into Fe-'free' medium in order to diminish the cellular Fe levels; reduced cell yields in this culture indicated that *A. tamarense* was Fe-deficient. In the first experiment, a series of culture tubes containing different amounts of ferrihydrite were inoculated from the Fe-deficient *A. tamarense* stock, and the tubes placed in a 16 °C water illuminated at 80 µEinsteins m^{-2} s^{-1} by fluorescent Vita® lights on a 14:10 h light-dark cycle. Growth of the cultures was monitored every 2 or 3 d by measuring in vivo chlorophyll fluorescence (excitation 465 nm; emission 675 nm) with a Perkin Elmer 204 spectrofluorometer adapted for the culture tubes. Measurements of in vivo fluorescence accurately reflect cell numbers of *A. tamarense* (Watras et al. 1982, Schenck 1984). The second and third growth experiments used smaller ferrihydrite additions to better define the Fe-regulated growth response. A separate culture series, with the same nutrient additions as for the bioassay tests, was used for labile Fe determinations (see below).

Field program – water samples. Water samples were collected approximately weekly from mid-April through October 1988 near the mouth of the Damariscotta River estuary at 6 stations situated opposite shoreline collection sites used by the Maine Department of Marine Resources for monitoring red tide outbreaks (Fig. 1a). Water samples of 500 ml were collected from 5 m depth using an all-teflon MERCOS (Hydrobios) sampler mounted on a nylon line equipped with a plastic-coated weight. This sampling device is suited for trace metal determinations in open ocean environments (Freimann et al. 1983). Surface water temperatures were measured from 'bucket' samples and Secchi depths were recorded at each station. Samples were transported to the laboratory and split for salinity, total Fe, and labile Fe determinations (see below). Sample processing was completed within 6 h of water collection.

Gulf of Maine waters were sampled at 10 m depth along a transect extending from Boothbay Harbor to Georges Bank (Fig. 1). For the collections, the research vessel was positioned into, or oblique to, the wind and the sampling device lowered by hand off the bow. The samples were processed for labile Fe determinations (see below) on board ship under a downflow filtered-air bench (Class 100); Fe analyses were completed onshore.

Field program – toxin levels. The Maine Department of Marine Resources monitors red tide outbreaks along the entire Maine coastline by measuring saxitoxin concentrations in tissues of the blue mussel *Mytilus edulis* L. and other bivalve species. To evaluate the relationship between Fe and red tides, blue mussel saxitoxin data from the study area (Fig. 1a) were compared with the concentrations of labile and total Fe

measured at the nearby water sampling sites. The blue mussel rapidly accumulates and eliminates dinoflagellate saxitoxins, making it an ideal indicator species (Yentsch & Incze 1979) as well as an integrator of the relative numbers of *Alexandrium tamarense* in the surrounding waters. Saxitoxin concentrations in whole body blue mussel homogenates were determined by the standard mouse bioassay used for paralytic shellfish poison analysis (AOAC 1965, Hurst & Yentsch 1981).

Fe analysis. Concentrations of chemically labile Fe were measured with a technique that utilizes the chelating agent 8-hydroxyquinoline (oxine) (Wells et al. in press). Briefly, 1 ml of purified 6.15 mM oxine was added to 100 ml of unfiltered sample water. The samples were buffered to pH 6 with 1 M sodium phosphate (monobasic). After 1 h, during which the oxine complexed labile (or reactive) Fe, the samples were drawn by vacuum through C_{18} columns (Sep-Pak®) which retained the oxine-Fe complexes. The complexes were eluted from the columns with 2 ml of distilled methanol and the Fe in the eluates measured by graphite furnace atomic absorption (GFAA). The C_{18} columns were precleaned to remove Fe contamination by repeated sequential washes with the oxine-buffer and methanol reagents, the methanol eluate of the final cleaning step being retained for a reagent plus column blank. Recoveries of acidified Fe spikes from seawater were 100 % ± 6 %. The detection limit for 100 ml of sample was ca 1 nM Fe.

In order to determine the extent to which naturally occurring organic-Fe phases were retained on the C_{18} columns, and thus would be enumerated as 'oxine-labile Fe', seawater samples buffered to pH 6 were processed without added oxine. The Fe concentration in these tests were < 10 % of those measured with oxine added. Similarly, when dense phytoplankton cultures were processed without added oxine, the organic-Fe fraction retained on the C_{18} columns was < 5 % of that measured with oxine. Because these values were within the level of precision for the oxine technique, no correction factors were applied to field measurements.

For total Fe determinations, 30 ml of unfiltered sample water were acidified to pH 1.6 with ultrapure HCl and aged a minimum of 2 wk at room temperature before analysis. Total (acid-solubilized) Fe was then determined by either direct injection GFAA, or by oxine preconcentration (as above) after adjusting the sample pH to 5 to 6 with ultrapure NH_4OH (Fe recoveries with oxine at pH < 4 are not quantitative). Intercomparisions showed a reasonable correspondence between these methods.

Ultra-clean techniques were employed throughout sample collection, processing and Fe analyses. All sample manipulations and oxine treatments were done under a downflow filtered-air bench (Class 100). The bottles were soaked a minimum of 24 h in 10 % (reagent grade) HCl, rinsed with deionized water, and resoaked in 5 % ultrapure HCl for a minimum of 12 h. The bottles were rinsed thoroughly with deionized water immediately before use. Reagents used in the Fe analyses were purified to minimize Fe contamination. Ultra-pure HCl and NH_4OH were prepared by subboiling distillation of reagent grade materials in a polypropylene still. Reagent grade oxine was purified by vacuum sublimation at 120 °C onto a cold finger (Honjo et al. 1978); the phosphate buffer was cleaned by repeated extractions with purified oxine. Glass-distilled methanol was used for cleaning the columns and eluting the samples. Reagents were prepared in small batches and stored in acid-cleaned polypropylene bottles until use.

RESULTS

Growth experiments

Growth of *Alexandrium tamarense* with different amounts of added ferrihydrite was preceded by a lag phase of about 10 d (Fig. 2a, b). There was little if any

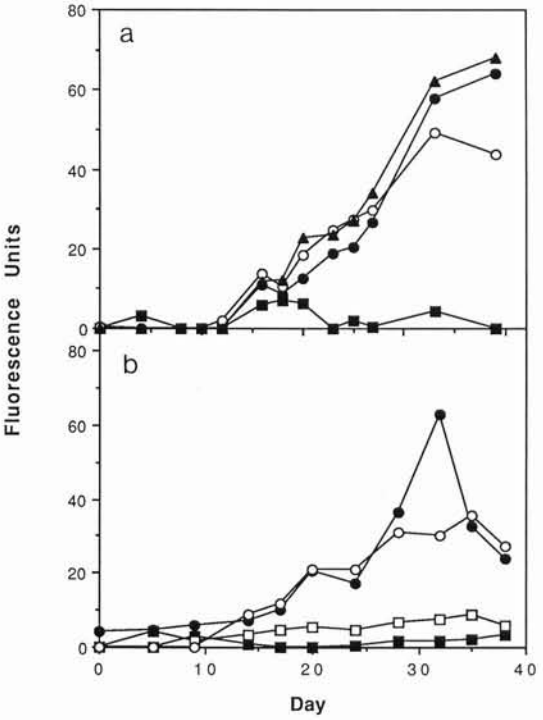

Fig. 2. *Alexandrium tamarense*. Growth response in sequential (a, b) batch cultures with added ferrihydrite. Fe additions: (■) no added Fe; (□) 5 nM ferrihydrite; (●) 10 nM ferrihydrite; (○) 25 nM ferrihydrite; (▲) 50 nM ferrihydrite

growth of *A. tamarense* without ferrihydrite added and growth improved only slightly with 5 nM ferrihydrite (Fig. 2b). Larger additions of ferrihydrite (10, 25, 50 nM) gave substantially better growth than did 5 nM ferrihydrite; however, growth differences among these (higher) Fe tests were small. Using fluorescence as relative index of cell number (Watras et al. 1982, Schenck 1984), the maximum slope of fluorescence increase in the 10 to 50 nM ferrihydrite tests corresponded with a growth rate of ca 0.5 divisions d^{-1}. Maximum densities of *A. tamarense,* estimated from a fluorescence-cell number regression (unpubl.), were on the order of 1×10^7 cells l^{-1}.

The organism's Fe-regulated growth response was calibrated with oxine-labile Fe (oxine-Fe) by measuring Fe concentrations in a separate series of culture preparations (Fig. 3). Oxine-Fe concentrations were

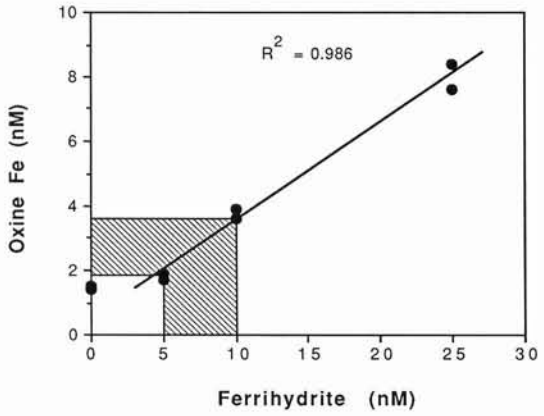

Fig. 3. Concentrations of oxine-Fe in the culture media with ferrihydrite additions. Hatched area denotes the threshold between Fe-replete and Fe-deplete conditions

measured immediately after the media preparation so the values in Fig. 3 represent those at the start of the bioassays (i.e. before significant ferrihydrite hydrolysis or cellular uptake of Fe). The concentration of oxine-Fe in the control (seawater enriched with N, P, trace metals, and vitamins but not Fe) was 1.5 nM Fe, essentially at the limit of analytical detection (1.0 nM). Oxine-Fe concentrations increased proportionally with the amount of ferrihydrite added, accounting for ca 35 % of the total Fe. Based on the growth response, conditions shifted from Fe-limited to Fe-replete between 5 and 10 nM of added ferrihydrite (Fig. 2a, b), corresponding to a limiting threshold value of ca 3 nM oxine-Fe (Fig. 3). While some growth occurred below this threshold value, more Fe was needed for sustained growth under these bioassay conditions (Fig. 2b).

Field survey – Fe

There was considerable range in total Fe concentrations in the study area, with values extending from 25 to 1445 nM (Fig. 4a). At stations where the tidal currents were relatively small (Stns 3 and 4), values showed some indication of a seasonal distribution, with higher concentrations occurring in the spring and fall than in summer (Fig. 4a). Spatial differences also

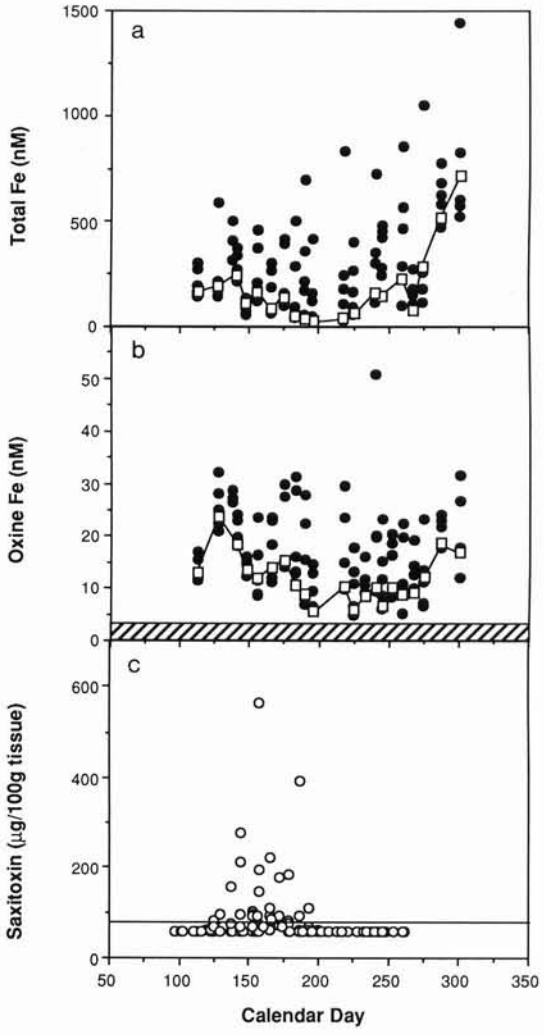

Fig. 4. Concentrations of (a) total Fe, (b) oxine-Fe, and (c) saxitoxin in blue mussel tissues, in the study area during 1988. For the most part, there were no seasonal trends in the total and oxine-Fe data with the exception of Stns 3 and 4 (Fig. 1) which showed some indication of seasonality; only data points from Stn 4 (□) are shown connected for simplicity. The hatched area in (b) shows the concentrations of oxine-Fe at which *Alexandrium tamarense* would be limited by Fe, according to the bioassay results. The detection limit for tissue toxin concentrations, measured by the standard mouse bioassay, was 58 µg (100 g tissue)$^{-1}$. The quarantine level (80 µg (100 g tissue)$^{-1}$) is marked in (c)

occurred among the stations on any given day, the total Fe concentrations being consistently higher at stations subject to strong tidal currents (Wells & Mayer in press).

Concentrations of oxine-Fe ranged from 5 to 50 nM Fe and also showed some indication of seasonality at Stns 3 and 4 (Fig. 4b). Oxine-Fe concentrations were not found to decrease below 3 nM, the threshold value indicated in the growth experiments to cause Fe limitation of *Alexandrium tamarense* (but values did come within a factor of 2 at Stns 3 and 4). As seen with total Fe, concentrations of oxine-Fe were consistently higher at the stations where tidal currents were stronger, probably because of sediment resuspension or enhanced vertical mixing of bottom waters rich in oxine-Fe and total Fe (unpubl.).

Oxine-Fe concentrations in the Gulf of Maine surface waters (July 1988) decreased rapidly with increasing distance from shore, with values changing from ca 8 nM oxine-Fe at the coast to near or below detection (ca 1 nM) within 45 km offshore (Fig. 5). Over Georges Bank (270 km, 30 m deep), the oxine-Fe concentrations were considerably higher (10.9 nM) than at the nearby deep water station (≤ 1 nM; 235 km, 223 m deep). A similar distribution pattern of total Fe was found; concentrations diminished quickly from inshore (49 nM) to offshore (≤ 2 nM; 45 km), remained low in the Gulf of Maine basin (≤ 2 nM), and increased again over Georges Bank (93 nM) (Wells & Mayer in press).

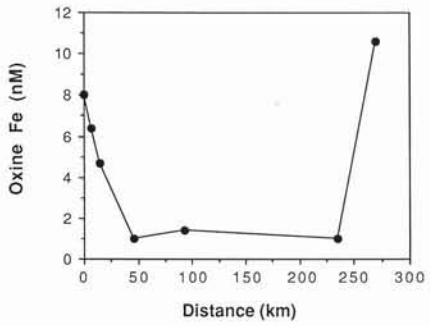

Fig. 5. Oxine-Fe concentrations at 10 m depth in the Gulf of Maine in July 1988 (Calendar Days 192 to 195). Station locations are shown in Fig. 1. Distances are those along the transect from Boothbay Harbor to Georges Bank (270 km). The shore station value (0 km) is the mean of oxine-Fe values measured at the headland stations (3 and 4; Fig. 1) on Calender Day 189

Field survey – tissue toxin

Red tides occurred in the study area during the late spring and early summer of 1988 (Fig. 4c). Tissue saxitoxin concentrations increased well above the quarantine limit (80 µg (100 g tissue)$^{-1}$) at all 6 mussel sampling stations on several occasions. Although all stations were affected, the timing and severity of the *Alexandrium tamarense* blooms differed slightly among stations (data not shown). There was no clear relationship between tissue saxitoxin concentrations and concurrent in situ measurements of oxine-Fe and total Fe (Fig. 6a, b). In contrast to most earlier years, no red tides developed during the fall in 1988, in spite of the apparent general increase in Fe availability in the study area (Fig. 4b).

DISCUSSION

Rigorous testing of the relationship between Fe and red tides has not been possible without methods for estimating biologically available Fe in seawater. Because Fe in seawater should occur principally in colloidal or particulate forms, Fe availability is likely

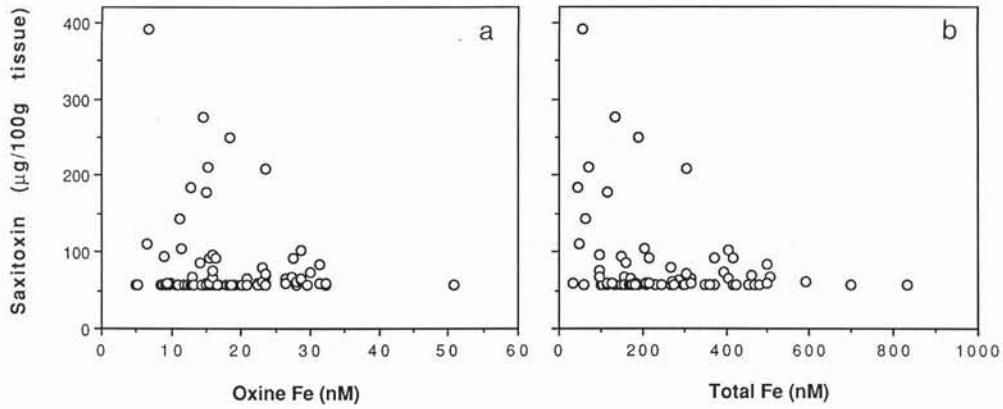

Fig. 6. Tissue saxitoxin concentrations of blue mussels vs Fe: (a) oxine-Fe, (b) total Fe. The data plotted are from the 6 tissue sampling stations and from 4 water sampling stations opposite these shore sites; data from water sampling Stns 2 and 4 are excluded here

dependent on dissolution kinetics which replenish the soluble pool of free (reactive) Fe used by phytoplankton. Presumably, a faster dissolution rate of solid Fe forms (i.e. more chemically labile) should lead to a greater potential supply of Fe to phytoplankton. Fe limitation should result when the phytoplankton demand exceeds Fe dissolution rates.

In the oxine technique, adding oxine in excess over total Fe simulates, in a broad sense, a high 'biological' demand for Fe through complexation ('uptake') of soluble Fe which, in turn, induces colloid and particle dissolution. This analogy is imperfect because oxine may additionally enhance dissolution by direct interaction with the solid Fe surface. While even highly crystalline materials (e.g. goethite, hematite) ultimately can dissolve in seawater, biologically available Fe would comprise only those forms dissolving on time scales relevant to phytoplankton generation rates, something crystalline materials apparently cannot do (e.g. Wells et al. 1983). We have found that only the more labile Fe forms, measured by 1 h of reaction time with oxine, appear to be proportional to Fe availability; 1 h oxine-Fe measurements correlate positively with the extent to which different Fe oxyhydroxides support phytoplankton growth in cultures (Wells et al. in press). Thus, the oxine technique offers a means for testing in situ the hypothesis that red tide outbreaks are initiated by increases in Fe availability.

Growth in the bioassays was preceded by a lag phase of about 10 d that likely reflected the poor physiological state of the Fe-depleted inoculum; for example, the normal lag time for the red tide dinoflagellate *Gymnodinium sanguineum* in culture (ca 3 d) increases to 7 to 8 d under Fe-deficient conditions (Doucette pers. comm.). Growth differences among the tests after the 10 d lag are interpreted as illustrating the relative state of Fe nutrition. The results indicate that the minimal Fe requirement of *Alexandrium tamarense* in batch culture is > 1.8 nM of oxine-Fe; although some growth occurred at this level of Fe, it was not sustained over the duration of the bioassay (Fig. 2b). By comparison, doubling the oxine-Fe concentration to 3.8 nM gave sustained growth for weeks at rates in the maximum range reported for *A. tamarense* in batch cultures (Watras et al. 1982, Anderson et al. 1984, Schenck 1984). Further, the maximum cell densities with ≥ 3.8 nM oxine-Fe were on the order of 10^7 cells l^{-1}, which is 3 orders of magnitude greater than concentrations shown to cause extremely toxic shellfish in nature (Yentsch & Mague 1979). It therefore seems that the 3 nM threshold of oxine-Fe is a reasonable first approximation for distinguishing Fe-deplete from Fe-replete conditions for *A. tamarense,* and that severe red tide outbreaks are possible at only moderately higher concentrations of oxine-Fe.

It is important to emphasize here the empirical and approximate nature of the 3 nM threshold value. Ferrihydrite is chemically unstable, the bond structure being altered over time by dehydration of the polymeric Fe(OH, H_2O) precipitate (Van der Giessen 1968, Murray 1979, Eggleton & Fitzpatrick 1988). These alterations lead to decreased solubility, lability and (hence) biological availability of the colloidal Fe (Wells et al. 1983, Wells et al. in press). The time-dependent maturation process, combined with the increasing Fe demand associated with greater cell abundances, should have diminished Fe availability as the bioassay progressed. The 3 nM oxine-Fe threshold (measured at time zero) may therefore overestimate the actual Fe requirement of *Alexandrium tamarense,* with sustained growth perhaps being possible with a smaller, but continuous, supply of Fe. Additional complication comes from the possibility of 'luxury' uptake of Fe by *A. tamarense*; most or all the Fe needed for growth may have been taken up within the first few days, before the rates of cell metabolism increased. Thus, the bioassay results are best employed to indicate the Fe reserve needed for bloom development if no new Fe was introduced to surface waters.

Oxine-Fe concentrations in the growth medium increased with additions of ferrihydrite at a consistent proportion (ca 35 %) of the total Fe added (Fig. 3). This proportion was roughly half of that measured for ferrihydrite in unenriched seawater (Wells et al. in press) indicating that one or more of the enrichment constituents rendered ferrihydrite less labile. One possible explanation would be phosphate adsorption or co-precipitation with ferrihydrite; adsorption of the added trace metals to ferrihydrite may also have contributed to the decrease in its lability. The effect of such indirect factors on Fe availability cannot be resolved from these data.

The coastal area chosen to test the relationship between Fe and red tide development is a region where red tides have been recorded frequently since monitoring began in 1972. These red tides are characteristic of those in other regions of the Maine coast, being patchy, temporally discontinuous events generally occurring between May and October. The outbreaks are believed to originate primarily by an autochthonous mechanism (excystment of resting stages overwintering in the underlying sediments), but the shoreward advection of offshore populations might be important at the headland site (Stn 3) on Pemaquid Neck (J. W. Hurst pers comm. 1988). There are no major freshwater inputs to the study area, although the region is influenced by runoff from the Penobscot River situated 100 km to the northeast. During 1988, red tide outbreaks occurred repeatedly in the study area and the bloom intensities, inferred from the saxitoxin con-

centrations in blue mussel tissues, were similar to those in earlier years (Thayer et al. 1983, J. Hurst pers. comm. 1988).

The change in blue mussel tissue saxitoxin concentrations did not correlate with either water temperature or salinity (data not shown), concordant with the findings of other studies (Mulligan 1975, Sweeney 1975, Martin & White 1988). Tissue saxitoxin concentrations also were not related to water clarity, as measured by Secchi depth. (Light data were not available for comparison.) Although these factors undoubtedly constrain the conditions suitable for red tide outbreaks, the results suggest that these variables did not initiate bloom development.

For changes in Fe availability to be a direct cause of red tide outbreaks it is necessary that *Alexandrium tamarense* normally experience Fe-limitation in coastal waters, such that increases in Fe availability initiate its rapid growth. However, at no time did oxine-Fe concentrations in surface waters decrease below the 3 nM threshold estimated to cause Fe limitation. Although values did come within a factor of 2 of this threshold (5 to 6 nM) at the headland stations (3 and 4) during summer, when water column stratification would be greatest, oxine-Fe concentrations at the other stations remained considerably higher, probably as a result of tidally-induced vertical mixing (unpubl.). Moreover, based on the high bioassay cell densities generated with 3.8 nM oxine-Fe, it appears that the availability of Fe in the study area was sufficient not only for rapid cell growth, but also for heavy bloom development throughout the sampling period.

With *Alexandrium tamarense* apparently not limited by Fe in these waters, there is little reason to expect that in situ concentrations of oxine-Fe would reflect its abundance. Indeed, there was no correlation between oxine-Fe and tissue saxitoxin concentrations, the index of *A. tamarense* populations (Fig. 6a). To test the possibility that pulsed changes in Fe caused a time-delayed growth signal (due to the relatively long generation times of *A. tamarense*), tissue toxin concentrations were compared with surface water oxine-Fe concentrations measured 1, 2, and 3 wk earlier, but still no association was found (data not shown). Measurements of oxine-Fe were therefore a poor indicator of red tides.

In drawing comparisons between oxine-Fe and tissue saxitoxin data we recognize that cellular toxin levels can be variable and are poorly defined in natural systems. As a result, blue mussel saxitoxin data may not have reflected consistently the abundance of *Alexandrium tamarense* cells. Nonetheless, because the primary goal of this study was to determine whether estimates of Fe availability could assist in predicting the occurrence of poisonous shellfish, correlations between bivalve toxicity and Fe were considered most meaningful.

It should be remembered that oxine-Fe analyses were performed on unfiltered water samples and thus the values comprise the oxine-lability of both dissolved (< 0.4 μm) and particulate (> 0.4 μm) Fe phases. Although biologically available Fe is generally considered to occur in the dissolved fraction, discrimination between dissolved and particulate fractions likely has limited relevance when considering Fe availability; by utilizing free (reactive) Fe, the phytoplankton ultimately draw upon all Fe forms regardless of substrate size. We have found, for instance, that a substantial proportion of oxine-Fe often resides in the particulate phase (Wells & Mayer in press). The summed lability of dissolved and particulate fractions should therefore provide a better estimate of the availability of Fe in seawater.

Offshore in the Gulf of Maine, oxine-Fe concentrations decreased below 3 nM beyond about 25 km from shore, and were near or below detection (1 nM) at the 3 basin stations (Fig. 5). Fe availability in the basin waters may therefore have been too low to support sustained growth of *Alexandrium tamarense.* Although Dinophyceans are often the second most abundant phytoplankton group in the Gulf of Maine basin, their numbers are generally low, and *Gonyaulax* spp. (which until recently included *A. tamarense*) are a small component of the dinoflagellates present (Marshall & Cohn 1983, Marshall 1984a, b). In contrast, oxine-Fe concentrations over Georges Bank (10.6 nM) exceeded even those measured in coastal waters, and there is some evidence that red tide outbreaks occur here (J. Hurst pers. comm. 1988). The distribution of *A. tamarense* populations is therefore consistent with the pattern of estimated Fe availability in the Gulf of Maine.

Outbreaks of *Alexandrium tamarense* in Maine coastal waters did not correspond with changes in total Fe concentrations (Fig. 4b), in contrast with blooms of the toxic dinoflagellate *Ptychodiscus breve* (formerly *Gymnodinium breve*) in Florida which correlated positively with total riverine inputs of Fe over a 25 yr period (Ingle & Martin 1971, Kim & Martin 1974). However, the latter correlation is equivocal. Firstly, total Fe concentrations were not monitored at the sites of bloom initiation off the Florida coast; Fe concentrations at these initiation sites might not have been a function of river runoff. Secondly, outbreaks may have instead been related mechanistically to other riverine constituents changing in concert with Fe (e.g. humic substances; Prakash & Rashid 1968). However, the unique importance of Fe for red tide development has been implicated directly in other bloom site studies (Matsunaga et al. 1984), in enrichment experiments (Wilson 1966, Martin & Martin 1973, Graneli et al. 1986) and in physiological studies (Doucette & Harrison 1990). The lack of correlation

between Fe and red tides reported here may indicate that *A. tamarense* (clone GT429) has a lower Fe requirement than other toxic dinoflagellates, rendering it less sensitive to changes in Fe availability; too few data on dinoflagellate Fe requirements presently exist to allow comparison among species. Alternatively, the differing results from Florida and Maine waters may be related to the sites of bloom inititation. Florida red tides usually initiate 18 to 74 km offshore and are transported inshore by winds, tides and currents (Roberts 1979); as many as three-fourths of these offshore 'microblooms' terminate before being advected inshore (Steidinger 1975), suggesting that nutrient conditions are usually inadequate for bloom development. By comparison, red tides in Maine coastal waters often appear to develop autochthonously within the inshore environment (J. Hurst pers. comm. 1988). Were they to initiate offshore instead, as in Florida, the oxine-Fe data suggest that pulsed inputs of available Fe could be critical for bloom development.

Because *Alexandrium tamarense* became Fe-limited at oxine-Fe levels that were measurable in cultures and in the field, we suggest that the oxine technique be used to determine whether other phytoplankton species are Fe-limited in seawater. In particular, the Fe requirement of *Ptychodiscus breve* should be determined in culture and compared with concentrations of oxine-Fe in waters off the Florida coast where blooms initiate. In this respect, it would be crucial that the oxine-Fe threshold for growth be determined with great accuracy because Fe concentrations in offshore waters are much lower and less variable than in coastal waters. Oxine-Fe organism growth rate calibrations could be refined by using continuous or semi-continuous culturing techniques, thus reducing some of the uncertainties associated with long-term batch methods. Still better would be to relate oxine-Fe measurements to short-term Fe uptake rates using ^{59}Fe. In this way, Fe uptake rates could be isolated from crop production factors and might prove to be a more direct index for estimating Fe nutrition in seawater.

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