BARIUM UPTAKE BY MARINE DIATOMS

by

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ABSTRACT

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The marine diatoms Skeletonema costatum, Thalassiosira

pseudonana, and Coscinodiscus rex were cultured in batch and

chemostat in an attempt to verify that diatoms act as carriers

of barium in its marine geochemical cycle. Various models of

uptake are developed. Removal in batch culture is negligible.

Removal in chemostat culture accounts for less than 10% of

the ambient barium, indicating the agency of these diatoms

is insufficient to explain the well-documented oceanic surface

depletion and deep water enrichment of barium, according to

standard models or even according to the most generous models.

Experiments to investigate the mechanism of uptake are outlined,

should a barium-accumulating diatom be found.

ACKNOWLEDGEMENTS

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INTRODUCTION

The interest in the geochemical cycle of barium and the possible role of diatoms in that cycle comes from various sources. One is the radioactive isotope ^{226}Ra produced in the sediments by the decay of ^{230}Th. Koczy [1] suggested its use as a time-tracer of the rate of mixing processes and of water mass movements. As it is present at lower concentrations in the surface layer than in the deep ocean, the relative concentrations compared to the decay rate ought to give the time required for mixing across the thermocline. Also, since ^{226}Ra has a bottom source whereas ^{14}C, the other major tracer of mixing rates, has an aerial input, the two should serve as independent checks on the system. Surface depletion of ^{226}Ra indicated rates of mixing across the thermocline that were at least an order of magnitude lower than those yielded by the ^{14}C method. This was ascribed to unknown non-radiological processes controlling the distribution of ^{226}Ra. In the case of ^{14}C, non-radiologic processes influencing the distribution of carbon can be corrected for exactly, by consideration of the isotope ^{12}C. Radium has no stable isotope, and so for this correction a chemical analogue must be found instead. Barium, occurring directly above radium in the alkali earth metals of the periodic table is such an analogue. Barium profiles with depth show surface depletion very similar to ^{226}Ra profiles, implying that non-radiologic processes are

primarily responsible for the low relative concentrations of ^{226}Ra at the surface. The non-radiologic component accounts for the lion's share of the ^{226}Ra signal, and as such the correction system must be well-founded and exact. The divergence from proportionality between radium and barium, inferred to be the radiologic component, is in fact so small that extracting time information on a large scale will have to await improvements in ^{226}Ra analytical technique [2].

The phenomenon of surface depletion relative to the deep water is most strikingly apparent for the nutrients silicate, phosphate, and nitrate, and for ΣCO_2 and alkalinity, but the same type of profile is observed in attenuated form for nickel [3], cadmium [4], and zinc [5], ~~Bruland, M & N~~ as well as for barium. Barium is the most abundant (about 40 n M/kg surface) of the trace constituents to show this relationship (surface values for nickel, cadmium, and zinc, are 3 n M/kg, .1 n M/kg, and .2 n M/kg respectively) and so can best be investigated as a model system for the other three.

For such disequilibrium between the surface and deep water to maintain itself, there must be removal at the surface and production (or regeneration) at depth. This is usually taken to indicate organisms at work. Herein lies the third interest in this work. Barium, because of its weight, among other considerations, is generally held to have no biological importance, and yet it seems to be handled and transported by living systems. How does an organism

deal with an element that is neither useful nor toxic? Does it pass through the system by default, or is it handled by enzymatic systems specifically designed for the safe passage or exclusion of trace metals in general?

The original evidence that barium is actively removed from the water column, was the enrichment of sediments in barium, and the existence of seemingly authigenic barite crystals in the sediment despite apparent $BaSO_4$ undersaturation in the overlying waters [6]. Barium was found to covary with the opal content of sediments (Ba/Si $\simeq$ 3.8 x 10^{-3}) [7] and with overlying high productivity zones, though there was dissent on the implication of these facts [9].

More striking evidence is the covariance of barium with silicate and specific alkalinity (representing production and dissolution of diatom and radiolarian frustules, and cal-careous coccoliths and foraminiferal tests respectively), al-though not with the nutrients nitrate and phosphate. This covariance obtains not only in individual profiles but also in barium's enrichment in Pacific deep water relative to Atlantic deep water in concert with most nutrients, but especially with silica and alkalinity.

To separate the comparators silica and specific alka-linity, which over most of the ocean covary, and determine whether opal or carbonate is the more likely carrier phase for barium and radium, Edmond [10] charted radium correlations

with each across the Anarctic circumpolar current boundary,
south of which carbonate productivity drops markedly with
respect to diatom productivity. The radium adheres closer
to the silicate across the boundary. More recent data, how-
ever, is more ambiguous about differentiating between opal
and carbonate carrier phases [2].

Redfield has described the relationship of the ratio of
nitrogen to phosphorous in plankton to that in seawater [11]. From
this he has concluded that whenever removal of two chemical
species is proportional, their concentration in seawater will
vary linearly with one another. Stallard [12] has taken the
inverse of this; that whenever two chemically reactive species
plot linearly against each other, and not against conservative
properties, their removal rate must be everywhere related by a
constant factor. Symbolically,

$$\frac{d[Ba]}{d[Si]} = k$$

where

$$[Ba] = k [Si] + C$$

This is roughly the case over most of the world ocean with the
factor being about 7×10^{-4} [13] mole barium per mole silicate.

The nature of this removal can at present only be inferred
indirectly. As an illustration of the relation of barium to
the nutrients, profiles of barium, silicate and nitrate, and
a plot of barium against silicate are presented for a Pacific

station in Fig. 1 and an Atlantic station in Fig. 2. The
Pacific station illustrates the closer link of barium with
silicate than with nitrate. Both barium and silica exhibit
maxima around 3500 m below the surface as opposed to nitrate's
shallow maximum at 1000 m. This suggests barium's incor-
poration into "hard" or refractory material that can avoid
destruction and dissolution until farther in the water column.
Materials in this category include the silicious tests of dia-
toms and radiolaria and the calcareous shells of foramnifera
and coccolithophorids. Materials contraindicated are cyto-
plasm, organic coats, aragonite and celestite. The property-
property relation is generally a straight line,

$$[Ba] = 22 + 7.1 \times 10^{-4} [Si]$$

but including some pronounced discontinuities. The two at
[Si] = 150 and [Si] = 50 are evident in the silicate profile
as well, but the discontinuity at low barium and silicate
represents a genuine divergence. The Atlantic profile is
dominated by the various cores of water riding over one an-
other at that station. Still, throughout the profile, and
the sections that represent different source regions, barium
follows silicate. The property-property relation is, at a
gross level, a straight line

$$[Ba] = 43 + 8.3 \times 10^{-4} [Si]$$

but can be broken down into a series of line segments whose

Figure 1   Chemical data for Geosecs Station 343, 14°N
            123°W Easter Pacific

            A.   Barium concentration with depth

            B.   Silicate concentration with depth

            C.   Nitrate concentration with depth

            D.   Property-property plot of barium against
                  silicate

Note discontinuities in the barium-silicate relation
correspond to discontinuities in the silicate profile

Figure 2   Chemical data for Geosecs Station 54   15°S

30°W Southwest Atlantic

A.   Barium concentration with depth

B.   Silicate concentration with depth

C.   Nitrate concentration with depth

D.   Property-property plot of barium

against silicate

Abbreviations indicate respective positions of Sub-Tropical

Underwater (STUW) Antarctic Intermediate Water (AAIW)

Mediterranean Intermediate Water (MIW) Labrador and North

Atlantic Deep Water (LANDW) and Antarctic Bottom Water

(AABW). Straight lines connecting these water masses in

the property-property relation represent simple mixing.

Barium nM/kg (top left): depth km vs Barium nM/kg, with labels STUW, AAIW, MIW, LANDW, AABW

Silicate μM/l (top right): depth km vs Silicate μM/l, with labels AAIW, MIW, LANDW, AABW

Nitrate μM/l (bottom left): depth km vs Nitrate μM/l, with labels AAIW, MIW, LANDW, AABW

Barium nM/kg vs Silicate μM/l (bottom right): with labels AABW, LANDW, AAIW, MIW, STUW

joints represent the centers of the various water masses. Again, the surface discontinuity is not reflected in the silicate data, although it is in the salinity data (not shown) [2].

Crystalographic data argue against calcite as the carrier phase. The radius ratio of transition from the calcite form of a carbonate to the aragonite form is 0.67 [14]. The ratio for calcium is 0.67 and so it can assume either form. Strontium, with a radius ratio of 0.75, is strongly excluded for calcite with respect to aragonite [15]. For barium, with a radius ratio of 0.87, the exclusion should be nearly complete.

Various analyses of marine plankton have been performed and usually significant amounts of barium have been found [9], [16], [17], [18]. Commonly, however, concomitant hydrographic work is not done; the planktonic phosphate, nitrate, carbon, silicate and carbonate values are seldom reported. All this would have helped fill in the picture, but even then, separation of the plankton so that the carrier organisms and the carrier phases can be determined is not generally practicable.

The most direct method for investigating this problem would seem to be controlled culture experiments. For my own work, three species of marine centric diatoms were grown in batch and chemostat culture in an attempt to verify the hypothesis of diatoms as agents of barium removal. In culture, a single species can be grown and observed. The composition of the growth medium can be monitored and manipulated to more rigorously determine uptake levels, laws, and mechanisms.

Through the determination of laws and mechanisms, insight can be gained to understand deviations from smooth correlation, for instance, in the Circumpolar Current, where certain features in the silicate profiles are only weakly reflected in the barium profiles. Cultures have the disadvantage, of course, of not being the ocean, which is the original object of interest. Still, what they may lack in fidelity they can make up in perspective and understanding.

Much work has been done with the interaction of trace metals and phytoplankton in culture. Most of it involves their toxic or salutory effects, or the amounts that can be accumulated. In this work by contrast emphasis is on the effect the phytoplankton have on the water chemistry, and the stoichiometry of uptake. To this end, high precision analysis of water, and a clean and defined system have higher priority than, say a wide phylogenetic diversity or a close approximation of the natural environment. Importance is also placed upon reference to models, so that results can be not only reported, but analyzed within the context of oceanographic data.

Edmond has proposed, by analogy to chemical coprecipitation, a model of barium uptake and emplacement into the opal frustule such that the ratio of barium to silica in the frustule reflects their ratio in the surrounding water through the fractionation constant α. Symbolically,

$$\frac{d[Ba]}{d[Si]} = \alpha \frac{[Ba]}{[Si]}$$

and at steady state concentrations of barium and silica

$$\left(\frac{x_{Ba}}{x_{Si}}\right)_{diatom} = \alpha \frac{[Ba]}{[Si]}$$

This is what the stoichiometry of uptake should look like, and although it has the appearance of chemical coprecipitation, it does not specify the mechanism of uptake. Still, ignoring the agency of the organism, it is logical that a contaminant should appear in proportion to its ambient concentration and in inverse proportion to the ambient concentration of the major constituent. This has been established for instance for the Sr/Ca ratio in mollusk shells [15]. Fresh water gastropods, grown under controlled conditions, precipitated shells whose strontium content reflected the Sr/Ca ratio in the surrounding water with a fractionation coefficient of 0.237 (compared to 1.12 for non-biologically precipitated aragonite) that was in-dependent of temperature, growth rate, absolute Sr levels, and species. This despite the separation of the site of shell for-mation from the ambient water by a biological membrane.

In chemostat culture, the manifestation of the α relation-

ship would be direct:

$$\left(\frac{x_{Ba}}{x_{Si}}\right)_{diatom} = \alpha \frac{[Ba]}{[Si]}$$

or in concentration parameters

$$\frac{[Ba]_i - [Ba]_a}{[Si]_i - [Si]_a} = \alpha \frac{[Ba]_a}{[Si]_a}$$

where i stands for inflow and a for ambient. Solving for α,

$$\alpha = \frac{([Ba]_i - [Ba]_a) [Si]_a}{[Ba]_a ([Si]_i - [Si]_a)}$$

Batch culture is more a closed system. If the diatom can also be seen as a "closed system" (only uptake and no dissolution) then the removal equation can be integrated

$$\int_i^f \frac{d[Ba]}{[Ba]} = \int_i^f \alpha \frac{d[Si]}{[Si]}$$

$$\alpha = \frac{\ln([Ba]_i/[Ba]_f)}{\ln([Si]_i/[Si]_f)}$$

Here i stands for initial and f for final. In batch culture change in barium is related to change in silicate by an exponent of α. In chemostat culture the two are related by a factor of α. Since α is less than unity, the same amount of change in silica would produce a vastly smaller effect upon barium in batch than in chemostat. This is one of the major advantages of chemostat over batch, despite the former's greater difficulty and complexity.

If the barium is incorporated within the frustule at its formation, then it must be handled by the cell's own machinery. The silica frustule construction occurs on the organic silicalemma on the newly formed wall that separates the daughter cells immediately following mitosis (see Fig.3). It takes place in the very center of the cells excluding the possibility of purely chemical coprecipitation. The process, from silica uptake to deposition involves a membrane-bound transport system [19], the expenditure of energy through ATP hydrolysis [20], protein synthesis, polymerization, coordination by microtubules [21], and precipitation onto the silicalemma [22]. Azam [19] has described such a membrane-bound enzymatic transport system for silica, taking as proof of its existence not only its adherence to Michaelis-Menten enzyme kinetics, but also its variation with temperature and its inhibition by germanium, which occurs below silicon in the periodic table. The germanium makes an instructive possible analogy with barium. In the range of [Ge]/[Si] = 0.01, it is incorporated into frustules without apparent deleterious effect. In the range of [Ge]/[Si] = 0.01, it is toxic to diatoms, preventing normal frustule formation [23]. The method of interaction appears to be competitive inhibition, in which germanium competes with silica for the same enzymatic sites, and can be described by the same sort of enzymatic kinetics, albeit with different constants. The equations are [24]

Figure 3   Cell Division and Silica Frustule

Formation in <u>Thalassiosira</u> <u>pseudonana</u>

Note discarded girdle, which could account

for 10% of the diatom's opal [33] (Guillard

personal communication).

silica girdle

cell membrane

silica valve

Mitosis

new girdle

connecting strands

discarded girdle

daughter cells

nascent valve

silicalemma

daughters separating

new valve

$$E \quad + \quad S \quad \underset{k-1}{\overset{k+1}{\rightleftharpoons}} \quad ES$$

$$ES \quad \underset{k-2}{\overset{k+2}{\rightleftharpoons}} \quad E \quad + \quad S$$

where E represents unbound enzyme, S the substrate (in this case ambient Si, Ge or Ba), ES the enzyme-substrate complex, P the product (in this case intracellular Si, Ge, or Ba), and E_T the total amount of enzyme, bound or unbound. Traditionally, k-2 is assumed to be negligible and so the rate equation takes the form

$$- \frac{d[S]}{dt} = k_{+2} [E_T] \frac{[S]}{\frac{k_{-1} + k_{+2}}{k_{+1}} + [S]}$$

The expression $\frac{k_{-1} + k_{+2}}{k_{+1}}$ forms the lumped constant K_m. In nutrient-uptake studies the further approximation is made that $k_{+2} \ll k_{-1}$ and so

$$\frac{k_{-1} + k_{+2}}{k_{+1}} \simeq \frac{k_{-1}}{k_{+1}} = k_s$$

which is effectively the dissociation constant for the enzyme-substrate complex. Setting these equations for barium and silica, and assuming that the same enzyme [system] operates on both.

$$- \frac{d[Ba]}{dt} = k_{+2 \, Ba} [E_T] \frac{[Ba]}{k_{s \, Ba} + [Ba]}$$

$$- \frac{d[Si]}{dt} = k_{+2 \, Si} [E_T] \frac{[Si]}{k_{s \, Si} + [Si]}$$

$$- \frac{d[Ba]}{d[Si]} = \frac{k_{+2 \, Ba}}{k_{+2 \, Si}} \frac{[Ba] (k_{s \, Si} + [Si])}{[Si] (k_{s \, Ba} + [Ba])}$$

The two features of enzyme kinetics, the rate at saturation,

and the substrate concentration at half-saturation are, under the given approximations, separable with respect to kinetic constants. The product formation rate constant, k_{+2}, determines the amount of barium incorporated, and the enzyme-substrate dissociation constant $k_s (= \frac{k_{-1}}{k_{+1}})$ determines the apparent stoichiometry of barium uptake with respect to silica (and also the inhibition of silica uptake by barium if such were the case). Four limiting cases present themselves:

1) $k_{s\,Ba} \gg [Ba]$ $k_{s\,Si} \gg [Si]$

$$\frac{d[Ba]}{d[Si]} = \frac{k_{2+Ba}}{k_{2+Si}} \frac{[Ba]}{[Si]} \frac{k_{s\,Si}}{k_{s\,Ba}}$$

2) $k_{s\,Ba} \ll [Ba]$ $k_{s\,Si} \ll [Si]$

$$\frac{d[Ba]}{d[Si]} = \frac{k_{2+Ba}}{k_{2+Si}}$$

3) $k_{s\,Ba} \gg [Ba]$ $k_{s\,Si} \ll [Si]$

$$\frac{d[Ba]}{d[Si]} = \frac{k_{2+Ba}}{k_{2+Si}} \frac{[Ba]}{k_{s\,Ba}}$$

4) $k_{s\,Ba} \ll [Ba]$ $k_{s\,Si} \gg [Si]$

$$\frac{d[Ba]}{d[Si]} = \frac{k_{2+Ba}}{k_{2+Si}} \frac{k_{s\,Si}}{[Si]}$$

The first case is substantially the α model. Barium removal with respect to silica reflects their relative proportions in the ambient medium. The second represents roughly a constant accumulation of barium per cell regardless of the ambient concentration of barium or silica. A stoichiometry of this sort might be interpreted as the cell's having a need for a specific amount

of barium, which it acquires and then stops. In culture,

the results from batch and chemostat should be similar with

the expression $\dfrac{[Ba]_i - [Ba]_a}{[Si]_i - [Si]_a}$ being a constant. Uptake of this

sort is in accordance with the implications of property/

property linearity without the need for further information as

to the relative proportions of barium and silica in surface sea

water. In possibility number three, barium per cell reflects

only the ambient barium concentration. There would be a dif-

ference between batch and chemostat only to the extent that

barium is depleted in the batch medium in which case the cellular

composition would reflect the exponential decline. The constant

expression would be

$$\frac{\ln\,(Ba_i/Ba_f)}{Si_i - Si_f}$$

In chemostat the constant expression would be

$$\frac{Ba_i - Ba_a}{Ba_a} \cdot \frac{1}{Si_i - Si_a}$$

Oceanographically, this model is compatible with the property/

property relation only in the case that surface barium levels are

roughly constant throughout the ocean. The other hybrid pos-

sibility, number four, is the inverse of number three, its

special property being that without the model it would be very

difficult to understand why barium uptake should be inversely

related to silica concentration and not at all to barium con-

centration. The difference between batch and chemostat would

be pronounced in case four because silica depletion is so much

greater than barium depletion. The constant expression for

batch is

$$\frac{Ba_i - Ba_f}{\ln (Si_i - Si_f)}$$

and for chemostat

$$Ba_i - Ba_a \times \frac{Si_a}{Si_i - Si_a}$$

Again oceanographically the property/property relation would hold if surface silicate levels were roughly constant.

Another feature of the enzymatic model is that it can be extremely species specific as it depends upon the affinity of an enzyme for an element which is neither useful nor toxic in concentrations normally encountered. On the other hand, the enzyme's affinity for barium could be related in some way to its affinity for silica. This might tend to discriminate between diatoms from different environments [25]. An enzyme's affinity for its substrate, or an unaccustomed substrate, is usually a function of its three-dimensional conformation. This in turn can be radically or subtly affected by single amino acid substitutions generated by a simple point-mutation in the genetic code. That is, given enzymatic uptake, incorporation of barium can be such a mutable characteristic that there is no reason to suggest, as Riley and Roth [17] have done, that if there were a species difference, there ought to be more similarity within a family than between families [26]. Environment and randomness probably hold greater sway than phylogeny in this model.

Non-specific adsorption and ion exchange are physical processes that could account for barium uptake by diatoms. If this were the case, one might expect to see barium removal near the sea floor by resuspended sediment, analagous, to ^{210}Pb. This is not observed, but neither is it necessary; resuspended sediment is not the same as living diatoms [27]. The stoichiometry of uptake would follow the Langmuir isotherm equation [28]

$$\frac{\theta}{1-\theta} = k^{-1}a_i$$

where θ is the fraction of the surface covered (or sites occupied) and a_i is the activity of species i. Rearranging,

$$\theta = \frac{k^{-1}a_i}{1+k^{-1}a_i}$$

an expression very like the Michaelis-Menten equation is obtained. Two limiting cases obtain, one where surface area (or number of sites) is limiting and one where it is not. In the first case the diatom is saturated with barium and the barium to silica ratio is constant (assuming the ratio of silica to binding site is constant) which is like case two of the enzymatic model. The situation of sites' not being limiting leads to barium to silica in the diatom being proportional to ambient barium concentration as in enzymatic case

three. The species dependence of physical processes would
be less marked although it might reflect the degree of sili-
fication, the texture and hence surface area that the frustule
presents, and the extent of the organic casing on the out-
side of the opal frustule. Silica is highly undersaturated
in surface waters, and so for opal frustules, unlike car-
bonate coccoliths, there is a strong tendency to dissolve.
Diatoms combat this tendency by covering the test inside and
out with an organic coating and, some have suggested, with
an authogenic clay [29] (for the barium to reside in the clay it
would have to be 14% by weight $BaSO_4$). Despite this, dis-
solution accounting for 10% of the weight of the frustule
has been measured for Thalassiosira pseudonana [30]. There
is evidence that this ten percent represents a discarded girdle
after division (Dr. R.L.L. Guillard, personal communication)
(see Fig. 3) and not general attrition. But if it is dis-
solution of the entire test then equilibrium would imply pre-
cipitation at the same time with the possibility of coprecipi-
tation of barium. If we postulate that the diatom constructs
a frustule of pure $SiO_2 \cdot nH_2O$, and once it comes in contact
with the outside, pure silica dissolves and silica mixed with
barium precipitates then the process can be seen as a race
between diatom growth and contaminant insinuation. With an
equilibrium constant for opal of $10^{-2.7}$ [28] and setting [Si]
to 10^{-6} M (slightly higher than culture conditions) and dis-
solution accounting for a fraction of 10^{-1} of the frustule,
at best $10^{-4.3}$ of the frustule is new material. Oceanographi-

cally Ba/Si in frustules should be about 7×10^{-4} [13]. This would make Ba/Si in newly emplaced material 14, a bit difficult to conceive of.

The fact of dissolution is felt as well in the analysis of batch culture data. If the α relationship and an open system is assumed, in which barium may leave the medium by incorporation into frustules and re-enter it by redissolution, the mass balance equation becomes

$$d[Ba] = \alpha \frac{[Ba]}{[Si]} d[Si] + ([Ba]_i - [Ba]) r \, dt$$

where r is the dissolution rate in units of inverse time, and the subscript i denotes initial values. When solved for α, there is still the undetermined parameter r. In fact r could only be determined directly for a given alga under given growth conditions by a silica (or analogue) isotopic experiment [26]. Otherwise silica dissolution is completely masked by uptake. (An alternative, certainly open to objection, is the measurement of dissolution of killed cells [31]). The correction term is only small when d[Si]/dt is large or when $[Ba] \gg Ba_{diatom}$, both conditions obtaining more towards the beginning of the growth curve, when $\Delta[Ba]$ is small and hence difficult to measure accurately. Failure to correct for dissolution leads to an overestimate of α. The situation is quite different for a chemostat. The mass balance equation for barium at steady state in a chemostat of volume V with flow rate Q is

$$\overset{\text{inflow}}{} \quad \overset{\text{outflow}}{} \qquad \overset{\text{uptake}}{} \qquad\qquad \overset{\text{redissolution}}{}$$

$$\frac{d[Ba]}{dt} = [Ba]_i \frac{Q}{V} - [Ba]_a \frac{Q}{V} - \alpha \frac{([Si]_i - [Si]_a)}{[Si]_a} [Ba]_a (\frac{Q}{V} + r) + r([Ba]_i - [Ba]_a)$$

At steady state when the left-hand side is set to zero, the expression $\frac{Q}{V} + r$ cancels everywhere. Solving for α

$$\alpha = \frac{([Ba]_i / [Ba]_a - 1}{([Si]_i / [Si]_a) - 1}$$

This is part of the attractiveness of the chemostat over-batch.

MATERIALS AND METHODS

The diatom species cultured were Skeletonema costatum, Thalassiosira pseudonana, and Coscinodischs rex. Skeletonema was grown in batch and in chemostat culture. Chemostat work with Skeletonema was abandoned partly due to low uptake, but mostly due to problems of sticking to the plastic container, causing non-steady-state conditions, self-shading, and a surface for bacterial growth. With Thalassiosira there was much less sticking and with Coscinodiscus there was none, and so these were grown in chemostat, although not in batch.

All three species are marine centric diatoms of the family Coscinodiscineae. Skeletonema costatum (Greville) Cleve clone Skel was isolated (and supplied) by Dr. R.R.L. Guillard (Woods Hole Oceanographic Institution) from Long Island Sound in 1956. It is mainly a coastal species, found in large numbers when it occurs [32]. It forms chains by spines which emanate from the periphery of the test. Chains consist of an average of six individuals in culture, depending on the degree of agitation. The individuals are the shape of an equidimensional cylinder, about 10μ on a side. Thalassiosira pseudonana Hasle and Heimdal (Cyclotella nana Hustedt) clone 3H was isolated (and supplied) by Dr. Guillard from Moriches Bay, Long Island in 1958. It is a coastal and estuarine phytoplankter, tolerating wide salinity ranges. It can form chains of up to four individuals in culture, connected by five to eight strands eman-

ating from slime pores about the margin of the cylindrical test (also about 10^μ on a side) [33]. Coscinodiscus sp (provisionally termed rex) was isolated (and supplied) by Larry Brand (Woods Hole Oceanographic Institution) from Nantucket Sound in 1977. It exists only as individuals (except for paired daughters) in culture. It is a more flattened cylinder 200^μ in diameter.

The medium used for culture experiments is Guillard's F/2 medium, [34] (see Table 1) which is a nutrient-enriched seawater medium. Most artificial media, besides posing greater difficulty in their preparation, also would have higher barium concentrations than natural surface seawater due to contaminants in the major salts. Further, F/2 has proven adequate to culture a wide variety of phytoplankton. Nutrient stock solutions are prepared, autoclaved, and stored in 125 ml polypropylene bottles, except for the vitamin stock which is kept frozen in .5 ml quantities in 1 ml ampoules. The nitrate and phosphate stock solution and the trace metal stock solution are made up to be 10^3 more con-centrated than the final medium, the vitamins 2×10^3. Sil-icate stock is approximately .01 M to avoid precipitation in the autoclave. There need be little concern that EDTA might lower the activity of barium. Competition from calcium and magnesium which have much higher affinities [35] and concentrations, keeps chelated barium to less than 10^{-5} of total barium (see Table 2).

TABLE 1

Composition of Guillard's medium F/2 [28]

$NaNO_3$	75	mg
$NaH_2PO_4 \cdot H_2O$	5	mg
$Na_2SiO_3 \cdot 9H_2O$	varying	
Na_2EDTA	4.36	mg
$FeCl_3 \cdot 6H_2O$	3.15	mg
$CuSO_4 \cdot 5H_2O$	.01	mg
$ZnSO_4 \cdot 7H_2O$	.022	mg
$CuCl_2 \cdot 6H_2O$	.18	mg
$Na_2MoO_4 \cdot 2H_2O$	.006	mg
Thiamine HCl	.1	mg
Biotin	.5	µg
B_{12}	.5	µg
Seawater	to 1 liter	

TABLE 2

The Upper Limit of Chelation

of the Species Ba, Ca and Mg*

Species	Total Concentration	Complex Formation Constant [35]	Concentration of bound species	Fraction of total bound
EDTA	1.5×10^{-5}M		1.5×10^{-5}	1
Ba^{2+}	5.0×10^{-8}M	5.8×10^{7}	8.3×10^{-14}	1.7×10^{-6}
Ca^{2+}	1.0×10^{-2}M	5×10^{10}	1.4×10^{-5}	1.4×10^{-3}
Mg 2+	5.4×10^{-2}M	4.9×10^{8}	7.4×10^{-7}	1.4×10^{-5}

*Iron was not considered as its concentration is affected

by the algae.

Batch cultures are grown in 1 liter polycarbonate erlen-
meyer flasks each with an inverted beaker over the top in
F/2 medium. The medium is prepared and filtered through six
autoclaved 250 ml capacity Millipore Sterilfil units with
0.4μ Nucleopore membrane filters. The contents of each unit
is poured into an autoclaved erlenmeyer flask and four of the
six are innoculated with 1 ml of actively growing algal cul-
ture. One blank and one innoculated flask are sacrificed
immediately. The rest are placed in a 20°C constant temper-
ature room beneath "cool white" fluorescent bulbs. Innocu-
lated flasks are sacrificed at intervals until the diatom
population plateaus, at which time the last innoculated flask
and the second blank are sacrificed. The purpose of this
arrangement is to monitor what the algae do to the barium
concentration over time and to determine if there is any
change associated with the seawater's simply sitting in a
polycarbonate flask beneath fluorescent lights. Sacrificing
is by centrifugation in four 50 ml polypropylene tubes, the
contents of which are combined in a polyethylene bottle for
analysis of dissolved barium and silica. The pellet is dis-
tributed between two tubes and washed twice in vycor-distilled
water. The washed pellet from one tube goes for particulate
barium analysis, the other for silica. All plastic in con-
tact with liquid is soaked in 1 N HCl and silicone rubber
is stored in 2% by weight EDTA.

Chemostat design (see Fig. 4) is basically taken from
Goldman [36] with modifications due to the constraints of

Figure 4   Chemostat assembly

1)   2 liter polypropylene medium bottle with melted-
on tubing, shielded from light

2)   Peristaltic pump squeezing shut silicone rubber
tubing.

3)   Polypropylene tubing held by nylon NPT fitting
against 47 mm Nucleopore Swin-Lok polycarbonate
filter holder with .4μ Nucleopore membrane filter,
and teflon gasket.

4)   Filter screws into built-up and NPT topped cap
of chemostat vessel fabricated from Nalgene
spherical bottom 250 ml polycarbonate centrifuge
bottle, and polycarbonate tubing.

5)   Silicone tubing leads to 500 ml amber polypropy-
lene bottle.

6)   Aquarium air pump.

7)   Drying tube packed with activated charcoal and
stoppered with pyrex wool.

8)   Swin-Lok filter holder with glass fiber filter.

9)   Constant temperature bath.

10)   Two "cool white" fluorescent lights.

this study i.e. low barium contamination, and silicate limitation. For instance, barium is near saturation at the levels used in the experiments and so sterilization of sea water can not be by autoclaving, which could radically change its chemistry. Instead, the seawater is filtered before the nutrients are added and there is an in-line filter just above the chemostat vessel. Clogging of the filter limits the amount of medium used and thus, in tandem, the size of the chemostat vessel and the duration of the experiment. Given the duration of the experiment, the amount of time involved in barium analysis, and the residence of sample in the overflow bottle for up to a day, it made most sense to analyze the entire contents of the chemostat vessel (as opposed to the overflow as is traditional in chemostat work). The algae were found to stick to the plastic vessel in the uniform flow field of constant magnetic stirring, and so simple bubbling in a round-bottom vessel suffices to keep them in suspension instead.

All components in the chemostat were chosen or constructed to minimize contamination by barium and by silica, to be autoclavable, and also to minimize substrate for bacterial growth. To avoid leaching of silica into solution, no glass was used in the system. Possible barium contamination also precluded glass, as well as metal or black rubber, and militated against the use of even silicone rubber whenever possible. Polyethylene and polystyrene do not survive autoclaving well, and Tygon tubing connections tend to either stick or

come loose in the autoclave. Further, Tygon tubing provides

a substrate for bacterial growth and so would have to be

changed daily [37], which is unacceptable from the stand-

point of contamination. The materials in contact with the

liquid are polypropylene, polycarbonate, teflon, and a mini-

mum of silicone rubber. Polypropylene, polycarbonate and

teflon are soaked in 1 NHCl for at least a day and until

used. Silicone rubber is soaked in 2% EDTA.

The equipment consists of the following: Medium is

stored in a two liter polypropylene bottle. This has a 1

1/2 meter length of polypropylene tubing melted on. The

tubing leads into 10 cm of silicone rubber peristaltic tubing.

A further short length of polypropylene tubing is held by

a nylon NPT fitting against a 47 mm Swin-Lok polycarbonate

filter holder (Nucleopore Co.) with a Teflon gasket, sili-

cone rubber O-ring, and a .4 μ polycarbonate membrane filter

(Nucleopore Co.). The filter screws into a built-up and

NPT-tapped polypropylene cap, which screws onto the chemo-

stat vessel. The vessel is fabricated from a 250 ml poly-

carbonate spherical bottom centrifuge bottle (Nalge Co.)

and 1/4" I.D., 3/8" O.D. polycarbonate tubing. The pieces

are machined, bent and joined with trichloroethylene (later

evidence indicates chloroform serves better). One tube

comes down the side and is joined at the bottom of the bottle

with a pinhole for bubbling air. The second tube is joined

at an angle near the top as an overflow tube. To the over-

flow is attached a piece of silicone tubing leading to an

amber polypropylene collection bottle. The air is sent by a small aquarium air pump through a drying tube packed with activated charcoal and pyrex wool and then through a glass fiber filter to the chemostat vessel. The vessel sits in a 20°C ($\pm$ 0.2°C) bath continually illuminated by two "cool white" fluorescent bulbs from the side.

Since the chemostat overflow is at the top, 3 cm away from where the fresh medium drips in, conceivable complete mixing might be a problem. That this is not the case is indicated by two observations. First comes from a dye experiment, quantitated by spectrophotometry in which dye concentration in the vessel decays with washout as predicted by complete mixing, within experimental error (see Table 3). Perhaps more convincing, the ambient silicate, as measured by sacrificing the entire chemostat vessel is not consistently lower than the silicate in the overflow (it is lower in three out of ten cases). If mixing were not complete, and part of the inflow medium, which is between one hundred and two hundred times more concentrated, washed out directly, then the silicate concentration of the overflow would certainly reflect this.

This lack of a consistent difference between instantaneous and collected silica alleviates another concern, that while in the overflow bottle the diatoms are either dissolving or assimilating more silica. There is more variation from day to day than between the two determinations. The process of dissolution or extra assimilation must be either small or

Table 3

DYE MIXING EXPERIMENT

The chemostat vessel is filled with dye $KMNO_4$ λ_Δ = 526 nm at a certain concentration and distilled water is added at a rate close to standard conditions while bubbling. After an interval of dilution the concentration of dye is measured spectrophotometrically in the chemostat vessel, the inflow, and the total overflow (normalized to the original concentration being equal to one).

Inflow (i) .008

Chemostat bottle (b) .440

Total overflow (f) .669

Original (o) 1

chemostat bottle capacity 238.2

Overflow 200.4

Mass balance $[dye]_o V_b =$

$$[dye]_b V_b + [dye]_f V_f - [dye]_i V_f$$

With complete mixing   238.4 $\sim$ 243.3

$$[dye]_b = [dye]_i + ([dye]_o - [dye]_i) e^{-V_f/V_c}$$

.440 $\sim$ .436   .9% off in the opposite direction from incomplete mixing.

balancing.

Filtered seawater, obtained from the Environmental Sys-
tems Laboratory, Woods Hole, Mass. is filtered again through
a glass fiber filter (nominal pore size 1μ) before storage
in a 6-liter plastic carboy. Seawater is filtered through
a .45μ cellulose nitrate filter into the autoclaved medium
bottle, and the nutrients are added to make F/2 medium. The
bottle is placed, sealed from light, about 1 1/2 meters above
the level of the chemostat vessel. The rest of the equipment
is rinsed in distilled deionized water and then glass dis-
tilled water. The chemostat vessel is attached to its air
and water filters and autoclaved, as is the peristaltic
tubing. When cool, the air source is connected and approx-
imately 70 ml of actively growing culture is poured in. The
vessel is set into the constant temperature bath, connected
to the peristaltic tubing and the medium bottle, and the
medium is dripped in at about the same rate as will be for
the duration of the experiment. In this way (as opposed
to innoculating a full vessel with a small amount of cul-
ture) the culture is almost at steady state by the time it
comes to volume and the possibility of auxospore formation is
minimized [38]. The daily monitoring of the experiment is
as follows: The collected overflow is filtered into a grad-
uate, the volume recorded and the filter discarded. A
sample of 25 ml is injected by syringe into a 100 ml poly-
ethylene bottle, acidified, and stored for silicate analysis.

Cell counts are done microscopically on samples obtained by
tipping the vessel. The time required for five drops of
medium to fall is recorded as a check on the stability of the
flow rate. At a washout rate of 1 day^{-1} and 2 liters of
medium, the experiment can last a maximum of six days. At the
end, in addition to the daily monitoring regimen, the vessel
is detached from the medium filter at the cap, and with the
air source still connected, the contents are poured into a
Millipore Sterilfil unit with a pre-weighed .4µ Nucleopore
filter. Part of the filtered liquid rinses the 500 ml
polyethylene sample bottle and then goes for silicate analysis.
The rest of the liquid is poured into the bottle and spiked
for barium analysis. Another 500 ml sample bottle is placed
below the pre-chemostat-vessel filter to catch inflow medium
at the same rate as it had been delivered during the course
of the experiment. In this way, the difference between the
chemostat vessel sample and the inflow medium sample should
be the residue from innoculation, the final filtration, the
bubbling air, the residence in the polycarbonate vessel, and
the activity of the phytoplankton.

The filtration process and the container have been
determined to not present significant sources of contamination.
The residue from innoculation should not be significant
after several generations have passed, as the innocula
are grown in polycarbonate flasks and in the same medium.
The bubbling of air can affect the barium concentration in
that it causes some evaporation of the medium and hence in-

creases the total ion concentration. The extent to which this
occurs depends on the rate of bubbling, the room temperature
and humidity. In the later runs corrections have been made
to changes in chlorinity where significant (> 1%).

The "reactive" silicate is analyzed according to the re-
duced silico-molybdate colorimetric method of Strickland and
Parsons [39] with several modifications suggested by Fanning
[40]. A 25 ml sample is injected into a 100 ml polyethylene
bottle by syringe and acidified with two drops 6 NHCl for
storage. At the time of analysis 10 ml of ammonium para-
molybdate is injected into the bottle by syringe. After 20
minutes the metol-sulphiteoxalic sulfuric acide reducing
agent is added. The interval is approximate but is main-
tained exactly the same for samples and standards by stop-
watch. Standards consist of a distilled water blank, a
blank based on synthetic seawater and a standard made up
in synthetic seawater of either 5μ M or 50μ M depending on
the concentration range of the samples. After 6 to 24 hours
absorbance at $810n$ M is read in either 1 cm or 10 cm cells
depending on the concentration and the instrument.

As a check on the precision of the method, a set of
standards from $.1\mu$ M to 5.0μ M in distilled water were run
(see Table 4). The standard deviation of the values from the
linear regression line was $.006\mu$ M. This standard deviation
is extremely small (and difficult to believe) but it was
obtained under ideal conditions and so cannot be considered
generally applicable. It serves to demonstrate, however, that

Table 4

Silicate Standard Curves

Standards made in distilled water

Concentration	Absorbance	f(absorbance) based on linear regression	Residual
0	.020	.0014	.0014
.1	.031	.1034	.0034
.25	.47	.2519	.0019
.5	.072	.4839	.0161
2.5	.291	2.5159	.0159
5.0	.558	4.9935	.0065

$$\frac{\sqrt{\Sigma r^2}}{6-2} = .0060$$

the "method" errors, e.g. contamination or insensitivity, are
very low. "Careless" error, e.g. inaccuracy of standards,
timing or reagent volume error, should be proportional to
concentration. This indicates that measurements of low
silicate values (between .1μ M and 1μ M) are as reliable
as those of high values (between 20μ M and 80μ M). The
major problem is a biological one, that at low growth rates,
the concentration that the diatoms take the silica down
to is small but unstable. Some compensation must be made
for the fact that the individual frustules suspended in
the chemostat at any given moment were formed at different
times and hence different ambient silica concentrations.
A formula for "adjustment for history" is given in Table 5.
It is basically a weighted average of recorded silica values
where the weighting factor is the probability that a frustule
formed at the time of the recorded silica value is still
present by the end of the experiment. For comparison
of ambient silicate with growth rate, however, (see Table 6)
such an adjustment was not made. Instead an estimate of
the approached "steady state" silicate concentration was
used.

The analysis of dissolved barium is by the method of
Bacon and Edmond [13]: a sufficient amount of spike solu-
tion, enriched in the isotope ^{135}Ba is added volumetrically
by microburette to yield a ^{138}Ba/^{135}Ba ratio of about one.
The barium is separated from other salts by cation exchange,
concentrated down by evaporation and loaded on the filament

Table 5

A Typical Run T13
Innoculation 11-17 13:00

Sec/drop	Time	Days to the end	Overflow volume (ml)	Cell Counts cells/ml		[Si]μM
32,6	11-18 14:30	4.3 (3.3)	436	$532/.2mm^3$	2.62×10^6	.22
33.8	11-20 16:45	2.2 (1.6)	266	$466/.2mm^3$	2.33×10^6	.41
33.6	11-21 23:00	1.0 .5	208	$430/1.5mm^3$	2.87×10^6	.24
32.6	11-22 22:15	0				
Final chemostat vessel		0	235			.25

For cell counts

$\hat{\sigma}$ = Poisson-type counting error = 4.6%

S = 10.3%

Flow rate

$\overline{Q}$ = 210.5 mZ/day

$\overline{Q}/V$ = .896/day

Ambient silica adjusted for history

$$[Si] = \frac{\sum_i Wi[Si]_i}{\sum_i Wi} \quad \text{where } Wi = 2^{-[(\text{days to the end})_i \times (Q/V)]}$$

Table 6

Skeletonema costatum chemostat particulate silicate

	Run 5	Run 6
$[Si]_i - [Si]_f$	41.0μM	21.7μM
particulate analysis	45.3μM	19.9μM
% difference	10.5	-8.3

of a mass spectrometer. From the ratio of the two isotopes ($R_{138/135}$) the original concentration of barium is calculated according to the relation

$$nM\ Ba/kg = \left(\frac{R_{138/135} - .0389}{10.928 - R_{138/135}}\right) \left(\frac{nM^{135}Ba}{ml}\right)_{spike} \times \frac{spike\ vol.}{0.0656}$$

$$- \text{column blank} \times \frac{sample\ weight}{column\ weight} /sample\ weight$$

the constants involved having to do with the isotope ratios in nature and in the spike solution.

Particulate silica was determined by dissolution in Na_2CO_3, neutralization with H_2SO_4, and colorimetric analysis as with dissolved silicate. In this method, the plankton is transferred into a 125 ml polypropylene bottle which is filled with 5% by weight Na_2CO_3, tightly capped and set in an 85°C oven for at least 10 hours. After cooling, the bottle is reweighed and the bicarbonate neutralized with concentrated H_2SO_4 to below the alkalinity endpoint pH = 4.5 (2.7 ml H_2SO_4/100 ml Na_2CO_3). The blank and standard are made up in heated and neutralized Na_2CO_3 in the same way as the sample. For later runs this procedure was abandoned in favor of estimating particulate silica by difference between inflow and outflow concentrations. The latter method compares well with direct analysis of the particulate (see Table 7), is probably more precise, and involves no handling of the filtered diatom material, which is best as regards barium contamination.

Table 7

Chemostat silica data

Thalassiosira	Experimental weight mg	[Si] µM	[Si] µM	$[Si]_i - [Si]$ µM	%Opal by Weight	Cell count #/mℓ	Si/cell µM/cell	dry weight/ cell mg/cel
7		48.4	.26	48.1		3.3×10^6	1.5×10^{-8}	
8		30.2	.30	29.9		2.0×10^6	1.5×10^{-8}	
9	3.86	47.3	.21	47.1	21.9	2.0×10^6	2.4×10^{-8}	8.0×10^{-4}
10	2.68	35.2	.17	35.0	23.5	1.4×10^6	2.5×10^{-8}	8.0×10^{-9}
11	2.63	38.9	.38	38.5	26.3	1.7×10^6	2.3×10^{-8}	6.4×10^{-9}
12	1.71	24.3	.35	24.0	25.3	6.2×10^6	3.9×10^{-8}	11.5×10^{-9}
13	3.99	56.1	.27	55.8	25.2	2.9×10^6	1.9×10^{-8}	5.7×10^{-9}
14	2.06	31.4	.33	31.1	27.2	1.2×10^6	2.6×10^{-8}	7.2×10^{-9}
Coscinodiscus								
1	.29					2.4		
2	2.23	62.5	4.0	58.5		230		
3	3.04	31.9	.7	31.2		250 (for T.P.) (T.P.9-14)		
$\bar{x}$	2.82			38.7	24.9	1.6×10^6	2.6×10^{-8}	7.8×10^{-9}
estimated method error)								
$\hat{\sigma}$	.02	2	.2	1	1.1	$.5 \times 10^6$	$.9 \times 10^{-8}$	2.4×10^{-9}
S					1.9	$.78 \times 0^6$	$.7 \times 10^{-8}$	2.0×10^{-9}
S%					7.6	47	26	26

Particulate barium is analyzed as follows: After filtration of the contents of the chemostat vessel, the Nucleopore filter and the sides of the filter funnel are washed four times with vycor-distilled water. The osmotic pressure from the distilled water washed causes most of the cells to lyse with some removal of soluble cellular components into the wash water. If it had been important, cells could have been maintained intact by washing with ultrapure iso-osmotic NH_4Cl (residue NH_4Cl crystals would have sublimed during later sample preparation) but as it was, the distilled water wash gave consistent gravimetric results; that is, the biomass on the filter is tightly correlated to silicate removal from the medium (see Table 7). Any mass balance shortfall between inflow barium and the sum of ambient and particulate barium could be ascribed to soluble cellular barium, and interesting result in itself (or else to weakly adsorbed barium which would have washed away with NH_4Cl as well as with distilled water).

The filter is removed and stored in a Millipore petrislide at room temperature until well dry, and then it is weighed. Fifty μℓ of ^{135}Ba-enriched spike are loaded onto the filter and a blank filter (approximately a five-fold excess as estimated from barium removal fro two reasons: smaller volumes would have a large percentage uncertainty, and if a significant quantity of barium is irreversibly bound in the sample preparation, then starting with a good quantity of barium will guarantee enough at the end to give a traceable signal

on the mass spectrometer). The spike is dried down in a
filtered air stream and each filter is folded into the bot-
tom of a 5 ml vycor beaker. The beaker is covered with an
inverted 10 ml pyrex beaker and ashed at 600°C for 15 hours.
The ash is transferred and the vycor beaker rinsed twice with
the vycor-distilled HNO_3 into a 5 ml Teflon beaker. The
HNO_3 is dried away and approximately five drops of ultra-
pure HF are added (a hundred-fold excess with respect to
the quantitative formation of the volatile SiF_4, but just
enough to cover the bottom of the beaker). This rests for
a day, covered with parafilm. The HF is dried away on a
hot plate followed by five drops of ultrapure $HClO_4$ to
drive off the fluorine. This is heated by hot plate and heat
lamp until fuming stops (approximately two hours). A sec-
ond application of $HClO_4$ is driven off in the same way, but
more thoroughly; after the fuming stops the heat lamp
is moved closer to the beakers until the fuming stops again.
A few drops of vycor-distilled HNO_3 are added and dried
down to convert the $BaCl_2$ to $Ba(NO_3)_2$. The beaker is cov-
ered with parafilm to later enter the regular barium analysis
procedure at the step of loading the sample on the fila-
ment of the mass spectrometer.

RESULTS AND DISCUSSION

If removal of barium from the water is related to the removal of silica, then presentation of the silica data is a logical prerequisite to the appreciation of the barium data. Skeletonema costatum, growing in batch culture, increased its biomass by a factor of almost one thousand and lowered the ambient silicate concentration from 140μ M/l to .7μ M/l (see Table 8). From day six to day eight the silicate concentration remained relatively constant while the population continued to increase by 25%. This would tend to support the idea that dissolution occurs concomitant with growth, although it does not serve to quantitate it. Thalassiosira pseudonana in chemostat culture took an inflow medium concentration of about 40μ M/l silica to about .3μ M/l (see Table 7). The progress of a typical run is displayed in Table 5. For comparison, the deep Atlantic has silica concentrations around 35μ M/l and the deep Pacific around 160μ M/l. Surface values are around 2μ M/l. One reason for lower inflow silica values in chemostat than in either batch or in deep water is to limit the density of the algae. An overdense culture is prone to problems of self-shading, which makes interpretation of nutrient limitation difficult [41], and to takeover by bacteria. The low ambient silica values are produced by the particular alga at their low growth rates. The average for Skeletonema was .45μ M Si/l and for Coscinodiscus 2μ M Si/l.

Table 8

Skeletonema costatum Batch Culture experiment No. 6

Description [Ba](nM/kg) [Si]μM Cell Volume ($\mu^3/m\ell$)

Description	[Ba](nM/kg)	[Si]μM	Cell Volume
Blank 1	65.48	141	0
Day 0	63.75	140	4×10^5
Day 4	63.92	101.5	6.14×10^7
Day 6	64.83	.8	2.25×10^8
Day 8	65.49	.7	2.82×10^8
Blank 2	65.53	140	0

mean 64.83

s.d. .82

maximum possible ΔBa = 1.78 nM/kg

maximum Si_i/Si_f = 140/.7 = 200

$$\alpha = \frac{\log(Ba_i/Ba_f)}{\log(Si_i/Si_f)} \leq .005$$

Several lines of evidence lead to the conclusion the
Thalassiosira was silica limited. One is that silica con-
centration was so low. Paasche [42] determined .7$_\mu$ M Si/l
to be the concentration below which Thalassiosira pseudonana
ceased completely to take up silica (this result is in
direct contradiction to my data, which comes from sampling
the chemostat itself, as opposed to the overflow as did
Paasche). Nelson et al [30] found no such threshold con-
centration and estimated the half-saturation constant for
silica uptake by Thalassiosira pseudonana to be between
2.3μ M Si/l and 0.8μ M Si/l depending on the physiological
state of the diatom. If all other requisites for growth are in
surplus, a nutrient whose concentration is chronically
below K_s is considered limiting. Classically, a nutrient is
considered limiting if an increment in its concentration
produces a proportional increment in biomass. Comparison of
inflow silica concentration with diatom dry weight (see
Table 7) shows this to be the case. Depletion of the cel-
lular content of a certain nutrient compared that of well-
supplied cells is commonly taken as symptomatic of limita-
tion [43]. Indeed, a plot of the fraction of diatom weight
accounted for by opal against ambient silica for Thalassiosira
(see Fig. 5) shows this depletion, even within the small
range of ambient silica values encountered. This effect
has been noted by Paasche [42] for Thalassiosira pseudonana

Figure 5   Percent Opal (per dry weight)

versus Ambient Silicate (Adjusted*)

% Opal = 1.875 $[Si]_a$ + 19.6

*See Table 5

and Davis [41] for <u>Skeletonema</u> <u>costatum</u>.

A curious phenomenon, noted in other chemostat work [42], [43], is that at low flow rates (flow rate, at steady state, being equivalent to growth rate), the concentration of the "limiting" nutrient increases (see Fig. 6). If growth rate-versus-nutrients saturation kinetics were obeyed, the concentration would drop linearly with growth rate. Other models predict organisms' giving up altogether at low concentrations, which would then make the ambient concentration constant at low growth rates. The most obvious explanation for the observed contrary behavior is that at low growth rates, a second, unmeasured, parameter such as nitrate is depleted, to become more limiting [45]. Soeder [44] instead forms an explanation based solely (albeit indirectly) on the concentration of the limiting nutrient (phosphorous in his case), that is, making no reference to any other parameter, nor even to time. This is curious as mathmatically growth rate can not be considered a function of the nutrient's concentration in this range, since it can take on either of two values. (Strictly speaking, the growth rate is set by the washout rate to which the ambient concentration adjusts itself. From the perspective of an individual alga, however, growth rate depends - at steady state - on the immediate environment.) Another possibility is that at low growth rates the cells are physiologically impaired and lose silica to the medium during the stress of filtration. It is doubtful whether this phenomenon can be demonstrated to

Figure 6   Steady-State Silicate Concentration

versus Flow Rate for <u>Thalassiosira</u>

<u>pseudonana</u>

$$[Si]_a = {}^-0.287 \ Q/V + .51 \quad corr. = {}^-.88$$

ever obtain in nature, however, so it is likely to remain

a curiosity.

The most salient feature of the barium data is the

paucity of removal. The batch experiments with Skeletonema

can effectively be considered blank experiments, as if the

algae had never been innoculated and allowed to grow to

density. The variance measured in the experiment (see Table

8), since it follows no logic, can be taken as measurement

of the experimental methods' precision. The average barium

concentration is 64.83 n M/kg and the standard deviation

is .82 n M/kg or 1.3%. This seems reasonable compared to

the estimated precision of 0.7% for the barium analysis

[46], noting also the additional error accrued in exper-

imental handling. Barium removal in the chemostat is the

difference between inflow and ambient concentrations and

so the associated variance is the sum of the variance of

each, yielding a standard deviation of 1.16 n M/kg. For

Thalassiosira, five out of eight experiments exhibit barium

removal within or near two standard deviations of zero (see

Table 9). The results for the other species are similar

(see Tables 10 and 11). These results are small with regard

to analytic precision, but the story is worse compared to

oceanographic models. The average fractional removal of

barium in chemostat culture is 2.7%. Taking an estimate

of α based on oceanographic data of .03 together with the

average extent of silica removal of 99.2%, the fractional

Table 9

Thalassiosira pseudonana chemostat culture experiments 7-14

Experiment	$[Ba]_{ambient}$ nm/kg	$[Ba]_{inflow}$ nm/kg	$\dfrac{[Ba]_i - [B_2]_a}{[B_2]_a}$	Si μM	Si_i μM	$\dfrac{[Si]_i - [Si]_a}{[Si]_a}$	$\hat{\alpha}$
7	70.39	72.90	.0357	.34(.26)*	48.4	141	.0025
8	69.17	71.85	.0387	.51(.30)*	30.2	58.2	.00066
9	73.51	81.05	.1026	.21	47.3	224	.00046
10	69.82	70.76	.0134	.17	35.2	206	.00007
11	76.52	73.77	.036	.38	38.9	101	.00036
12	73.24	73.00	-.003 (.0478)**	.35	24.3	68	-.00004 (.00023)*
13	152.4	153.9	.0098 (.0213)**	.25(.27)	56.1	207	.00005
14	160.5	159.4	-.0068	.44(.33)	31.4	94.2	-.00007

$$\frac{[Ba]_i - [Ba]_a}{[Ba]_a} = .000344 \frac{[Si]_i - [Si]_a}{[Si]_a} - .0197 \quad r = .57$$

*Silicate values adjusted for history, see Table 5.

**Barium concentration normalized to chlorinity see p. 43.

Estimated method error ±1.16 nM/kg Ba

Table 10

Skeletonema costatum chemostat culture experiments 5 & 6

Description	[Ba] (nM/kg)	[Si] (μM)	Cell count (individuals/mℓ)
Run 5 inflow	75.34	41.6	
Run 5 ambient	67.73	.59 (1.3)*	6.99×10^5
Run 6 inflow	72.35	23	
Run 6 ambient	65.00	.28 (.71)*	4.67×10^5

$$\alpha = \frac{Si_a}{Si_i - Si_a} \frac{Ba_i - Ba_a}{Ba_a}$$

= (Run 5) .0016 (.0036)*

= (Run 6) .0014 (.0036)*

*Silicate values adjusted for history. See Table 5.

Table 11

Coscinodiscus rex chemostat culture experiments 2 & 3

Description	[Ba] (nM/kg)	[Si] (μM)	Cell count (individuals/mℓ)
Run 2 inflow	50.61	31.9	
Run 2 ambient	54.60	.88 (1.10)*	256
Run 6 inflow	49.68	62.5	
Run 6 ambient	51.18	4.13 (4.48)*	406

$$= \frac{Si_a}{Si_i - Si_a} \quad \frac{Ba_i - Ba_a}{Ba_a}$$

$= -.0020 \ (-.0026)*$

$= -.0021 \ (-.0022)*$

*Silicate values adjusted for history. See Table 5.

removal of barium would have been 81%. Taking instead the
model of a constant ratio of barium per silica, and an
oceanographic estimate of that ratio of 7 x 10^{-4} [13], the
fractional barium removal would have been 40%.

Still, an attempt was made to find a pattern to barium
removal and to relate it to other culture parameters, and
the results of other workers. The α relationship, a plot
of the ratio of barium removed to ambient barium against
the ratio of silica removed to total silica (see Fig. 7)
yields a slope, which is the estimate of α, of .0003 and
an intercept of -.0197. The correlation coefficient is
.57. A plot of barium removal versus silica removal, the
model of constant ratio uptake (see Fig. 8) yields a slope
of .19 n M Ba/μ M Si and an intercept of -4.6 n M Ba. The
correlation coefficient is .60. The hybrid enzymatic models
of barium per silica in the diatom being either proportional
to ambient barium or inversely proportional to ambient
silica (not depicted) fared less well as regards correlation
coefficients. Two tighter relations, for which no model
is obvious: fractional barium removal against growth rate
(corr. = 0.79, 0.93 without T 10 see Fig. 9) and fractional
barium removal against ambient silica (corr. = -0.63,
-0.96 without T 10 see Fig. 10). The first is not consis-
tent with observation of batch cultures, in which most in-
dividuals exhibit maximal growth rates, having lower barium
removal than chemostat. The second relation is difficult
to explain in that it makes no reference to the amount of

Figure 7   Fractional Barium Removal versus

Fractional Silica Removal for <u>Thalassiosira</u>

<u>pseudonana</u>.   The α relationship.

$$\frac{[Ba]_i - [Ba]_a}{[Ba]_a} = 0.000344 \ \frac{[Si]_i - [Si]_a}{[Si]_a} - 0.019 \quad corr.=.57$$

Figure 8   Barium Removal versus Silica Removal

for <u>Thalassiosira</u> <u>pseudonana</u>. The

constant ratio relationship.

$\Delta[Ba] = 0.20 \ \Delta[Si] - 5.0$ corr. = .61

Figure 9   Fractional Barium Removal versus

Chemostat Flow Rate for <u>Thalassiosira</u>

<u>pseudonana</u>

$$\frac{[Ba]_i - [Ba]_a}{[Ba]_a} = 0.146 \; Q/V - 0.089 \quad corr. = .79$$

$$= .187 \; Q/V - 0.113 \quad corr. = .93$$

$$without \; T \; 10$$

Figure 10   Fractional Barium Removal versus Ambient

Silicate (Adjusted*) for <u>Thalassiosira</u>

<u>pseudonana</u>

$$\frac{[Ba]_i - [Ba]_a}{[Ba]_a} = - 0.36 \ [Si]_a + 0.129 \quad corr.=-0.63$$

$$= - 0.71 \ [Si]_a + 0.243 \quad corr.=-.96$$

without T 10

*See Table 5.

$$\frac{[Ba]_{\bar{a}}[Ba]_i}{[Ba]_a}$$

nM/kg

0.1

0.05

0.0

• T9

• T13

• T8

• T7

• T14

• T10

• T12

0.2   0.3   0.4

$[Si]_a \; \mu M/l$

T11 •

diatom present.

The barium particulate analysis (see Table 12) could account for no more than 1.5 n M Ba/kg, and the results were not well correlated with barium removal. It is therefore suggested that the bulk of the measured barium represents contamination and the bulk of the barium associated with the diatoms was either adsorbed, and then washed off in the distilled water wash, or else dissolved intracellular and was lossed when the cells lysed from the osmotic presence of being in distilled water.

Despite any patterns discernible in the uptake of barium by Thalassiosira pseudonana, the level of uptake is too small to be quantitatively important to the cycle of barium in the ocean. That is, if an oceanful of Thalassiosira, or barium concentrators of that order, were sending to the depth all the barium they had managed to accumulate in their lifetimes, without any dissolution in the mixed layer, they still would not produce the surface depletion/bottom enrichment profiles that we see. The proof involves comparing the ratio of particulate barium to carbon production in the chemostat, and the ratio of barium removal from the mixed layer to carbon production in the world ocean. (In this case the chemostat is seen to be not only like the ocean, but like the entire ocean.) It should be stressed that barium removal is ratioed to carbon production not because

Table 12

Particulate Barium Analysis

	138/135	nMBa	nMBa-blank	nM/kg	α
Blank 2	.09624$\pm$1.31%	.139$\pm$2.2%		(.58$\sim$.8%)	
Thalassiosira 12	.12760$\pm$.94%	.216$\pm$1.4%	.077$\pm$.004	.32$\pm$.02	5.8×10^{-5}
13	.09533$\pm$.79%	.137$\pm$1.3%	-.002$\pm$.004	-.008$\pm$.015	
Cocinodiscus 2	.2306$\pm$.24%	.472$\pm$.3%	.333$\pm$.003	1.39$\pm$.01	7.3×10^{-4}
3	.06389$\pm$1.01%	.061$\pm$2.6%	-.078$\pm$.003	-.33$\pm$.01	

Using T12 .15 nM Ba/mgC insufficient by a factor of 50

for the "worst case" model

2×10^{-5} g Ba/g"ash", which is identical to

Guillard's data

of any supposed intrinsic relationship between the uptake
of barium and the biochemistry of carbon fixation. Rather
it is because this model deals with a worst case. If
barium is removed from the mixed layer by primary pro-
ducers, then what is needed is a parameter that measures
their productivity.

The average barium uptake including those experiments
for which ambient barium was nearly four times average
surface seawater values, is 2.7 n M/kg or .64 n M Ba. The
average yield of cell material is 2.8 mg, which using .3 g
C/g dry weight [47] expresses flux from the mixed layer
by particles as the difference between input from rivers
and upwelling deep water and removal by downwelling water,
or

$$P_{Ba} = k_{DM} \, [Ba]_D + I_R \, [Ba]_R - k_{MD} \, [Ba]_M$$

where P_{Ba} is the flux of particulate barium from the mixed
layer, k_{DM} and k_{MD} are the exchange coefficients between
the mixed layer and the deep ocean, I_R is the flux of con-
tinental waters, and $[Ba]_D$, $[Ba]_R$, and $[Ba]_M$ are the barium
concentrations in the deep ocean, the rivers, and the mixed
layer respectively. Rearranging,

$$P_{Ba} = k_{DM} \, [\frac{I_R}{k_{DM}} \, [Ba]_R + [Ba]_D (1 - \frac{[Ba]_M}{[Ba]_D})]$$

Values of $k_{DM} = k_{MD} = 7.2 \times 10^{17}$ l yr^{-1}, $I_R/k_{DM} = .05$,

$[Ba]_M/[BA]_D = .3$ [48], $[Ba]_R = 450$ n M kg^{-1}, $[Ba]_D = 100$ n M kg^{-1} [2] have been assigned, leading to

$$P_{Ba} = 7.2 \times 10^{19} \text{ n M yr}^{-1}$$

Oceanic carbon production has been estimated at 40 billion tons CO_2 per year [49] or 9.9×10^{18} mg C yr^{-1}. The ratio n M Ba/mg C for the world ocean is then 7.3 or nearly a factor of ten greater than that for the chemostat work.

Since this is a "less than" argument, assumptions that enter into it are of two types: one which, if it does not hold, only makes the inequality stand out in greater relief, and the other, which can critically affect the inequality. Assumptions of the latter type are that the environment seen by each individual phytoplankter, tumbling within the chemostat, is sufficiently similar to that encountered in the open ocean (as regards, for instance, nutrient concentrations and availability, light, temperature, etc.) that the mode and level of barium uptake is substantially similar. Further, the phytoplankton themselves are assumed to be in a similar physiological state. These may not hold, but the only alternative is for laboratory behavior to be sufficiently well described that extrapolation to field conditions is unambiguous. All the assumptions of Broeker's box model apply here as well.

The more "robust" assumptions are that all marine photosynthesis is carried out by barium concentrators like Thal-

lasiosira, and that once emplaced, the barium is not re-
dissolved until the alga falls below the mixed layer.
Either of these assumptions being violated, the argument
is made only more forceful. If there were barium non-
concentrators photosynthesizing in the ocean, the barium
uptake of the concentrators should be higher than the oceanic
average. If some barium redissolved in the mixed layer,
then the measured uptake would be greater than that esti-
mated from oceanographic considerations.

It turns out that this model agrees well with the α
model of barium removal. (Note that this is only an inter-
comparison of models. No new information about nature
arises from their combination.) In the α model barium is
seen to follow silica about the ocean. Much as in the
"worst case", with barium concentrators performing all
marine photosynthesis, silica concentrators, diatoms, do
account for the bulk of marine photosynthesis. Also in
the "worst case" barium is not recycled in the mixed layer
and in fact silica is not recycled much in the mixed layer.
According to Broeker's model, the number of times an
element is reused before sinking below the thermocline is
estimated as the ratio of its uptake rate to its removal
rate. Using oceanic productivity measurements, and an
Si/C ratio by mole fraction of .2 [50], it is estimated
that silica is cycled through the mixed layer 1.7 times;
it is permitted little more than one pass. (By much
the same model, phosphate makes nearly ten passes before

disappearing from the surface waters.) This also indicates that any model of incongruent dissolution, in which barium plummets while most of the silica is regenerated will not help the story much (except perhaps to explain why barium flux to the sediment is proportionally greater than that of silica.) The similarity in results between the "worst case" and α models is further reflected in the two orders of magnitude difference between the laboratory estimate of α, .0003 and that from field data, .03. One order of magnitude comes from the factor of ten difference between Ba/C in the lab and the field. The other order of magnitude comes from ambient silicate's being nearly an order of magnitude lower in the chemostat than in most surface sea water. That is, if the silicate concentration in culture had been equal to that in nature, and had the same barium removal been measured, my estimate of α would have been .003 instead of .0003. Conversely, if the α model is a real phenomenon, then the factor of ten higher ambient silica would have been reflected in a factor of ten lower barium removal and hence a lower Ba/C by the same factor of ten.

The barium uptake of Thalassiosira may be physiologically interesting, and conceivably its mode of uptake could be similar to that of greater concentrators, but in terms of the oceanic cycle of barium it is not significant.

Comparisons with the data of other workers are difficult to make directly, for one because the conditions of growth employed and encountered by one group are not stated

or measured with the intent of fitting into the models formulated by another group. Someone using my data, for instance would be frustrated attempting to make use of the parameter of barium per cell. Cell counts were not considered of primary importance and reflect their priority in their reliability. In the same way, other laboratories do not have the facilities to measure dissolved barium accurately, and silica values are seen as irrelevant. But even in something so basic as stating the amount of particulate barium, one can find it ratioed to cell number, to dry weight, to wet weight, to ash weight, to carbon, etc. frequently without inclusion of the species-dependent conversion factors. Still, by estimating some of these factors, comparisons can be made.

Using a conversion factor of 0.34 ($\pm$ 0.13) for ash weight to dry weight for Thalassiosira pseudonana [51] the average barium removed in my experiments was 93 ppm in the ash. This is compared to 27 ppm for Thalassiosira pseudonana for Guillard [51] and up to 500 ppm in the ash for some dinoflagellates and cryptomonads in culture. Black Sea diatoms have ascribed values from 20 ppm to 3×10^4 ppm [18]. Riley and Roth [17] have measured up to 1700 ppm in a cryptomonad cultured in synthetic medium and 540 ppm in the ash of Irish Sea plankton. Martin and Knauer [16] report about 100 ppm in the ash of "microplankton" collected at 150°W, 26°N near Hawaii. My estimate

of α for Thalassiosira is .0003. Assuming standard F/2

silica concentrations of 103μ M Si/l and harvesting at 10

μ M Si/l and Woods Hole surface seawater with 50 n M Ba/l,

the estimate of α from Guillard's data for Thalassiosira

pseudonana is .012. The molar ratio of barium to silica

is 1.8 x 10^{-5}. Based on barium and silicate measurements

during different seasons, (Edmond unpublished) Black Sea

diatoms show an α of from .0001 to .11. The barium to

silica molar ratio ranges from 1.3 x 10^{-5} to 1.3 x 10^{-2}.

The Martin and Knaur data on "microplankton" when related

to nearby GEOSECS Station 204 yield an α of .08 with a

barium to silica molar ratio of 5.8 x 10^{-4}. With respect

to the "worst case" model, all of Guillard's phytoplankton

are insufficient, with the nearest contender being Platymonas,

at 2.5 n M Ba/mg C (7.3 being considered sufficient). Riley

and Roth's Irish Sea plankton and cryptomonaid are nearly

sufficient. The Black Sea diatoms and Martin and Knaur's

"microplankton" are more than sufficient. (It should be

noted that given the α model of uptake, species that seem

insufficient in batch culture could prove sufficient in

chemostat.)

If a phytoplankter that does concentrate oceanically

significant amounts of barium could be found and cultured,

then several more elaborate experiments suggest themselves.

One of the questions to unravel concerning barium uptake

by the phytoplankton is whether the process is a physical

one or whether it is under biochemical control. For

instance, it has been shown that surface adsorption and enzymatic uptake can exhibit similar stoichiometry. The features that distinguish biochemical control are that the rates versus, say, adsorption are slower, the reactants are enzymatically bound, the variation with temperature is different [19] and the reaction is thermodynamically irreversible within an individuals' lifetime. Therefore if the barium isotopic composition of the medium were to change suddenly, adsorption/ion exchange would reflect the medium's isotopic composition well within seconds, (isotopic fractionation for the pair ^{138}Ba-^{135}Ba should be miniscule) whereas biochemical uptake and emplacement would, in the case of an unsynchronized culture, approach equilibrium as a decaying exponential, with a time constant of the order of the cell specific growth rate. The case for a synchronized culture should be even more favorable. It is well established [20] that silica uptake and frustule formation occur at specific points within the cell cycle, and that this cell cycle will, for most diatom species, synchronize itself with the light/dark regime (although the preferred "time of day" for these events is not the same from species to species). If barium uptake were correlated to silica uptake, then performing the experiment at a time of minimum silica uptake should produce no equilibrium in the biological case and near-complete equilibration in the physical case. In the extremely contrary case of just happening to hit the interval of Ba uptake, then the degree

of equilibration would be less than or equal to one half

in the nature of biological semiconservative reproduction.

That is

$$.5 > \xi = \frac{V_{ch}}{V_s} \frac{x_{135n}(\frac{^{138}Ba}{^{135}Ba})_{d} - x_{135n}}{x_{138s} - x_{135s}(\frac{^{138}Ba}{^{135}Ba})_{d}}$$

Where the subscripts Ch, s, n and d refer to chemostat,

spike, natural, and diatom respectively; V stands for

volume, x for mole fraction of total barium. The para-

meter measured is the isotopic composition of the diatom

$(\frac{^{138}Ba}{^{135}Ba})_{d}$, Further, if it were indicated by other lines of

evidence that incorporation into the silica frustule was

not the case, then it might be possible, using 30 ml sam-

ples (flow rate = 240 ml/day, a sample every three hours)

to monitor ambient barium levels and determine during which

phase of the cell cycle barium uptake is greatest. In

greater detail, the experiment would proceed as follows:

The chemostat would be assembled and innoculated in the

normal way, but under 14 hours light 10 hours dark (insul-

ation from ambient room light would have to be stricter).

After at least four generations [52], reactive silicate

concentration would be measured on each 10 ml (= one hour)

of chemostat overflow for at least 24 hours. The level of

silicate uptake during any interval (in μM [Si] $l^{-1}hr^{-1}$,

or if more useful, further normalized to cell number) can

be approximated by the expression:

$$Si_{uptake} = (\frac{23}{24} [Si]_{k-1} + \frac{1}{24}[Si]_i - [Si]_k)Q/V$$

where $[Si]_k$ refers to the overflow silicate concentration at the kth hour (or interval), $[Si]_i$ is the inflow silicate concentration. The period of lowest uptake would be chosen for spiking and then sacrifice within minutes thereafter. The spike would consist of the standard ^{135}Ba-enriched $Ba(NO_3)_2$ at a concentration of 12 n M ^{135}Ba/ml except made up in distilled water rather than 6 N HCl as is usual. The filtered diatoms would be prepared and analyzed in the normal way save for the spike, as the critical information is the $\frac{^{138}Ba}{^{135}Ba}$ ratio in the diatoms at the time of sacrifice. If the $\frac{^{138}Ba}{^{135}Ba}$ ratio is the same as the natural ratio, this implies thermodynamically irreversable, biochemical uptake. Conversely, a $\frac{^{138}Ba}{^{135}Ba}$ ratio reflecting that of the ambient water after the spike would imply surface adsorption or ion exchange.

In order to determine if barium is sequestered in "soft parts" or "hard parts" a method could be divised to separate the two components cleanlv without contamination and without effecting transfer of barium between them. (Acid cleaning, the standard method for preparing diatom frustules [32] would leach metals from the frustules into the bulk phase. Disruption by glass beads has been used to prepare chemically unaltered frustules and cell walls, [22] but the suspended glass produced

would certainly be contaminating for barium. A likely
candidate for this separation is sonication in ultra-
pure H_2O_2.

In detail, the diatoms are harvested by centrifu-
gation in four 50 ml polypropylene centrifuge tubes and
resuspended in ultrapure NH_4Cl, 27.4 g/l (isoosmotic to
32.0°/$_{oo}$ sea water). The suspensions from the four tubes
are mixed together and then distributed equally between
two tubes. The diatoms in each tube are spun down and
washed with NH_4Cl again. After one more centrifugation the
pellets are poured and the tubes rinsed with vycor-distilled
water into two 5 ml vycor beakers. One beaker is run for
particulate barium in the normal way, entering the flow chart
at the combustion step. The other beaker is subjected to
the separation procedure. Most of the liquid is dried away
at room temperature. Then 3 ml ultrapure hydrogen peroxide
are added and the beaker is placed in a sonicator bath for
a sufficiently long time such that more time would not effect
further oxidation of organic carbon (to be determined). Care
is taken that the water temperature in the sonicator bath
does not climb too high, as silica solubility increases
rapidly with temperature. After the oxidation sonication,
frustules are diluted to 50 ml with vycor-distilled water
and separated from the bulk solution by centrifugation
(1600 g's depending on the species). The supernatent is
analyzed for silicate so that the amount of barium assigned
to "hard parts" can be increased in proportion to the

silica lost (assuming congruent dissolution). The pellet
is run for particulate barium, again entering the standard
procedure at the combustion step. Analysis of the frustule
for residual organic carbon is incompatible with barium
analysis and so would be done in an earlier dry run of the
experiment (where all plasticware is replaced by combusted
glass).

Another possibility for "hard parts"/"soft parts"
separation is bacterial digestion, which would simply in-
volve storing a container in the dark, covered but not sealed,
and perhaps slightly warm, for several weeks. The diatoms
would probably carry a sufficient innoculum of the correct
type of bacteria with them. The centrifugation would pro-
bably proceed less clearly and so this method is a latter
resort.

We now come to the problem of modelling natural phe-
nomena. Levins, in his 1966 paper [53] discusses three
characteristics of models: reality, generality, and pre-
cision. The hypothesis that arises from field evidence has
generality and precision. For the greater part of the world
ocean, knowing the silica concentration will immediately
tell the barium concentration. The evidence from the lab-
oratory forms a model of great precision and reality
(to the molecular level for those of prodigious energy).
The laboratory model has the quality of reality because a
hypothesis can be cleanly rejected. But it has no general-
ity. It is hoped that the summation of the two would endow

the model with reality, precision and generality. Rigorously,
this can not be so. In practice it is the only way to pro-
ceed.

If the laboratory experiments corroborate the hypo-
thsis born of the field, then that hypothesis becomes stron-
ger (and perhaps more adaptable to varying field conditions,
if the mechanistic insight to be gained in the lab can be
used for extrapolation) and yet not proved. If, on the
other hand, evidence from the laboratory does not corrob-
orate the field's hypothesis, little is gained. The hypo-
thesis is shown to have less than perfect generality, but
then such was never expected. To actually disprove the
hypothesis, either an alternative hypothesis would have
to eclipse it, or the detail of nature would have to be
reproduced in the laboratory. In this case, that means
culturing each constituent of the biological community in
conditions closely approximating those of the open ocean
where the phenomenon is observed. Not only is this phys-
ically impossible, but in doing so the reductionist, con-
trolled character of laboratory experiment would be lost.
And with it the quality of reality.

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