SI UPTAKE BY THE CENTRIC DIATOM, CHAETOCEROS GALVESTONENSIS

by

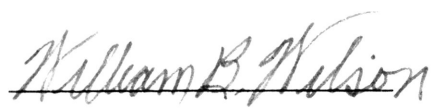
THOMAS RALPH TURNER, JR.

Marine Biology

Submitted in Partial Fulfillment of the Requirements of the
University Undergraduate Fellows Program

1977-1978

Approved by:



William B. Wilson

April 1978

ABSTRACT

Si Uptake by the Centric Diatom, Chaetoceros galvestonensis

(April 1978)

Thomas Ralph Turner, Jr.

Moody College

Faculty Advisor: Dr. W. B. Wilson

Chaetoceros galvestonensis was first classified in 1962 by Collier and Murphy at the A&M Marine Lab in Galveston. It, like other planktonic, centric diatoms, has extensions or setae projecting from the silicified cell wall. The four setae of C. galvestonensis were described by Collier and Murphy as being 2 μ in length. However, the length of the setae were seen to vary between isolates of organisms believed to be C. galvestonensis. These isolates were from Galveston and Alligator Harbor, Florida, and the reason for the variation in setae length was unknown, but were believed to be caused by differences in Si concentration in the water of the two locations. This study found that cells cultured in Si concentrations of 0.067, 0.120, 0.350, 0.558 and 0.866 mg Si/l showed no distinct variations in setae length. On the other hand, the initial concentration of Si in the media did have an effect on the final cell number and the amount of Si per cell of organisms cultured at that concentration. As the initial amount of Si in the media increased, the final cell number, as well as the amount of Si/cell, also increased.

ACKNOWLEDGEMENTS

The author is very greatful to Dr. William B. Wilson for his help and guidance throughout this study and for his helpful critical review of this paper. He also wishes to thank Ms. Linda Medlin for her advice assistance in the EM work and Ms. Anita Aldrich for her assistance in the preparation of the culture media and analysis reagents. He is also grateful to Mr. Osmund Brown and NOAA for their allowing him access to their transmission electron microscope. Finally, he wishes to thank the University Undergraduate Fellowship Program for furnishing him the opportunity to conduct this research and providing him with the funds to do so.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Diatoms are unicellular, golden-brown algae belonging to the Class Bacillariophyceae. Their cell wall is composed of organic material impregnated with silicious deposits. The amount of silica in the walls varies among the diatom genera. In the larger, more silicified cells, 50% of the dry weight is silica (Vinogradov 1953), while the content in other cells, such as Navicula pelliculosa, may be as low as 4% of the dry weight (Lewin 1957). Due to this silicification of the cell wall, diatoms have been referred to as living in "glass houses". These "houses" consist of two valves, the epi- and hypotheca, held together by silicious bands, the girdle. This girdle can be changed in shape to contract or extend the valves.

Many planktonic, centric diatoms have processess that project from the valves. These processess or setae are extensions of the cell wall and, like the wall, they have many minute pores which provide contact between the external environment and the cell. The exact function of these setae is unknown, but the most widely accepted theory is that they help to keep the cell suspended in the water column by increasing its surface area. The cell protoplasm can be extended into the hollow setae and by extension and withdrawal, it may enable the diatom to maintain its orientation in the water column (Wilson personal communication).

The style and format followed in this paper are those of the Journal of Phycology.

Chaetoceros galvestonensis was first classified by Collier and Murphy (1962) at the Texas A&M Marine Lab in Galveston. This small, centric diatom was isolated from the surface waters of the Gulf of Mexico near Galveston. The species has four setae, two on each valve, that are about 2μ in length (Fig. 1). The length of the cell is 3μ while the width is 1.5μ. The Collier and Murphy study indicated the species had a rapid reproduction rate, even under conditions of minimal nutrient supply, and could quickly deplete an adequate nutrient source.

Wilson (personal communication) noted that, in isolates of what was believed to be C. galvestonensis from locations near Galveston and Alligator Harbor, Florida, there was a variation in seatae length.

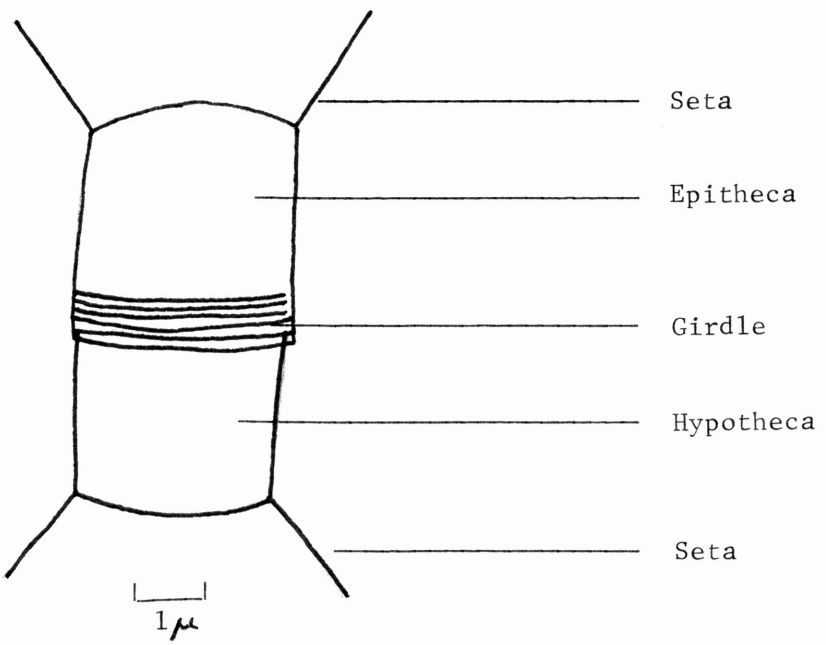


Fig. 1. Schematic diagram of the diatom, Chaetoceros galvestonensis.

Setae length varied from about 2 μ to about 40 μ. The factor(s) causing these differences is unknown, but it was considered that setae length may be related to the amount of silica in the water.

Werner (1977) found that the amount of silica in diatom cells can vary because of: 1) limitations of silica in the medium; 2) variation of other culture conditions (light, temperature, etc.); 3) variations in cell diameter and surface area; 4) the stage of the mitotic life cycle; and/or 5) variations in the size of special silica structures, such as setae. Under reduced Si conditions, the amount of Si per cell is reduced in some centric diatom species such as Skeletonema costatum (Harrison 1974) and Thalassiosira pseudonana (Paasche 1973). These species, like C. galvestonensis, are planktonic and continue to divide even after the Si supply is depleted by forming thinner valves. As the division rate slows within a culture due to nutrient depletion, the amount of Si per cell in the newly formed organisms also decreases (Lewin 1957).

The initial amount of Si in the media also effects the number of cells in that culture (Jørgensen 1955; Lewin 1955). The final number of cells in a given medium is proportional to the initial amount of Si in that medium.

The purpose of this study was to determine the effect of the Si concentration in the medium on the length of the setae of C. galvestonensis. Studies were also conducted on the effects of Si concentration on the amount of Si per cell and the growth of C. galvestonensis.

MATERIALS AND METHODS

Culture Methods

In order to eliminate the possibility of Si contamination, plastic (polycarbonate) culture vessels were used. Each had a volume of 75 mls. Boro-silicate glass tubes were not used because they may be a possible source of Si for diatoms either by extraction of Si from the glass by the cells or the leaching of Si from the tubes into the culture medium (Wilson personal communication). Therefore, if glass tubes were used, the diatoms would not be totally dependent on the medium alone for their Si supply.

The base medium used was a standard artificial seawater medium - NH15 (Gates and Wilson 1960) - prepared without the silicate addition. Sodium silicate, in the form of $Na_2SiO_3 \cdot 9H_2O$, was added to portions of this medium in amounts that resulted in media that contained 0.0625, 0.125, 0.25, 0.375 and 0.50 mg Si/l.[1] Four, 50 ml replicate portions with each concentration were placed in the culture vessels and autoclaved. The use of glassware to prepare the media was kept at a minimum to reduce the chance of Si contamination.

Three of the four replicates were inoculated with 0.1 ml of a 1 in 100 dilution of a stock culture which contained 4.63×10^6 cells/ml. This week-old culture was incubated in NH15 medium (salinity 28ppt) at 24C and in a light intensity of 1000 fc. The inoculum for this culture was from a bacteria-free clone, thereby reducing the chance

---

[1] The Si concentrations, as determined by analysis were 0.067, 0.120, 0.350, 0.558 and 0.866 mg Si/1, respectively.

of genetic recombination and mutation. The fourth replicate of each concentration was not inoculated and served as a control for the Si analyses to insure that other factors were not altering the Si concentration.

Test portions were incubated for 8 days at 24C and 1000fc. Hence, the only known difference between the stock and test cultures was the Si concentration. The length of the incubation period was based on previous observations. Growth of the cultures was not measured, but the cultures were examined periodically during the incubation period to verify that they were developing.

Cell Counts

All cell counts were made using the 0.1mm hemocytometer. Guillard (1973) considers this method to be the most accurate for counting cells the size of C. galvestonensis. Live cell counts of the inoculum were made twice to insure accuracy. Cells from the test portions were fixed with iodine and counts were made at a later date. Two counts were made of each inoculated test portion, for a total of 6 counts at each concentration. The mean number of cells in medium with each Si concentration was calculated.

Silica Analysis

Twenty-five mls of culture was removed from each test portion at the end of the 8 day incubation period and filtered through Type HA Millipore filter paper (pore size 0.45μ) to remove the cells. The filtrate from each portion was stored in a polycarbonate tube in the dark until the Si analyses were performed. The amount of Si in each of the four replicates of each concentration was determined by the

method outlined by Strickland and Parsons (1968). The difference of the mean final Si concentration of the 3 inoculated tubes and that of the uninoculated portion with the corresponding initial Si concentration was considered to be the amount of Si taken-up by the cultures. In other words, it was assumed that this decrease in the Si concentration represented the amount used by the diatoms for thecal silicification.

Setae Length

The iodine-fixed cells were rinsed 4 times with distilled water to remove the dissolved salts. A drop from each test portion was pipetted onto seperate grids, coated with an 0.3% Formvar solution, used in electron microscopy. The grids were air-dried for one day and another drop pipetted onto them to insure a high concentration of cells on each grid.

The grids were examined on the Hitachi transmission electron microscope at a magnification of 2100. Photomicrographs were made of 3 sections of each grid (Figs. 3a,b). Setae of the organisms were measured (in cms) by examination of the negatives on a light table and the use of a standard centimeter ruler. The lengths were converted to microns using a conversion factor as follows: for every 10,000 magnification, 1 cm = 1 μ. If the setae were displaced or distorted, the image was projected on a screen to increase magnification so that it could be determined whether the setae were complete or not.

The means and standard deviations of the setae lengths were calculated for organisms that grew in each Si concentration.

Silica Per Cell

To determine the amount of Si in each organism, the decrease in the amount of Si in the medium for each concentration used was divided by the increase in the number of cells in the portions with that concentration during the incubation period.

Statistical Analysis

Means and standard deviations were calculated for the number of cells, the decrease in the amount of Si and the length of the setae at each test concentration. Linear regression was used, in conjunction with the correlation coefficient (R), to determine the relationship between Si concentration and the number of cells in culture, setae length and the amount of Si per cell.

RESULTS

Cell Counts

The final number of cells in the test portions depended on the initial Si concentration of the medium (Table 1). The correlation between the amount of silica initially in the medium and the number of cells in that solution is good (R = 0.9798), as evidenced by the regression line's fit to the data points (Fig. 2). The standard deviations of the cell numbers are not large if the total number of cells per unit volume of medium are considered.

Conc. (mg Si/l)	Mean Number of Cells ($X10^4$ cells/ml)	Standard Deviation
0.067	98.00	± 11.00
0.120	112.83	± 9.17
0.350	171.33	± 21.18
0.558	242.83	± 8.09
0.866	272.50	± 9.16

Table 1. Number of cells at different concentrations of Si.

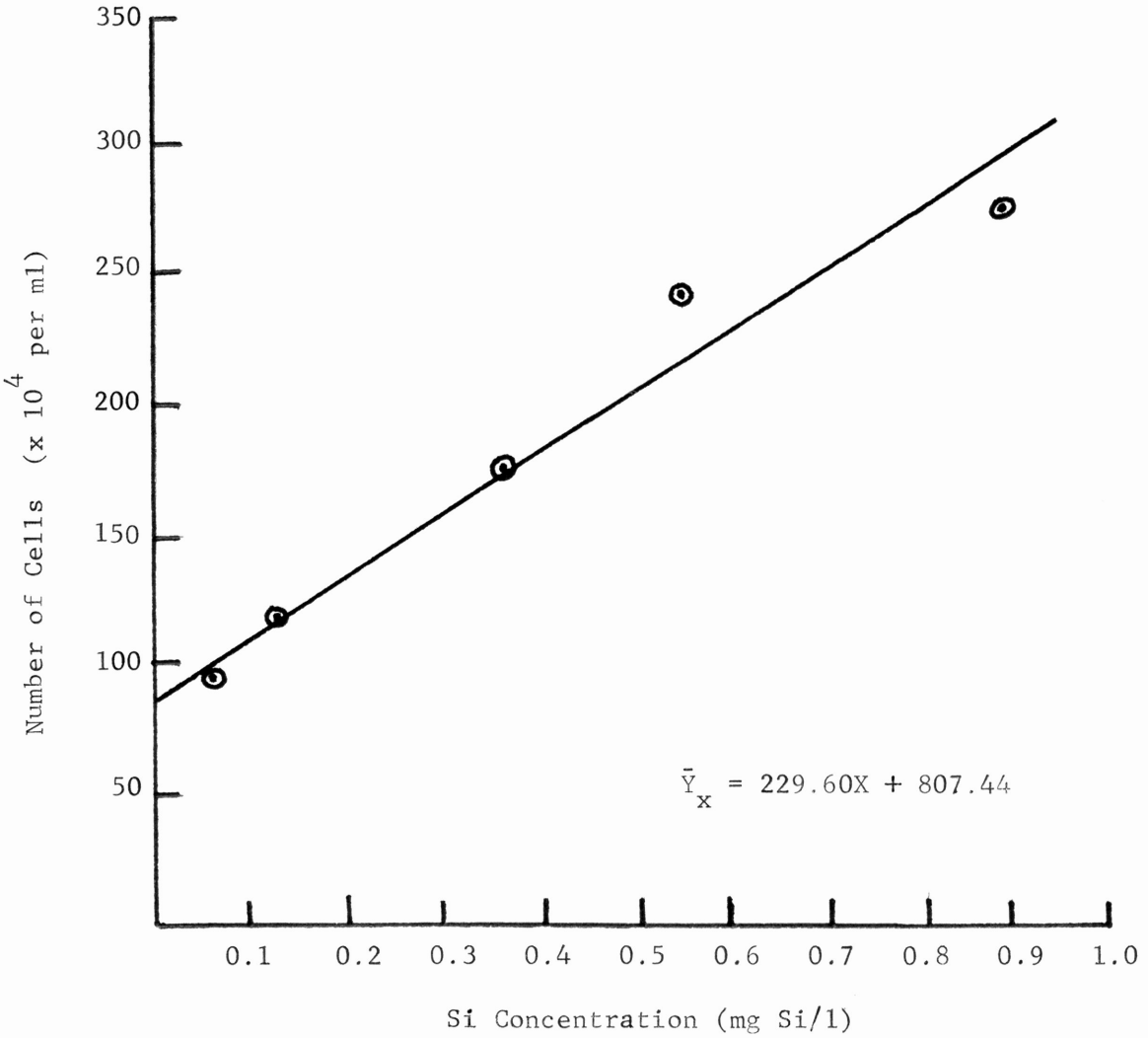


Fig. 2. The effect of Si concentration on the number of cells.

Silica Analysis

The Si analysis revealed that no Si remained in solution at concentrations of 0.35, 0.558 and 0.866 mg Si/1 (Table 2). Some Si may have remained in solution but, due to the lower limitation of the analysis method (0.1 μg-at Si/1), it was not detectable. However, Si uptake by the diatoms at the 2 lower concentrations was not complete, i.e. small amounts of Si were detected, by analysis, in the

Initial Conc. (mg Si/1)	Final Conc. (mg Si/1)	Standard Deviation
0.067	0.017	± 0.007
0.120	0.013	± 0.000
0.350	0.000	± 0.000
0.558	0.000	± 0.000
0.866	0.000	± 0.000

Table 2. The initial and final concentrations of Si in the five sets of replicates.

media.

Setae Length

There was no relationship between the amount of Si in the media initially and setae length (Fig. 4). The R value of 0.0361 indicates the poor correlation. No cells or cell fragments were observed on the transmission EM on the grids prepared with cultures that grew in media with 0.067 and 0.120 mg Si/1 (Table 3).

Silica Per Cell

The amount of Si per cell was found to increase as the initial

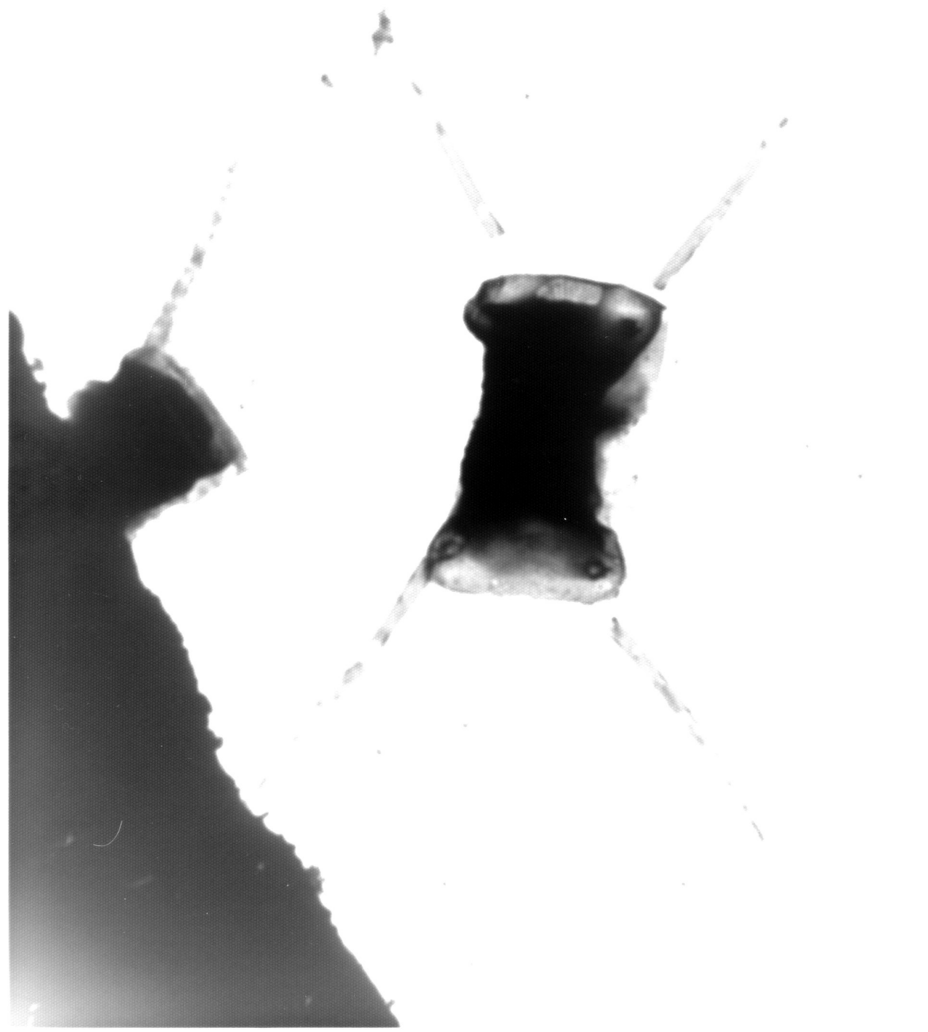


Fig. 3a. Photomicrograph of C. galvestonensis cultured in Si concentration of 0.558 mg Si/ 1.

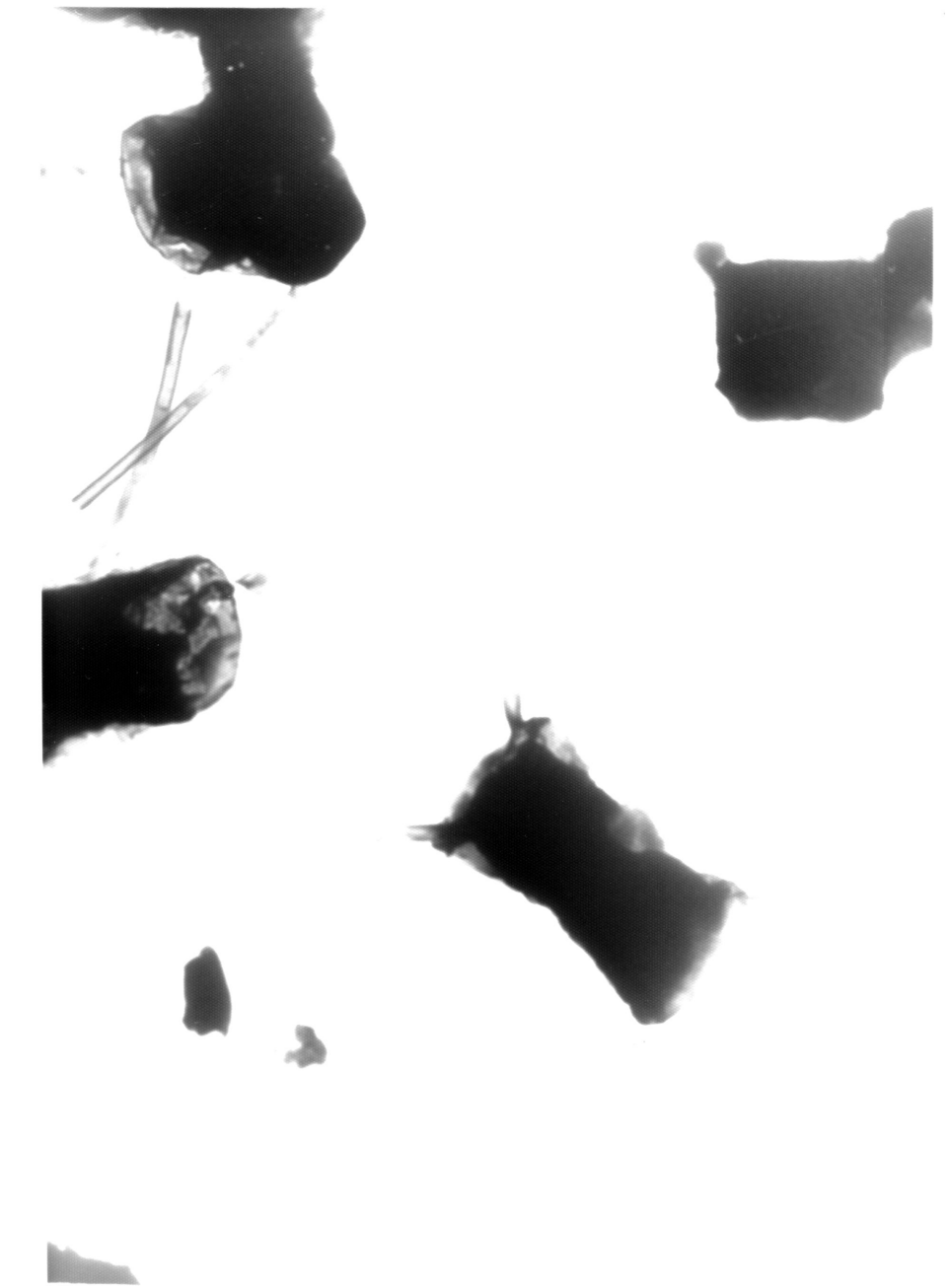


Fig. 3b. Photomicrograph of C. galvestonensis cultured in Si concentration of 0.866 mg Si/1.

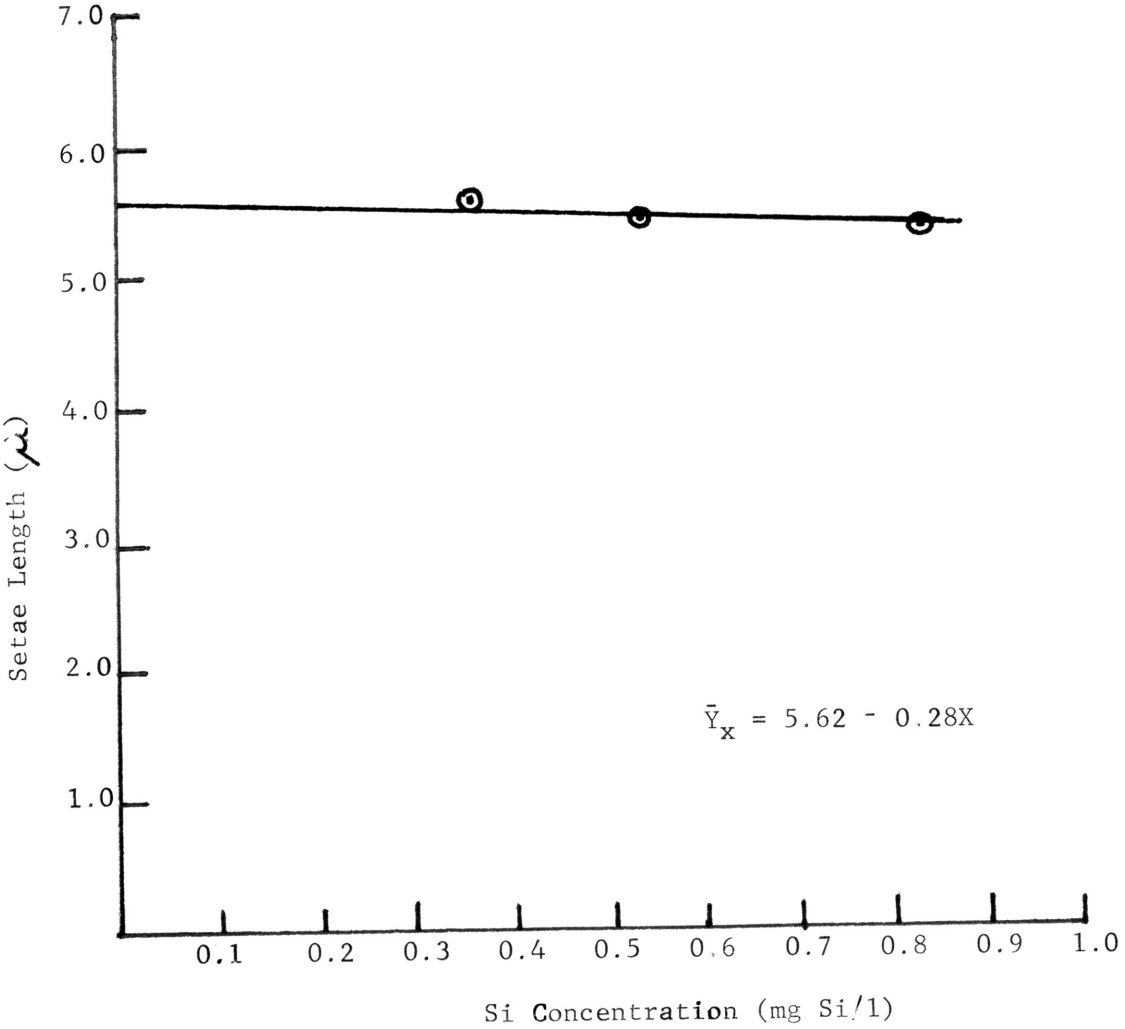


Fig. 4. The effect of Si concentration on setae length.

Conc. (mg Si/l)	Setae Length (μ)	Standard Deviation
0.067	------	------
0.120	------	------
0.350	5.52	$\pm$ 0.46
0.558	5.48	$\pm$ 0.48
0.866	5.38	$\pm$ 0.69

Table 3. Setae length at different Si concentrations.

concentration of Si in the media increased (Table 4). The correlation between Si concentration and Si per cell is good (Fig. 5), with an R value of 0.9761. The Si per cell values obtained in this study for C. galvestonensis are of the same magnitude (10^{-8} mg Si/cell) as those found for similar-sized diatoms (Lewin 1957; Paasche 1973).

Si Conc. (mg Si/l)	Si per Cell (10^{-8} mg Si/cell)
0.067	0.513
0.120	0.943
0.350	2.037
0.558	2.302
0.866	3.183

Table 4. Si per cell at different Si concentrations.

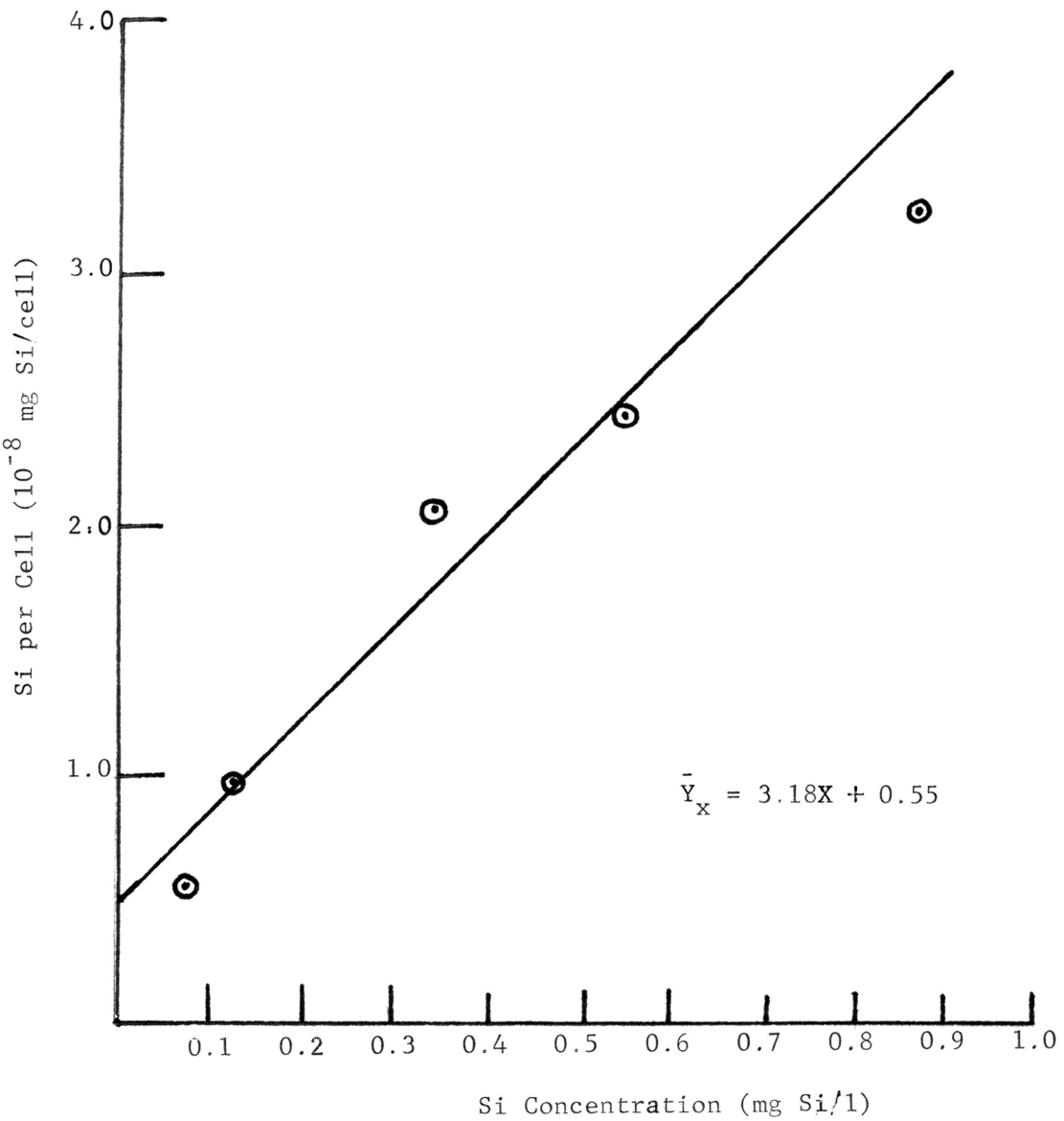


Fig. 5. The effect of Si concentration on Si per cell.

DISCUSSION

If adequate amounts of other nutrients are present, the final number of cells in a culture is proportional to the initial Si concentration of the medium (Lewin 1957). C. galvestonensis cultures in this study grew according to this relationship, although the final cell numbers were not directly proportional to the differences in initial Si concentration. The medium with the lowest Si concentration used (0.067 mg Si/l) contained 98.00×10^4 cells/ml at the end of 8 days of growth, whereas the medium with a Si concentration of 0.866 mg Si/l contained 272.50×10^4 cells/ml at this time. This indicates a slower growth rate at the lower Si concentration in C. galvestonensis. Similar results were reported by Lewin (1957) in N. pelliculosa.

If nutrients other than Si become limiting, the cells may cease to divide but the uptake of Si may continue (Lewin 1957). This Si is deposited in the existing valves and, thus, the amount of Si per cell increases. With such conditions, the total amount of Si in the medium may be depleted. This may have been the case at the 3 higher test Si concentrations where, at the end of the 8 day incubation period, no Si was detected in the media by analysis.

The addition of Si even after division has stopped may have occurred at the 0.350, 0.558 and 0.866 mg Si/l concentrations. The amount of Si per cell at these concentrations was larger than the 2 lower concentrations (2.037, 2.302 and 3.183×10^{-8} mg Si/cell, respectively, compared to 0.513 and 0.943×10^{-8} mg Si/cell), even though more cells were present at the higher concentrations.

Earlier studies have shown that more rapidly dividing cells deposit thinner valves than the slow dividing ones (Lund 1950; Jørgensen 1955; Lewin 1957). However, if this were the case in this study with C. galvestonensis, the higher concentrations, which should have been dividing faster than those at the lower concentrations since the net gain of cells over the same period of time was larger at the higher Si concentrations, would produce cells with a smaller amount of Si per cell than the lower concentrations. Since this condition was not found, it may be assumed that, at the higher Si concentrations (0.350, 0.558 and 0.866 mg Si/1), division had stopped before the end of the 8 day incubation period. However, the cells in these concentrations continued to take up Si, resulting in more silicification of the valves (more Si per cell).

In media with low Si concentrations (0.067 and 0.120 mg Si/1), the growth rate was slower and cell division continued through the 8 day incubation period. Thus, instead of more silicification of the existing valves, the cells were using the Si remaining in the medium to form new valves (through division). To verify this, portions of each culture would need to be analyzed daily during the incubation period for cell counts and Si amount.

If the cells at the higher concentrations were depositing Si in existing valves, there was no evidence found in this study to indicate that they were using this Si to increase the length of their setae. In fact, as the initial concentration of Si increased from 0.350 to 0.866 mg Si/1, there was a slight decrease, from 5.52 to 5.38 μ, in setae length. Since no cells were observed in the EM preparations and

no setae were measured of cultures grown in media with Si concentrations of 0.067 and 0.120 mg Si/1, the premise of Si concentration not having a definite effect on setae length cannot be verified. There might have been a lengthening of the setae from 0.120 to 0.350 mg Si/1, but without data from the concentrations below 0.120 mg Si/1, this cannot be concluded.

Due to the small amount of Si per cell at 0.067 and 0.120 mg Si/1, the addition of iodine to the cultures before the EM preparations were made may have caused the weakly silicified, thin valves of the cells at these concentrations to lyse. This lysing provides an explaination for the abscence of cells at these concentrations on the electron microscopy grids.

The variation of setae length of the different isolates observed by Wilson may have been caused by genetic differences, isolation of different species or physiological strains, or a variation of environmental factors such as light, temperature and Si concentration. The possibility of variation due to genetic recombination is slight since the amount of asexual reproduction greatly exceeds the amount of sexual reproduction in diatoms. The isolation of different strains is a distinct possibility since the isolates came from different geographical areas. More work needs to be done on the effects of the interaction of different environmental factors and Si concentration on setae length. However, this study indicates that Si concentration alone has no effect on the length of the setae in C. galvestonensis.

In conclusion, this study indicates that, with the culture conditions and Si concentrations used, the number of cells in a culture

and the amount of Si in the valves of those cells did depend on the initial Si concentration of the medium. On the other hand, there was no relationship between Si concentration and setae length in the 3 concentrations for which setae were measured.What probably occurred in the study was, after initial inoculation, the new cells formed at each concentration were all similar in the amount of Si they contained. Division stopped in the higher concentrations as other nutrients became the limiting factor due to the rapid growth rate at these concentrations. When this occurred, Si was deposited by the cells in the existing valves, thereby increasing the amount of Si per cell. This process was progressive from highest concentration to lowest so that each concentration was in a different stage of "development" when the experiment was terminated after 8 days. Since the amount of Si available to each cell was greater the higher the initial concentration, the likelyhood that there would be an increase in the amount of Si per cell at the higher concentrations was greater. Lewin (1957) found similar results when working with N. pelliculosa. The content of Si in each cell remained about the same regardless of the initial Si content of the medium as long as exponential growth was occurring. However, after this phase had ceased, the amount of Si per cell varied, depending on the amount of Si initially in the medium. The higher the initial concentration of Si, the more Si per cell was found.

LITERATURE CITED

Collier, A. & A. Murphy. 1962. Very small diatoms: Preliminary notes and description of Chaetoceros galvestonensis. Science. 136:780-82.

Gates, J. A. & W. B. Wilson. 1960. The toxicity of Gonyaulax monilata to Mugil cephalus. Limnol. Oceanogr. 5:171-74.

Guillard, R. R. L. 1973. Division rates. In Stein, J. (ed.) Handbook of Phycological Methods, Cambridge Univ. Press, London, 289-311.

Harrison, P. J. 1974. Continuous culture of the marine diatom Skeletonema costatum (Grev.) Cleve under silicate limitation. Ph.D. dissertation, University of Washington, Seattle. 140 pp.

Jørgensen, E. G. 1955. Variations in the silica content of diatoms. Physiologica Pl. 8:840-45.

Lewin, J. C. 1955. Silicon metabolism in diatoms. III. Sources of silicon for growth of Navicula pelliculosa. Pl. Physiol. 30:129-34.

_____ 1957. Silicon metabolism in diatoms. IV. Growth and frustule formation in Navicula pelliculosa. Can. J. Microbiol. 3:427-33.

Lund, J. W. G. 1950. Studies on Astrionella formosa Hass. III. Nutrient depletion and the spring maximum. J. Ecol. 38:15-35.

Paasche, E. 1973. Silicon and the ecology of marine planktonic diatoms. I. Thalassiosira pseudonana (Cyclotella nana) growth in a chemostat with silicate as limiting nutrient. Mar.Biol.

19:117-26.

Strickland, J. D. H. & T. R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can., Ottowa, 167, 311 pp.

Vinogradov, A. P. 1953. The Elementary Chemical Composition of Marine Organisms. Memoirs II. Yale University Press, New Haven, 325pp.

Werner, D. 1977. Silicate metabolism. In Werner, D. (ed.) The Biology of the Diatoms, University of California Press, Berkeley, 110-149.

APPENDIX -

RAW DATA

APPENDIX

Cell Counts

Conc. (mg Si/l)	Replicate	Count (x 10^4 cells/ml)
0.067	1	104
		89
	2	97
		88
	3	93
		117
0.120	1	117
		113
	2	114
		125
	3	97
		111
0.350	1	209
		150
	2	170
		162
	3	157
		180
0.558	1	251
		247
	2	230
		238
	3	241
		250
0.866	1	276
		280
	2	265
		270
	3	284
		260

Setae Length

Conc. (mg Si/l)	Lengths (cm)
0.350	1.2, 1.2,1.1, 1.1, 1.3, 1.1, 1.1, 1.35, 1.3, 1.3, 1.2, 1.3, 1.25, 1.1, 1.2, 1.1, 1.1, 1.2, 1.1, 1.2, 1.15, 1.2, 1.2, 1.1, 1.2, 1.3, 1.2, 1.1, 1.3, 1.1, 1.2, 1.2, 1.1, 1.1, 1.1, 1.1, 1.1, 1.2, 1.2, 1.3, 1.25, 1.2, 1.0, 1.0, 1.15, 1.15, 1.1, 1.0, 1.1, 1.4, 1.1, 1.2, 1.2, 1.4, 1.15, 1.2, 1.1, 1.3, 1.0, 1.15, 1.0. 1.1, 1.2,

Conc. (mg Si/l)	Lengths (cm)
	1.1, 1.3, 1.1, 1.2, 1.25, 1.3, 1.1, 1.1, 1.2, 1.0, 1.0, 1.1, 1.2, 1.15, 1.1, 1.1, 1.0, 1.05
0.558	1.2, 1.3, 1.3, 1.1, 1.2, 1.2, 1.35, 1.1, 1.05, 1.1, 1.15, 1.25, 1.1, 1.1, 1.1, 1.1, 1.2, 1.1, 1.1, 1.1, 1.3, 1.0, 1.3, 1.2, 1.2, 1.1, 1.2, 1.15, 1.0, 1.1, 1.2, 1.15, 1.2, 1.1, 1.0
0.866	0.9, 0.9, 1.0, 1.1, 1.3, 1.2, 1.2, 1.2, 1.1, 1.3, 1.1, 1.15, 1.2, 1.15, 1.1, 1.0, 1.2, 1.15, 1.0, 1.0, 1.0, 1.1, 1.2, 1.05, 1.15, 1.1, 1.05, 1.25, 1.3, 1.1, 1.25, 1.25, 1.1, 1.0, 1.3, 1.3, 1.2

Silica Analysis

F = 98.9

Blank reading = 0.01

Conc. (mg Si/l)	Replicate	Reading (% absorbance)
0.067	1	0.015
	2	0.021
	3	0.025
	4	0.033
0.120	1	0.015
	2	0.015
	3	0.015
	4	0.053
0.350	1	0.010
	2	0.010
	3	0.010
	4	0.135
0.558	1	0.010
	2	0.010
	3	0.010
	4	0.200
0.866	1	0.010
	2	0.010
	3	0.010
	4	0.300