A Marine Resource Management Internship Report

THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, INC.

AND

POLLUTANTS ACCUMULATION IN CORAL SKELETON

Submitted in Partial Fulfillment
of the Requirements for the
Master of Science Degree in Oceanography
Marine Resource Management Program
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by

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1- INTERNSHIP PRELIMINARIES

During the first semester of 1984, I started looking for a Marine Pollution course to take during the summer of that year. I asked for some information from the Bermuda Biological Station for Research, Inc. (it will be referred to as Biostation or simply BBS) in Bermuda. After a couple of weeks I had a phone call from Dr. Susan Cook, Director of Education at BBS, and I was offered a scholarship to take the class. I accepted it and started getting ready to travel. I read some research papers from the staff at BBS and I became interested in the M.A.P. (Marine and Atmospheric Program) research, program under the direction of Dr. Anthony Knap. After some letters and telephone calls between Drs. Cook and Knap and myself, we arranged for me to stay on after the class, to work as a Research Assistant Volunteer.

I arrived in Bermuda on July 14th, 1984 and left November 20th, 1984. These four months have been my best experience as an oceanographer, working at sea as well as in the laboratories everyday.

2- HISTORY AND GEOGRAPHY OF THE BERMUDA ISLANDS

Centuries ago, the 150 islands that comprise Bermuda were known as the "Isles of Devil". Its infamy was recorded for posterity by no less a writer than William Shakespeare whose

"Tempest" tells of "the still vex't Bermoothes". Today, these very same islands are accorded all the most favourable adjectives and each year are the vacation destination of about 500,000 visitors.

The history of Bermuda began in the 16th century. To Spaniard Juan de Bermudez goes the credit of discovering the islands. He visited there in 1503, but failed to claim them for his country. It was not until 1609 - more than a hundred years later - that British Admiral Sir George Somers' flagship "Sea Venture", on its way to the infant and struggling colony of Virginia with desperately needed supplies, came to grief upon Bermuda's reefs. It was a mishap which led to the British colonization in 1612 of what is known officially today as the "Bermudas" or "Somers Islands". For more than three and three quarter centuries, Bermuda has remained under the flag of Great Britain.

Bermuda rests on a shallow platform rising 4,500m (15,000 feet) from the abyssal depths of the Atlantic. Situated at 32° 20' N and 64° 40' W, it is about 700 miles off the east coast of the United States, almost equidistant from all coastal points between northern Florida and Nova Scotia.

Arranged in the shape of a fish-hook, the islands have a land area of 53.35 square kilometers (20.59 square miles). By comparison, the land area of New York City is 15 times larger. From east to west, Bermuda is about 34.5 km (22 miles) long with a maximum width of almost 3.21 km (2 miles).

The Bermuda platform is volcanic, mid-ocean origin, whereas

the islands themselves consist of consolidated eolian carbonate sand dunes. The surrounding Sargasso Sea provides a protective shield from northern winters: air temperatures range from 13 to 29°C (55-85°F) and water temperatures from 17 to 28°C (62-82° F).

In the absence of a continental shelf, the deep sea begins just off shore: depths increase to 3,600 m (12,000 feet) within 20 miles. A wide spectrum of marine biota is easily accessible, ranging from rocky and sandy shores to mangroves and coral reefs, to the Sargasso Sea itself. The reef areas extend out at varying distances. Because of these characteristics, Bermuda offers unmatched opportunities to gain information in the deep sea on day trips within a short distance, and to study the ecologies close inshore.

3- THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, INC.

3.1- History

The Biostation has an excellent location for basic ocean research as well as for research on modern problems associated with concentrated pollution, in the form of oil and sewage. Established in 1903, through the efforts of scientists from Harvard University, New York University, and the Bermuda Natural History Society, BBS is one of the oldest U.S.--founded marine laboratories. It was incorporated in New York in 1926 as an independent non-profit organization. In 1932, the

Bermuda Government and the Rockefeller Foundation joined forces to provide facilities and a modest endowment, and the Biostation opened at its present location on Ferry Reach.

The Biostation supplements the natural assets of Bermuda with up-to-date research facilities. Since 1969, it has operated year-round with an increasing variety of programms related largely to environmental quality.

Over the years, the Biostation has collaborated closely with major research institutions such as Woods Hole Oceanographic Institution, Lamont-Doherty Geological Observatory and the Smithsonian Institution; with Canada's Natural Research Council and the Bedford Institute of Oceanography; with U.S. government agencies such as the National Science Foundation, National Oceanographic and Atmospheric Administration, Environmental Protection Agency, Department of Energy, National Institutes of Health and Office of Naval research; and with the Bermuda government.

3.2- Facilities

- The Main Building. A former hotel built in 1910, the Main Building fulfills a multitude of functions. It contains laboratory space for both research and teaching, seawater aquaria, a culture room, darkroom, and living quarters for scientists and students.

- The Redfield Building. The E.L. Mark Library, which is aknowledged to be the finest island station library in the world is located on the first floor. Administration offices and a

lecture hall seating 80 are on the second floor.

- Conklin Laboratory. It was built in 1965, and contains 15 laboratories used by visiting scientists and the Biostation staff.

- Dock Facilities. Three vessels are available for work in- and off-shore: workboats of 9.8 m (32 feet) and 12.5 m (41 feet) which accommodate up to 33 people, and a 20 m (65 ft) ship, the R/V Weatherbird, equipped for open ocean research.

- Scott Laboratory. It can accommodate up to 30 students. Beneath it is a "wet" laboratory and lockers having a SCUBA compressor and diving gear.

- Research Services Building. The core of it is woodworking and machine shops to mantain and repair scientific equipment.

3.3-Research and education

In fulfillment of its mission the Biostation serves a scientific staff and visiting scientists from institutions around the world.

What is believed to be the longest running series of deep sea observations in the world has been that of the BBS Hydrostation S at 32° 10' N Latitude and 64° 30' W Longitude, 15 miles southeast of Bermuda. Since 1954 it has been the site of standardized measurements of water temperature, dissolved oxygen and salinity to depths of about 3,220 meters. Hydrostation has been providing oceanographers with a database for their measurement standards.

According to "A Quantum Leap", a brochure published by BBS,

"It may require another three decades before these measurements can be converted into confirmed cycles and patterns. Such must be the patience of the oceanographer. The patience, however, is being severely tested by the world's urgent need for firm data on the increase of carbon dioxide in the atmosphere and its correlation with the temperature of the ocean waters - the "greenhouse effect" . If a carbon dioxide buildup can be confirmed, it will be evident in rising ocean temperature; if, on the other hand, a significant portion of this surplus is being absorbed by the oceans, this too will become apparent. Hydrostation S will provide a database long enough to identify a trend. Should a "greenhouse effect" be developing, Hydrostation S may be the world's early warning outpost."

Because of manmade threats to the atmospheric and oceanic environment, many BBS efforts are directed along those lines. Scientific investigations include:

- Long range transport and fluxes of manmade pollutants, such as acid rain, to the ocean.

- Analysis of oil spills.

- Effects of oil and oil dispersants on reef-building corals.

- Biochemical studies of the accumulation and removal of pollutants in marine organisms.

- Trace metals and trace organics such as pesticides in the open ocean and in marine organisms.

- Development of environmental disaster plans with the Bermuda Government.

- Pharmacological investigations of marine fauna and flora.

- Eye structure and optics of marine animals and the chemistry and physiology of fish retinas.

- Coral reef ecology and fish census.

- Sea level history and volcanic origins of Bermuda.

- Preparation of reference works on Bermuda's natural history and marine life.

- As a sidelight to pollution work the bodies of stranded whales have been analyzed to determine the amount of polyclorinated biphenyls (PCBs) in them.

- In 1980 and 1984 international calibration exercises for measuring selected marine pollutants was held at the BBS (PANCAL).

During June, July and August advanced courses in the marine sciences are held at the Biostation. The faculty is drawn from American and European universities. The courses are:

- Tropical marine invertebrates.

- Tropical marine ecology.

- Analysis of marine pollution.

- Oil spill contingency planning.

These courses are aimed at advanced undergraduate, graduate, and post-graduate students.

4- MY WORK AS A VOLUNTEER

In order to pay my room at the Main Building and three meals

a day I had to work for different people at the Biostation as a volunteer. I will briefly describe five of the principal jobs that I was involved with.

4.1- The "Aguila Azteca" case

In early september 1984, the oil tanker "Aguila Azteca" from Manzanillo, Mexico, ran aground on coral reefs ten miles from the Biostation. The tanks were full with approximately 200,000 barrels of Bunker-C. The Marine Police, the Department of Agriculture and Fisheries, the U.S. Navy Station, and the BBS were alerted of the possibility of a major oil spill.

My job was to borrow from Esso Co. a pump and showers, and to adapt them to one of the Biostation's boats to spray oil dispersant in case of an oil spill from the tanker. After having everything ready, we were on stand by, and an Hercules airplane in Massachussetts was ready to go to Bermuda to spray oil dispersant too. A big tug boat was asked from Curacao and it was going to take it 3-4 days to be up the Sargasso Sea. A storm was near the islands and it could make the ship break very easily if it hit it. The situation was not a good one, a large amount of oil would eventually hit the coast and as a result, the tourism (first industry in Bermuda) would have declined. On the second day, Robert Smith, a coral reef ecologist from BBS, and I went SCUBA diving at the accident site. We made a survey at 70 feet depth of the reef situation, we took notes and photographs of the corals of the area. The pictures would have been used in court to prove the damages that would have occurred as a consequence

of oil in the environment. Because it would take between 100 and 200 years for a coral reef ecosystem to regenerate, the fines applied in this kind of situations are very high and well documented evidence is required.

Fortunately, the storm did not hit the area. Small tankers pumped the oil from the "Aguila Azteca" making it lighter and easier to be towed away from the reef.

4.2- Hydrostation S

Hydrostation requires a whole day of work at sea every two weeks. We left the dock at 0800 hs. and came back approximately at 1800 hs. We took water samples of the column of water down to 3,200 m with different intervals, using Niskin bottles. We measured temperature in situ and brought samples back to the laboratory to measure salinity and dissolved oxygen. Sometimes samples for trace matals were taken for studies at the Biostation and other institutions.

4.3- Inshore water sampling

Every fifteen or twenty days samples of surface inshore waters are taken in seven different locations around Bermuda: two in Hamilton Harbour, two in North Lagoon, one in Castle Harbour, one in Harrington Sound, and one in Saint George's Harbour. We measured transparency of the water with a Secchi disk, and temperature in situ. We brought samples back to the laboratory to analyze primary productivity, nutrients and salinity.

4.4- Rain water sampling

There are three rain water collectors located in Harbour Radio, Saint George, together with a machine that records amount of rain as well as starting and ending time of rain. These collectors had to be rinsed everyday if it did not rain. If it rained, we had to take the samples back to the laboratory. These samples were analyzed for trace metals, and pHs were taken for acid rain studies.

4.5- SCUBA diving

During September and October there were only two certified SCUBA divers at the Biostation so I was working underwater at least two or three times per week. Sometimes looking for lost equipment (plankton nets, chains, sampling bottles), other times taking sediment cores or coral samples, or taking photographs.

5- MY RESEARCH AT BBS

5.1- Introduction

My research took place at the BBS's M.A.P.(Marine and Atmospheric Program) laboratories. I worked with Timothy Jickells (Research Associate) and Anthony Knap (Associate Director). The work consisted of analyzing coral skeleton samples for trace metals (Cu and Zn) and for phosphorous (total and available).

For the trace metals analysis I had to set up a methodology to extract the metals from the $CaCO_3$ matrix. For the P analysis,

I modified the one used by Susan Boyd at BBS a couple of years before.

Coral skeletons have frequently been proposed as sources of chemical records of water quality for a variety of parameters(see review by Howard and Brown, 1984). Recently, their potential has been demonstrated for preserving phosphorous (Dodge et al., 1984) and lead (Dodge and Gilbert, 1984) records.

A Montastrea annularis coral colony was collected in February 1984, at about 5 m depth from reefs offshore Fort Lauderdale, Florida. Its age was determined by R. Dodge at Nova University, Florida, based on the skeleton bands (Fig. 1). The skeleton was cut year per year avoiding any contact of the metal part of the saw with the coral. Then packed and shipped to BBS to be analyzed.

Pilot tests were done during the first month, using different coral samples to determine the methodology to be used. The preparation of the samples and the analysis of the data took two more months.

5.2- Zinc and Copper analysis in coral skeleton

A number of trace contaminants appear to be introduced to nearshore marine waters as a result of different activities. Cu and Zn are present in seawater in higher concentration than naturally due to anthropogenic input. As a result of vessel-related activities, Cu and Zn have been introduced in the ocean because they are used in antifouling paints.

Zn is an important constituent of ship bottom primers and it is

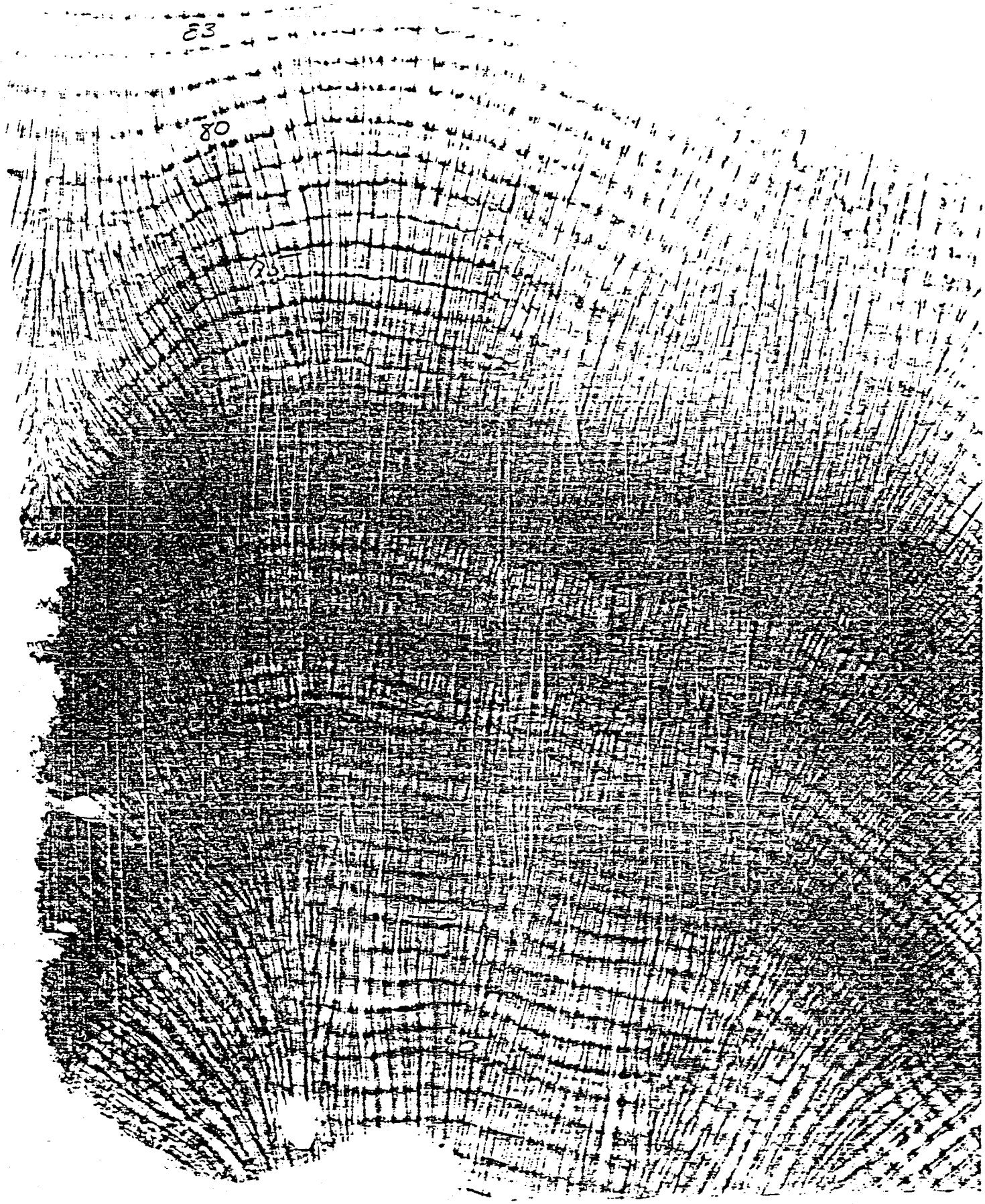

Fig. 1- X-ray of the age bands of the analyzed Montastrea annularis

utilized in sacrificial anodes attached to vessel bottoms to prevent corrosion of metal parts. Another possible source is the use of Zn compounds such as Zn chromates in vessel paints.

Cu is used as an antifouling agent in vessel bottom paints. We do not have numbers for Florida, but we do for southern California. Approximately 300,000 liters of such paints, containing an average of 600 g/l of Cu are applied annually to recreational, commercial and naval vessels along this coast. The corresponding estimated application rate of Cu via this mode (180 m tons/year) is more than one third the annual emission rate from major municipal wastewaters (510 m tons/year), and twice the estimated input from surface runoff and dry deposition (70 m tons/year). All the data are for southern California.

In Florida, the percentage input by antifouling paints should be higher because there is a higher density of boats than in southern California, and the sewage inputs are smaller because the urban centers are smaller.

Another input of Cu into the ocean is by the atmosphere. In the North Atlantic westerlies it has a concentration of 3.5 ng/m^3 and in the North Atlantic easterlies (in Sahara dust at times) as high as 15 ng/m^3 (N.A.S., 1975).

Wastewaters carry Cu into the sea from different sources. The principal of these sources are: the electroplating industry, spent Cu pickle liquors, the textile industry, and possibly the printed circuit and chemical industries.

It is very difficult to talk about trace metal pollution in coral reefs. Pollution strictly implies a deleterious effect on

an ecosystem which is usually assessed with respect to the biota. Detrimental effects of metals in corals would result in impairment of physiological processes. Metals in the deposited skeleton may be considered as being detoxified as they are isolated from, and thus unlikely to influence the living tissue. This is not to say that incorporation of metals into the skeleton may not lead to structural weakness of the aragonite.

Soluble metals in seawater probably represent the most obvious and direct route of metal uptake available to corals, although this pathway may not be the primary contributing factor to their metal status. The feeding activities of corals may represent a significant input of metals. Corals have three principal feeding mechanisms (Lewis & Price, 1975), one of which is the tentacular capture of zooplankton. Copepod exoskeletons (chitin) have been shown to accumulate Zn, Cu and other metals (Martin, 1970).

A second method is the utilization of mucous nets which trap not only zooplankton but fine particulate material, the mucous and entrained material being periodically ingested. The third important method of feeding outlined by Lewis & Price (1975) involves the extrusion of mesenterial filaments into the surrounding substratum presenting the possiblity of metal uptake directly from contaminated sediments. In areas exposed to elevated soluble and particulate metal concentrations, there may be enrichment of metals through the food chain.

Zooxanthellae may be involved in the direct uptake of metals in cases where potentially toxic metals are metabolically

substituted for vitally essential elements (Pilson, 1974). This direct influence of zooxanthellae on the host coral may have a direct influence both in terms of inclusion of metals in the skeleton and possible growth inhibition.

5.2.1- Glassware, reagents and standards preparation

a) Cleaning of 20 ml vials and 250 ml beakers:

1-soap solution

2-acetone

3-soaked in 50% HCl

b) Reagents preparation:

1- Pure NH_3- commercial NH_3 isothermally distilled; put a jar of distilled deionized water(DDW) and another with commercial NH_3 in a closed system for two days.

2- Glass redistilled reagent grade HNO_3.

3- Clean Ammonium Acetate by extraction with APDC/$CHCl_3$.

4- 3% APDC- 1 g of APDC + 30 ml of DDW. Clean it with chloroform in a liquid separation funnel. Prepare it every time before using it, do not keep it for a period longer than one day.

5- Clean chloroform- commercial $CHCl_3$ cleaned by acid extraction in liquid separation funnels.

c) Standards:

1- 0.5 ppm Zn:

0.2 ml Zn (1,000 ppm) + 2.5 ml acid + DDW up to 250 ml

2- 1.0 ppm Zn:

0.4 ml Zn (1,000 ppm) + 2.5 ml acid + DDW up to 250 ml

3- 2.0 ppm Zn:

0.8 ml Zn (1,000 ppm) + 2.5 ml acid + DDW up to 250 ml

4- 4.0 ppm Zn:

1.6 ml Zn (1,000 ppm) + 2.5 ml acid + DDW up to 250 ml

5- 1 ml Cu (1,000 ppm) in 100 ml DDW = 10 ppm Cu

1 ml (10 ppm)/100 ml DDW = 0.1 ppm Cu

1 ml (10 ppm)/250 ml DDW = 0.04 ppm Cu

1 ml (10 ppm)/1,000 ml DDW = 1.0 ppb Cu

6- 0.25 ppm Cu:

0.1 ml Cu (1,000 ppm) + 2.5 ml acid + DDW up to 250 ml

5.2.2- Samples preparation

There are 18 steps in the samples preparation:

1- Rinse the coral sample in 10% HNO_3 for 3 seconds.

2- Put 5 g of coral in a 250 ml beaker. Take note of exact weight.

3- Add 9 ml of HNO_3 (or sample weight x 1.8 = ml of HNO_3).

4- Dry it on a hot plate. Control the heat to avoid spilling.

5- Add 1 ml of HNO_3.

6- Dry it on the hot plate. Take it out at the point when a white foam is formed, no later because if we get a crust it will be more difficult to dissolve it.

7- Add enough DDW to dissolve the sample.

8- Add 1 ml of buffer solution (Ammonium Acetate). Shake it.

9- Add 0.8 ml of NH_3. Shake it.

Steps 8 and 9 are to bring the pH of the sample between 4 and 5, so the APDC can work in optimum conditions. Adjustements in these

amounts could be required.

10- Add 1 ml of 3% APDC. Shake it.

11- Put the sample into a 60 ml liquid separation funnel. Rinse the beaker with DDW and put it into the funnel to get all the sample.

12- Add 3 ml of $CHCl_3$.

13- Extract it into a 20 ml vial.

14- Repeat steps 12 and 13 for the same sample (double extraction). Rinse the funnel with DDW in between samples.

15- Add 0.1 ml of HNO_3.

16- Dry it on a hot plate. Now we have to be more careful than in step 4 because it is easier to have a splash from a 20 ml vial than from a 250 ml beaker.

17- Add 10 ml of 1% HNO_3.

18- Cover the vial with "parafilm".

Cautions:

1- Avoid any kind of contamination with metals. Always use plastic tools and wear polyethylene gloves.

2- All the steps should be done under a fume cupboard.

3- Make all additions using automatic pipettes to be accurate. It is important in the pH of the sample and in the blank values.

5.2.3- Data analysis

a) Some samples were run with spikes. The "Standard Addition Method "was used. This method is explained in Appendix I based on Beaty's manual "Concepts, Instrumentation and Techniques in

Atomic Absorption Spectrophotometry".

b) Curves of Absorbance vs. Concentration were determined for different coral samples.

c) Samples were taken randomly to be processed and analyzed.

d) Each day, a batch of 7 samples, 3 spiked samples, and 2 blanks were prepared and analyzed.

e) Table I:

sample # (year)	weight (g)	HNO_3 added (ml)
XX	X.XX	X.X

f) The samples for Zn were run in a Perkin-Elmer 460 Flame Atomic Absorption Spectrophotometer. Table II:

sample # (year)	Absorbance (AA-BG-Blank)

1- From the graph of Absorbance vs. Concentration, the best fitting line was calculated and we got a factor in this way:

if Abs. = .400, and Conc. = 1.01 ppm, then

Abs./.400 x 1.01 = Abs. x 2.525 (factor) to get a concentration in ppm from Absorbance values.

2- Once we got the concentration values in ppm, we transformed them into ug/g:

ppm x 10 ml / g of the sample = ug/g

The 10 ml value is the sample volume in the vial. So Table III will be:

sample # (year)	wt (g)	ppm	ug/g

3- These values were transformed once more into nmol/g :

ug/g x 1,000 / 65.38 .

Values for Zn in nmol/g:

year	concentration
35	2.83
36	2.20
37	2.17
38	[4.66]
40	2.25
41	2.28
42	0.32
43	1.24
44	0.99
45	2.54
46	0.69
47	1.53
48	2.00
49	1.13
51	[0.12]
52	1.07
53	0.46
54	0.76
55	0.83
56	1.02
57	1.12
58	[0.31]
59	1.19
60	0.73
61	1.45
63	2.66
64	4.21
65	1.02
66	[13.66]
67	1.99
68	8.84
69	7.88
70	1.02
71	1.73
72	0.83
73	1.61
75	2.14
76	1.99
78	0.87
79	[5.77]
80	2.81
81	0.96
82	2.95

The values in between brackets were not used in the graph and calculations. They probably are due to contamination in the

laboratory work. See the graph of concentration vs. time (years) in Figure 2, and the best fitting line in Figure 3.

Some statistical data from the Zn values are :

- Number of pairs computed: 38
- Mean: 1.95 nmol/g
- Standard deviation: 1.75
- Range: 8.52
- Coefficient of variation: 89.74%
- Mid-range: 4.58
- Median: 1.73
- Modes: 1.1(4); 1.0(3); 2.0(3); 2.2(3)
- Correlation coefficient (r): 0.16
- Slope: 0.02
- Intersection: 0.78

The percentage precision for these values is 11.5%. To get the 99% of precision (in the % precision values), we used three times the standard deviation and the media . This value (average from all the batches) is 35.5%. It is taken only from individual days of work, it does not mean anything if it is taken from all the days of work.

In Figure 3, the line shows a very small increase of Zn with time. This line would be almost horizontal (r=0) if the 1968-69 peak was not present.

g) The samples for Cu were run in a Perkin-Elmer 373 Graphite Furnace Atomic Absorption Spectrophotometer.

1- Three replicates of each sample were run using the

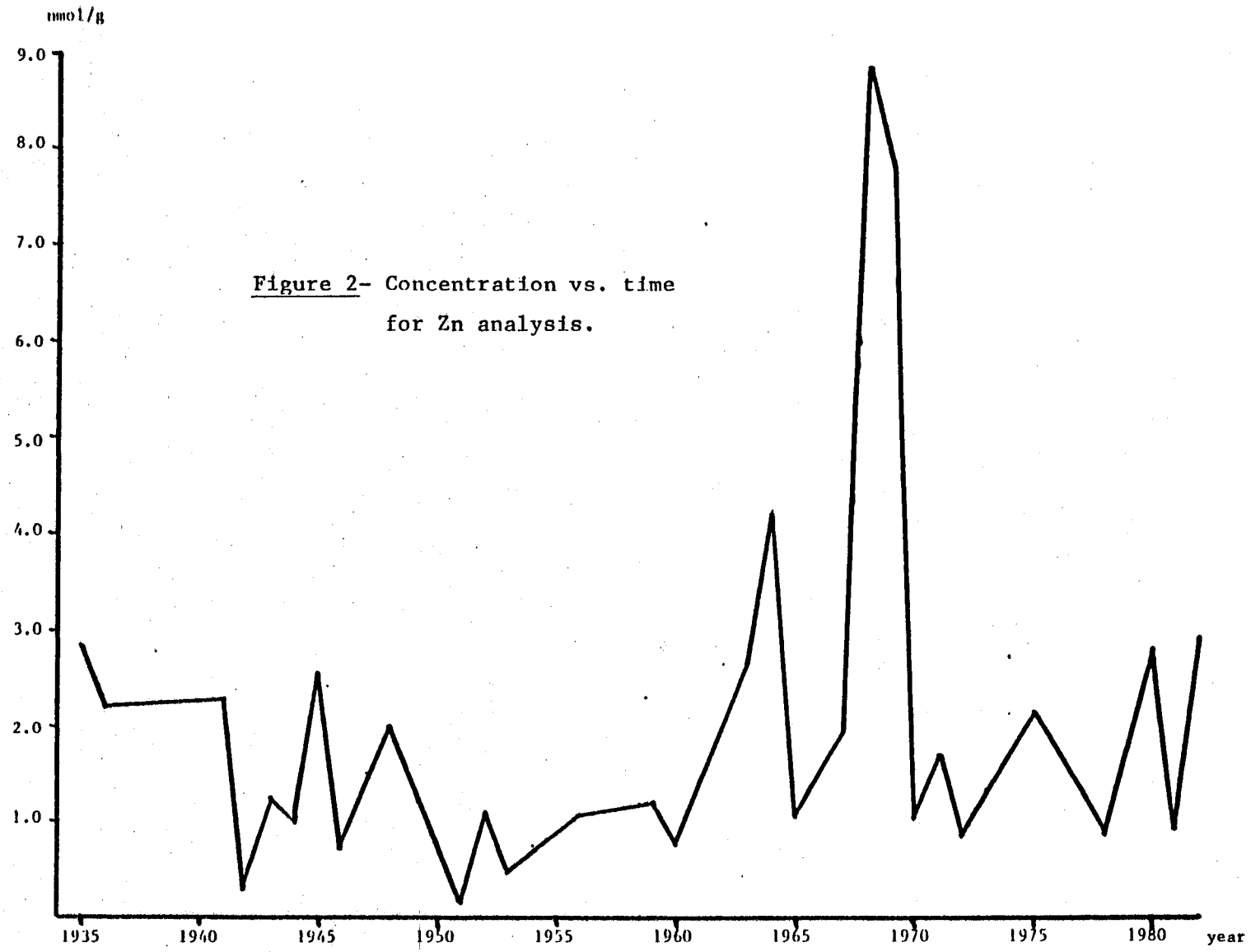

Figure 2- Concentration vs. time for Zn analysis.

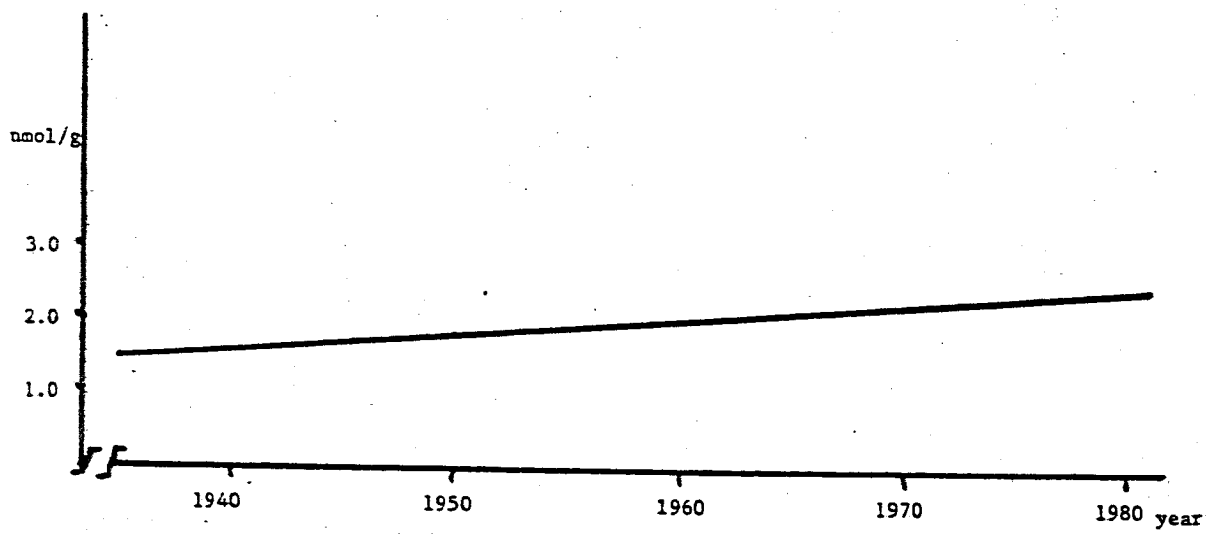

Figure 3- Best fitting line for the graph shown in Fig. 2.

following program:

		Wavelength:	324.7 nm	slit:	0.7 nm
Temperature	80	110	900	2,000	2,400
Ramp. time	5	5	5	0	1
Hold. time	10	15	20	5	3
				Record Read	

2- The same steps (1-3) used for Zn samples analysis were followed for Cu.

Values for Cu, given in nmol/g:

year	concentration
35	[2.90]
36	0.74
37	0.66
38	0.52
39	0.46
41	0.19
42	0.27
43	1.48
44	0.64
45	1.07
46	[0.42]
47	0.91
48	1.02
49	0.41
50	0.68
51	0.96
52	0.70
53	0.67
54	0.85
55	0.69
56	0.60
57	0.41
58	0.27
59	0.83
60	0.90
61	1.62
62	1.56
63	1.09
64	0.80
65	0.20
66	0.30
67	0.82

year	concentration
68	[0.11]
69	0.74
70	0.36
71	[3.38]
72	1.10
73	0.63
74	0.55
75	0.90
76	[1.92]
77	0.83
78	1.53
79	1.43
80	[2.55]
81	1.21
82	1.73

The values in between brackets, as for Zn, were not included in calculations and graphs. Very high values are probably due to contamination in the laboratory, and very low values can be due to a low uptake of the metal by the APDC. In Figure 4, we can see the graph of concentration vs. time (years), and in Figure 5, the best fitting line.

Statistical data for the Cu values:

- Number of pairs computed: 41
- Mean: 0.81 nmol/g
- Standard deviation: 0.40
- Range: 1.54
- Coefficient of variation: 49.38%
- Mid-range: 0.96
- Median: 0.9
- Modes: 0.6(7); 0.8(5)
- Correlation coefficient (r): 0.36
- Slope: 0.01
- Intersection: 0.17

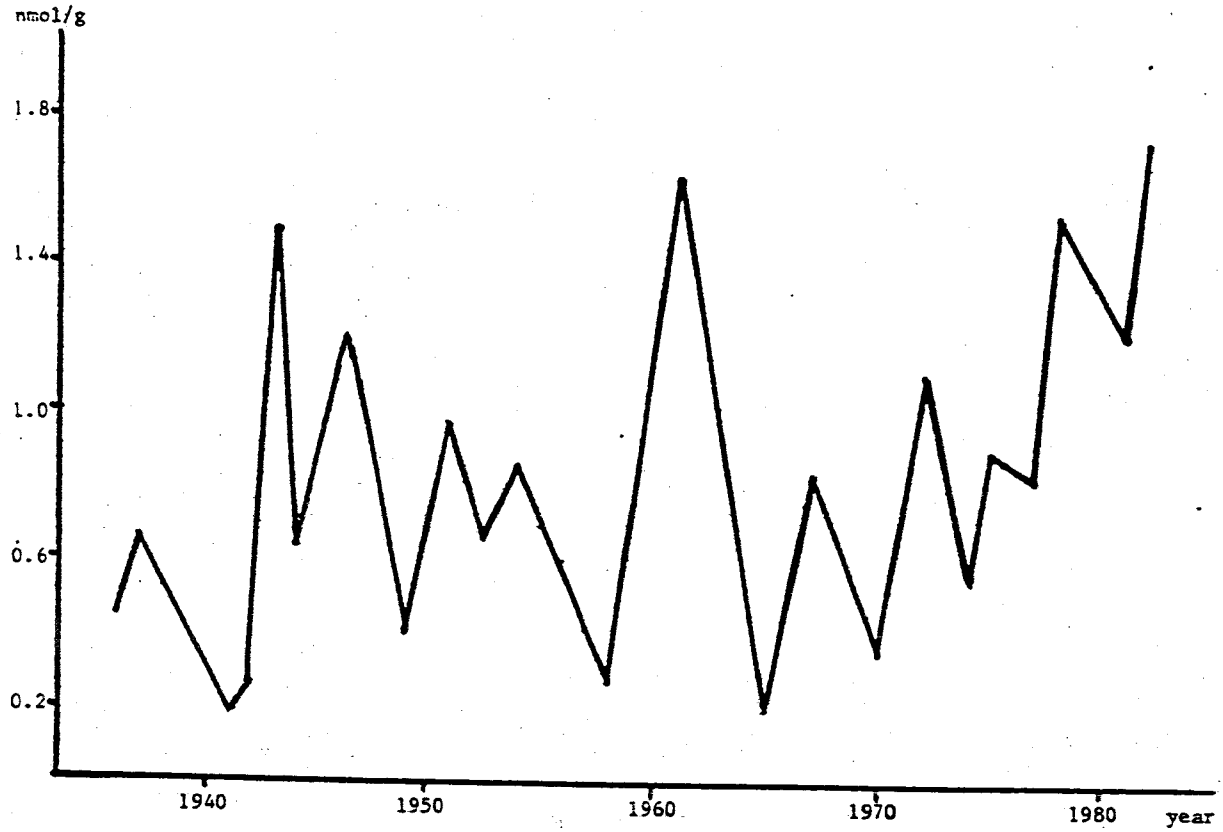

Figure 4- Concentration vs. time for Cu analysis.

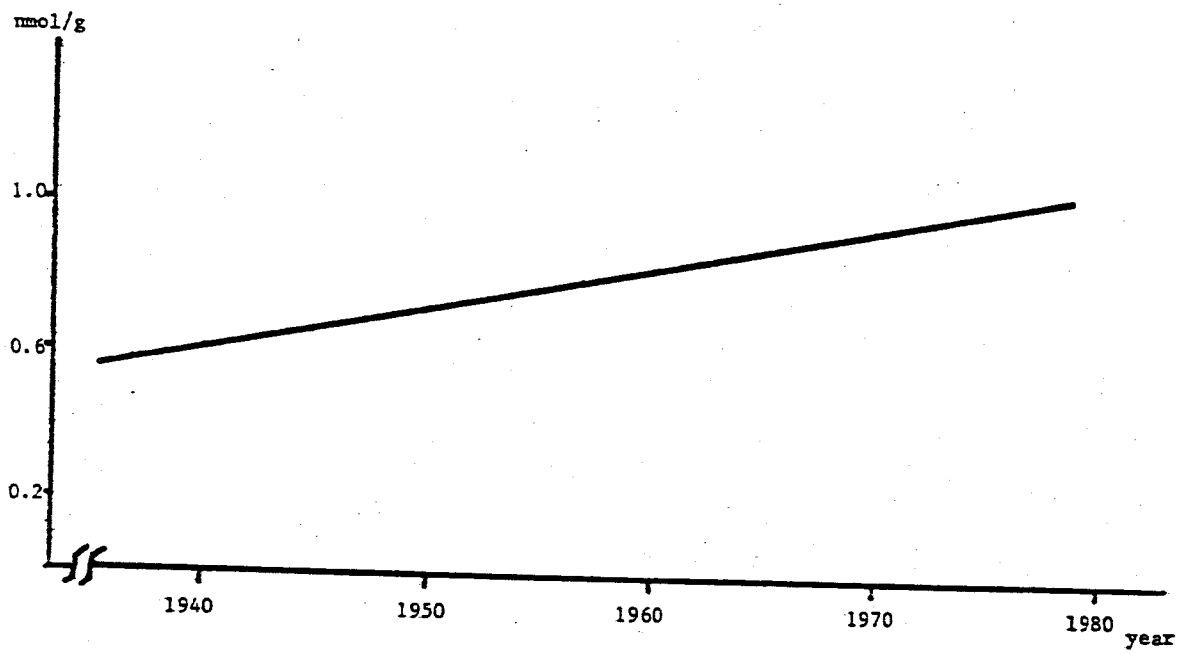

Figure 5- Best fitting line for the graph shown in Fig. 4.

The percentage precision for Cu data is 5.5%. The 99% of precision, as an average of all the daily batches, is 21.3%.

5.2.4- Discussion

In general, samples at the base and top of the skeleton showed elevated concentrations due to contamination with the tissue.

For Cu, the data reported here fall at the lower end of range of values quoted by Howard and Brown (1984). The record is quite constant from 1940 to 1970, apart from peaks in 1943 and 1960-61. There is another major peak in the early seventies and a slight increase through the 1970s. We believe this increase in the 1970s not to be the result of tissue contamination, since it is not seen in the phosphorous record (see 5.3.2 and 5.3.3), but rather due to an increase of Cu concentration in the water column probably due to boat traffic as reported elsewhere in Florida (Trefry et al., 1983). The cause of the 1943 and 1960-61 peaks is unknown.

The Zn data, which again falls at the lower end of the range reported by Howard and Brown (1984), shows a different pattern than that of Cu. As these data were generated from the same extracts, this suggests that neither pattern is an analytical artifact. The Zn data from 1940-1982 are dominated by two main peaks in the 1960s and a larger one in the late 1970s.

The knowledge concerning the effects of heavy metals on coral physiology is very poor. With the continuing growth of industry and exploitation of natural resources the encroachment

on nearshore coral reefs continues. Studies concerning the effects of realistically elevated metal concentrations on aspects of coral physiology, behaviour, and reproduction are needed to form a basis from which to minimize the ecological impact of metallic discharge to the tropical marine environment.

5.3- Phosphorous analysis in coral skeleton

It has been proposed that coral skeleton may contain a record of ambient phosphorous in the surrounding water (Dodge et al., 1984). The exact manner of incorporation of phosphorous into the coral skeleton has not been established. There are four possibilities. First, incorporation associated with detritus. Detrital particles have been reported within skeletal void spaces of coral skeletons (Barnard et al., 1974; Macintyre & Smith, 1974). It has been suggested that these particles may be incorporated via deposition on skeleton temporarily bared by tissue lesions, which are subsequently covered by new tissue which forms new skeleton over the previously bared area.

A second possibility could be an incorporation via occlusion of polyp tissue into the skeleton, or as skeletal organic matrix material. Organic matter concentrations within coral skeletons are considered to be generally low. If the material is occluded tissue or organic matter, it might be possible, assuming either (1) a relatively constant rate of occlusion of organic matter with varying P or (2) varying occlusion of organic matter with constant or regulated P, that the P concentrations of the coral skeleton would reflect ambient seawater conditions at the time of

incorporation.

Third, an inclusion as mineral phosphate. It is possible that coral skeletogenesis processes combine Ca with dissolved P to produce $CaPO_4$ directly into the skeleton (Dodge et al., 1984). This seems unlikely for several reasons. Phosphate has been reported as an inhibitor of calcification (Simkiss, 1964) where it acts as a crystal poison to aragonite. In addition, many crystallographic studies of coral skeletons have been performed to examine calcite versus aragonite ratios (Highsmith, 1981). No reports have been made, to our knowledge, of apatite within coral skeletons.

The fourth possibility would be an inclusion via endolithic algae. Endolithic algae pervade the coral skeleton and are present within the skeleton immediately below the living coral tissue (Jeffreys, 1968). An ambient nutrient supply rich in P may enhance the P content of the algae (or the algal biomass) which may in turn be reflected in our chemical analyses.

The inorganic P compounds are present mainly as orthophosphate in seawater. The majority of this orthophosphate is present as HPO_4^{2-} with the reminder consisting of $H_2PO_4^-$ (1%) and PO_4^{3-} (12%). The usual range in the open sea is 1-75 ug P/dm^3 (Johnston, 1976).

The main additions of P to the marine environment are considered to be sewage wastes, either directly discharged to the sea (including sludge dumpings) or via rivers into which treated wastes are discharged. Other sources are agricultural runoff and wastes from processing industries, but these are normally

considered to be small compared to the input by sewage. The relative size of contribution from river runoff and sewage has been illustrated on the basis of some calculations made on a hypothetical community of 10 million people covering an area of about 12,000 square miles, on a river discharging about 500 m^3s^{-1} (Johnston, 1976). The annual P contribution from sewage and the river was estimated to be 15,000 metric tons and 7 metric tons respectively.

5.3.1- Methodology

In the methodology that I set up along with T. Jickells there are 12 steps to determine available (inorganic) phosphorous and 13 to determine total phosphorous:

1- Clean vials as for trace metals analysis.

2- Break samples in small pieces using a mortar and a pestle.

3- If the samples look dirty do not use them. The P values are alterated by coral tissue. This problem is found in the last 2 or 3 years in the skeleton.

4- Run the first batch of samples in duplicate and check the precision. If the percentage precision is good (10% or less) it is not necessary to run the rest of the samples in duplicates.

5- Weigh approximately 0.2 g of sample and transfer it into a clean vial labeled with the year of the sample. Take note of exact weight. Add 3 clean vials in every batch of samples for blanks. Read a sample of DDW to measure the difference in absorbance in between both cells of the spectrophotometer. Take

two standards through the method: two clean vials with approx. 0.2 g of the same coral in both. Add to one of them 1 ml of working PO_4 and nothing to the other. The absorbance of the spiked standard should be approx. equal to the sum of the standard without spike plus the standard prepared in step #11.

6- Add 5 ml of DDW to each vial with automatic pipette. Then add 1 ml of 50% HCl.

7- Boil the vials on a hot plate until all the liquid has evaporated. Remove each sample from heat as it reaches this stage. The evaporation step should be done under a fume cupboard. Heat should be high enough to make the liquid simmer but not so high as to make it boil and cause problems by splashing.

Available (inorganic) phosphorous

8- Add 0.1 ml of NH_3 and boil to dryness. This step is to help the phosphate reagent's action that is retarded by low pH values.

9- Restore each sample to 20 ml adding DDW with automatic pipette.

10- Add 2 ml of phosphate reagent to each vial with automatic pipette. The reagent must be made up fresh each time and it is good at least for 6 hours. It is pale yellow and darkens over time. It reacts with phosphate to form a blue complex.

Reagent preparation (in order):

25 ml dilute H_2SO_4

10 ml Ascorbic Acid

5 ml Potassium Antimonyl-Tartrate

10 ml Ammonium Molybdate

11- Prepare standard: 1 ml of working phosphate + 19 ml DDW. Add 2 ml of reagent to it.

12- Read samples on the spectrophotometer at 885 nm wavelength after 15 minutes.

Total phosphorous

8- Add 0.5 ml of concentrated HNO_3 and boil to dryness.

9- Continue with steps 8-12 as for Available P.

We can avoid using NH_3. In this case, the developing time after the reagent addition should be at least one hour.

Table to use:

year weight(g) absorbance(885 nm) abs.-blank ug.at.P/g

To get the concentration of PO_4 in ug.at.P/g of coral:

[(abs.-blank) x F] / [weight x 50]

To get the F factor:

The concentration in the standard is: 1 ml x $6x10^{-2}$ ug.at. x 50 = 3 ug.at./l.

So, F = 3 / std.(abs.- blank)

5.3.2- Available P data

year	concentration(umol/g)
35	[0.11]
41	0.07
42	0.07
43	0.06
45	0.06
47	0.06
48	0.08
49	0.07
50	0.07

year	concentration(umol/g)
53	0.06
55	0.07
56	0.07
57	0.05
60	0.06
63	0.07
69	0.07
70	0.06
71	[0.03]
72	0.07
73	0.06
74	0.05
75	0.06
76	0.08
77	0.06
79	0.07
80	0.08
81	[0.11]
82	[0.13]

The values in between brackets have not been considered when graphing and analyzing statistically. The high values in 1981 and 1982 are due to contamination from the tissue. The 1935 value can be due to organisms that live between the coral and the substratum. There is no reason to believe that there is a decrease in the P amount in 1971; this 0.03 umol/g value is probably due to an analysis error.

See Figure 6 of concentration of available P vs. time, and Figure 7 is the best fitting line for these two variables.

Statistical data for the available (inorganic) P data:

- Number of pairs computed: 24
- Media: 0.06 umol P/g
- Standard deviation: 0.08
- Range: 0.03
- Coefficient of variation: 13.83%
- Mid-range: 0.01

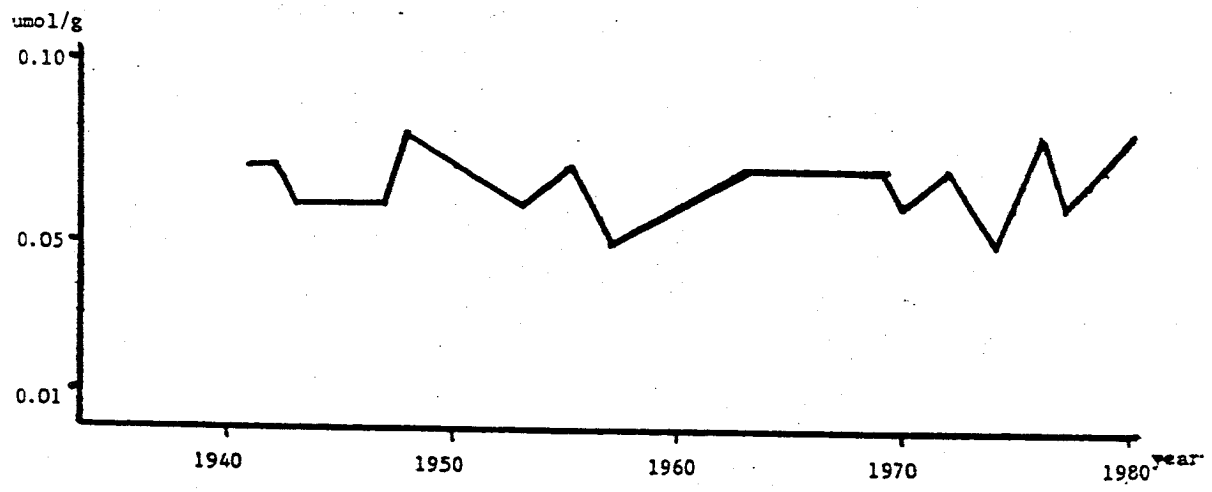

Figure 6- Concentration vs. time for available P analysis.

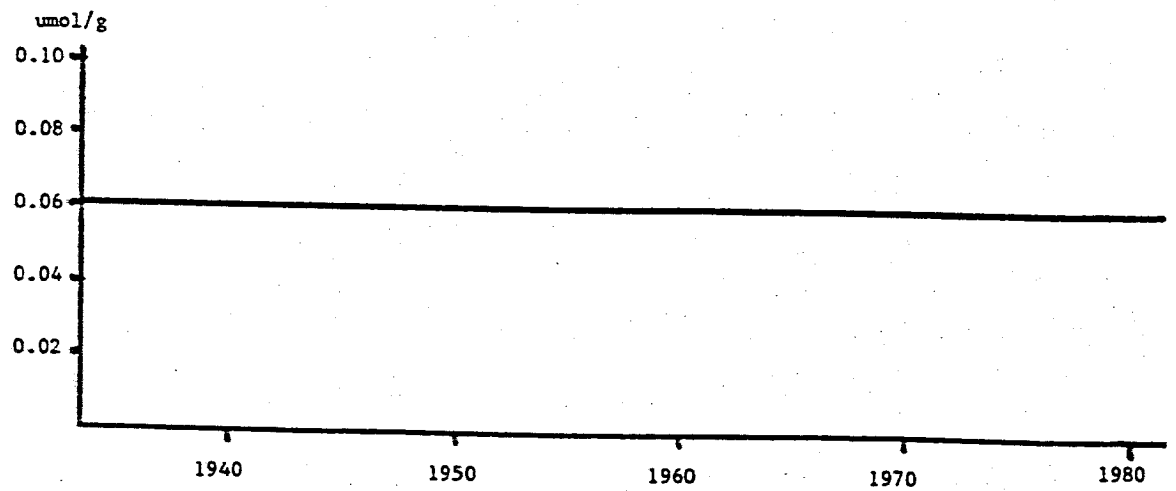

Figure 7- Best fitting line for the graph shown in Fig. 6.

- Median: 0.07
- Modes: 0.07(10); 0.06(9)
- Correlation coefficient (r): 0.02
- Slope: 1.19×10^{-3}
- Intersection: 0.17

The percentage precision for these samples is 6.5%.

5.3.3- Total P data

year	concentration(umol/g)
35	0.14
37	0.12
38	0.07
39	0.15
40	0.10
43	0.10
44	0.07
46	0.09
48	0.11
50	0.14
51	0.10
52	0.10
53	0.10
54	0.09
55	0.11
56	0.11
57	0.12
58	0.09
59	0.09
60	0.10
61	0.10
62	0.10
63	0.12
64	[0.16]
65	0.09
66	0.09
67	0.10
68	0.15
69	0.15
70	0.09
71	0.10
72	0.10
73	0.09

year	concentration(umol/g)
74	0.10
75	0.10
76	0.10
77	0.10
78	0.12
79	0.14
80	0.14
81	[0.16]
82	[0.25]

Again, the values in between brackets were not considered for calculations for the same reason that in available P samples.

These values are 25% higher of what we got from the analysis. We proved in 1984 and reconfirmed it in 1985 that the total P extracted by HNO_3 is 75% of the total P obtained by muffling. The problem with muffling is that we cannot "cook" the samples at temperatures higher than 600°C because the vials would melt, and it takes a lot of time. This differs from Dodge et al.,1984. They suggest that the two methods give identical results. In Figures 8 and 9 we have the graphs of concentration vs. time in years and the best fitting line respectively.

Statistical data for total P values:

- Number of pairs computed: 39
- Mean: 0.11 umol p/g
- Standard deviation: 0.02
- Range: 0.08
- Coefficient of variation: 18.88%
- Mid-range: 0.04
- Median: 0.10
- Modes: 0.10(15); 0.09(8)
- Correlation coefficient: 0.08

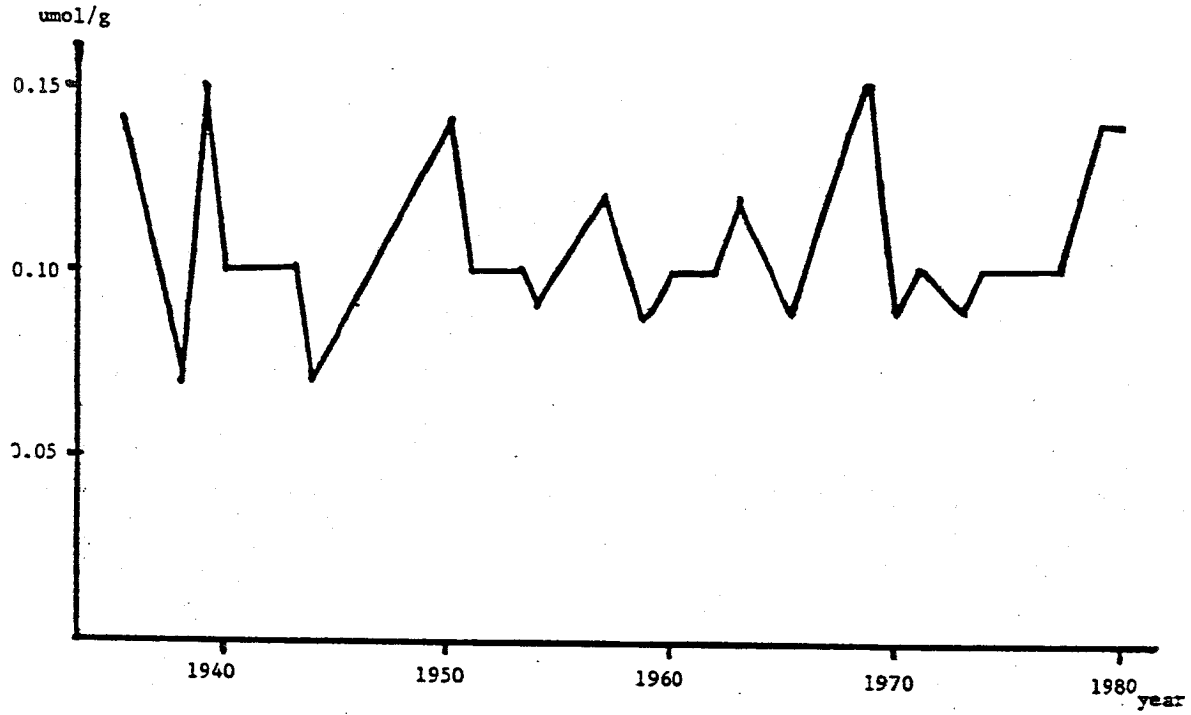

Figure 8- Concentration vs. time for total P analysis.

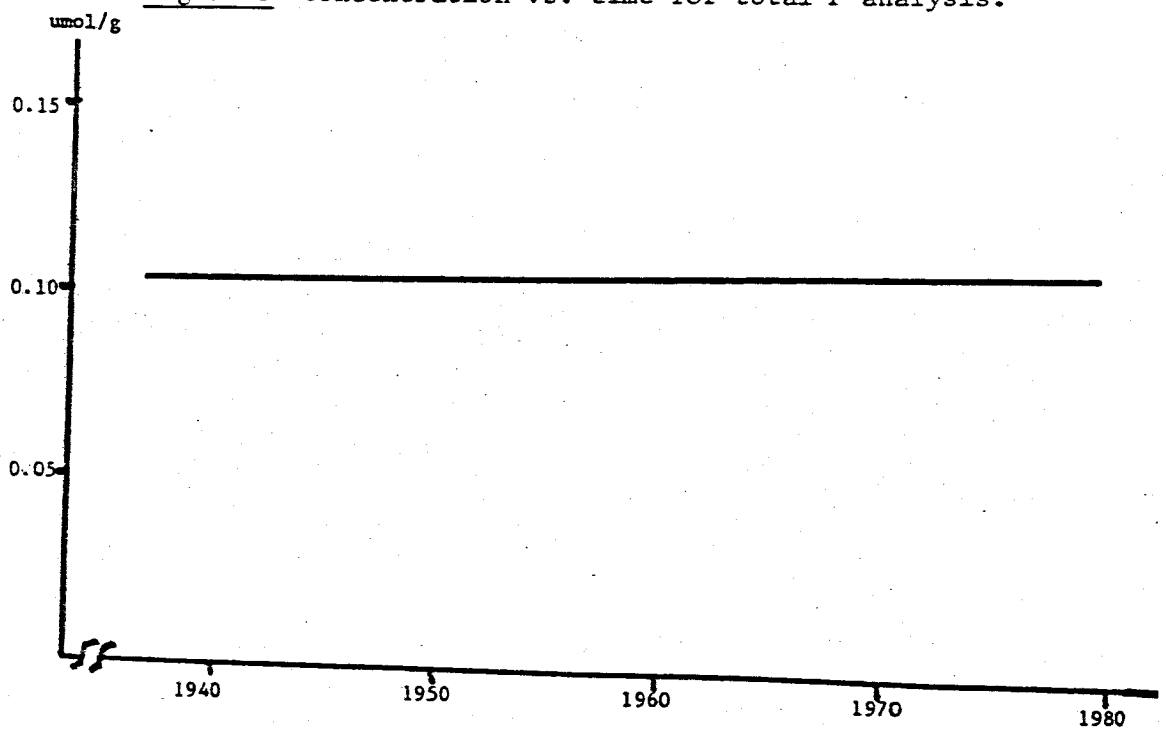

Figure 9- Best fitting line for the graph shown in Fig. 8.

- Slope: 0.0001
- Intersection: 0.10

The percentage precision for total P values is 4.6%.

5.3.4- Discussion

From the graphs in Figures 7 and 9 we conclude that there is not an increment in amount of P in the coral skeleton with age. So, if these values reflect the water conditions through time, we can say that P in waters out of Fort Lauderdale has been almost constant. Two large peaks in 1940 and late 60's and smaller ones in 1950, late 50's and mid-60's may reflect discharges of industries but we do not have data that confirm this.

The effects on reef systems can be detrimental (Kinsey & Davies, 1979; Walker & Ormond, 1982) and it is important to both establish baseline levels for comparison as well as to document enhaced eutrophication for prediction and management purposes.

5.4- Nickel and Cadmium analysis in coral skeleton

The same samples that were prepared for Cu and Zn analysis were used to analyze Ni and Cd. Standards were prepared and ran in the Graphite Furnace Atomic Absorption Spectrophotometer.

- There was no signal for Ni in any of the samples.
- There were signals for Cd, but the blank values were larger than the samples values. Obviously there are one or more interferences present. We think it was due to an interference with Ca. Some other methodology should be used for Cd analysis. It could be standard additions to

all the samples (appendix I). We could not try anything else because we did not have more samples from that same coral and the internship was almost over.

5.5 Lead analysis in coral skeleton

Lead is among the most pervasive of the pollutants man has introduced to the marine environment. Because Pb is frequently discharged directly into the atmosphere in the form of smelter and automobile exhaust emissions, all ocean surfaces probably receive more Pb as a result of precipitation and deposition of airborne aerosols than prehistoric times (Schaule and Patterson, 1981). In local coastal regions Pb pollution may also be present in sewage contaminated with industrial waste.

The magnitude of these increases is not easily evaluated. Pb concentrations in seawater, marine organisms, and polar ice and snow may be extremely low approaching the pg g^{-1} level (Settle and Patterson, 1980). Debate over the adequacy of analytical technique has brought into question data that would provide a historical record of Pb levels in the atmosphere and in the sea. For example, Pb determinations in core samples of glacial ice and snow as reported by Ng and Patterson (1981) are considerated by Jaworowski (1983) to be erroneously low because particles rich in Pb could have been lost during sample preparation.

In the marine environment a record of Pb pollution may be contained in the strata of bottom sediments (Ng and Patterson, 1982). However, slow sedimentation rates and biological reworking tend to obscure short-term variations in Pb levels. Improved

technologies of Pb additions to the marine environment are needed to assess the impact of changes in the availablity of Pb accurately (Dodge and Gilbert, 1984).

The same Montastrea annularis coral that I used for Cu, Zn and P analysis was used by R.E. Dodge and T.R. Gilbert, in Nova University and Northeastern University respectively, for the Pb analysis. They sent me the graph shown in Figure 10, from which some conclusions can be derived. The results from the Pb analyses confirm the value of corals as indicators of Pb pollution. The lead concentration shows an exponential increase from 1940-1970 and then shows a complex pattern which probably relates to the decline in usage of gasoline in the early 70s due to price increase, and the decline in the late 70s and early 80s with the use of unleaded gasoline. The concentration in more recent year bands are comparable to those reported previously for a coral from a polluted area in St. Croix while the concentration in the oldest year bands are comparable to the levels in a coral from an uncontaminated area of St. Croix (Dodge and Gilbert, 1984).

6- EVALUATION OF THE INTERNSHIP

Above all, my internship at the Bermuda Biological Station gave me valuable insights into how our marine resources are managed. It begins in the lab, where dedicated people often spend years trying to identify the cause and effect relationships between human activity and the marine environment. The frustrations faced by those trying to gain adequate funding for their research was a lesson in and of itself, for it showed me

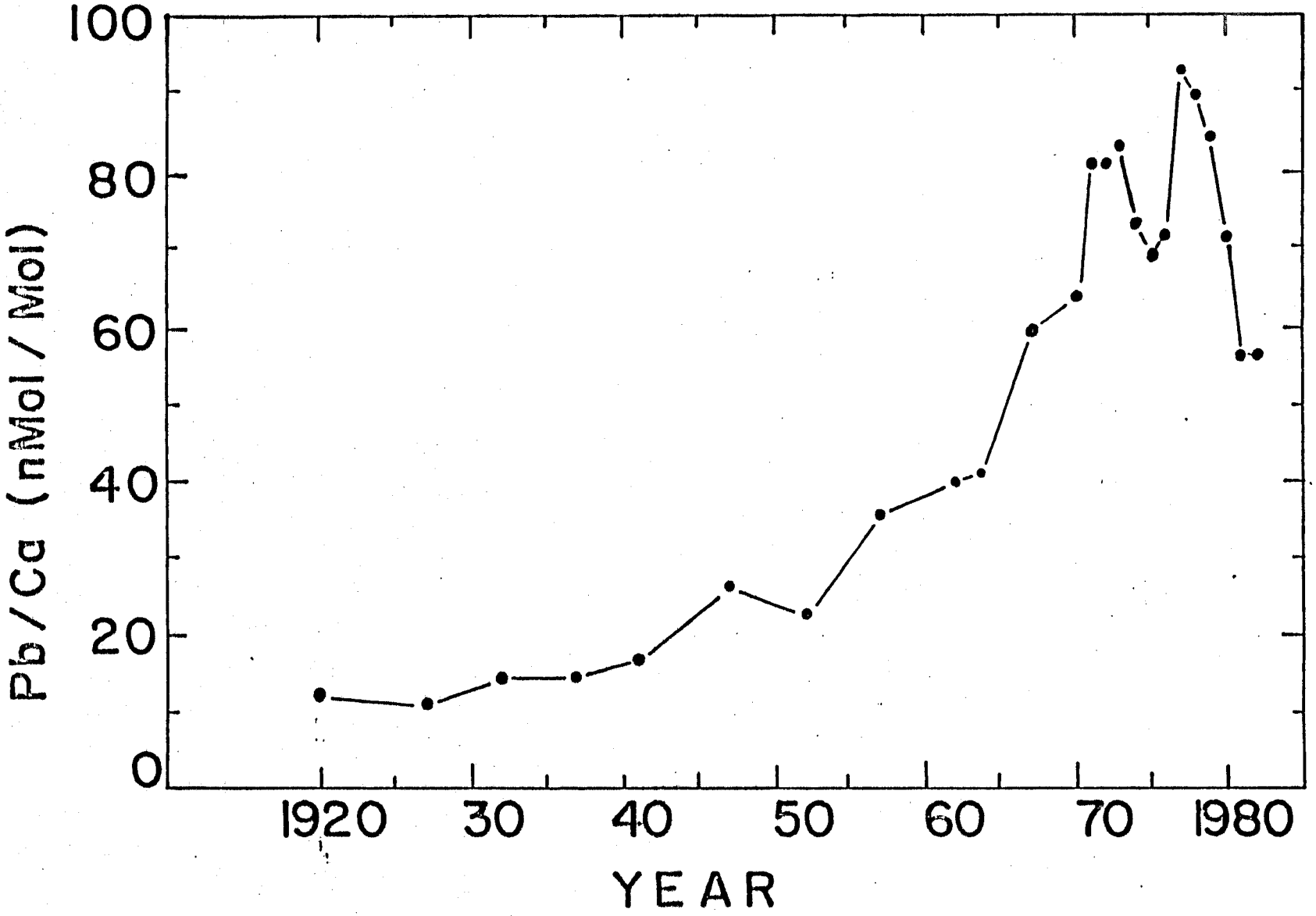

Figure 10- Concentration vs. time for Pb analysis.

the importance of proper planning, teamwork, and efficiency in conducting research studies. In addition, examining the management structure of the Bermuda Biological Station - from the director to the student volunteers - taught me how a successful research station is operated. The next step in resource management involves the application of research findings to the "real world."

Proper management in the real world is directed by scientifically informed, people-oriented individuals. Such people are able to understand scientific details and direct various assistants in order to achieve a variety of goals. Without such individuals, marine resource management would likely be far more complicated than it already is.

As I said in the first part of the report, working for four months at the Bermuda Biological Station for Research, Inc. was the best experience I have ever had as an oceanographer. At no other time during my formal education did I have the opotunity to deal with so many different aspects of the ocean studies. The work was not only in the laboratories but in the field as well, at sea or on the shoreline. The location of the Biostation is perfect for work in the marine sciences; the environment is beautiful, and the staff members (scientists, administrators and maintenance personnel) are professional, friendly and helpful people.

The visiting scientists and students go to the Biostation from all around the world. It is an excellent place to meet people and to be informed of the research that is taking place in

different laboratories, universities and institutions in the five continents.

During summer 1985 I went back to BBS as a Teaching Assistant for the Analysis of Marine Pollution course. After this, I continued to work for Dr. A. Knap as a Research Assistant in marine pollution, for two more months.

As an overall evaluation of the internship I can say that it was excellent and that it confirmed my interest in coastal pollution management. So much so that in the summer 1986 I will continue my studies towards a Ph.D. in Marine Resource Management with emphasis on Chemical Oceanography and Pollution Management.

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APPENDIX I

Interferences in AAS

There are three major interferences when working with Atomic Absorption Spectrophotometry. The first place in the flame process subject to interference is the very first step, the nebulization. If the sample is more viscous or has cosiderably different surface tension characteristics than the standard, the sample uptake rate or nebulization efficiency may be different between sample and standard. If samples and standards are not introduced into the process at the same rate, it is obvious that the number of atoms in the light beam and, therefore, the absorbance, will not correlate between the two. Thus, a "matrix interference" will exist.

A second place where the interference can enter into the flame process is in the atomization process. In this step, sufficient energy must be available to dissociate the molecular form of the analyte to create free atoms. If the sample contains a component which forms a thermally stable compound with the analyte which is not completely decomposed by the energy available in the flame, a "chemical interference" will exist.

There is a third major interference, however, which is often encountered in hot flames. The dissociation process does not necesarily stop at the ground state atom. If additional energy is applied, the ground state atom can be thermally raised to the excited state; or if thermal energy is sufficient, the electron may be totally removed from the atom, creating an ion. As these electronic arrangements deplete the number of ground state atoms

available for light absorption, atomic absorption at the resonance wavelength is reduced. When an excess of energy destroys the ground state atom, an "ionozation interference" exists.

Method of Standard Additions

Three major interferences (matrix, chemical, and ionozation) has been discussed. There is a very useful technique which often makes it possible to work in the presence of an interference without eliminating the interference itself, and still make an accurate determination of analyte concentration. The technique is called the "method of standard additions". Accurate determinations are made without eliminating interferences by making the concentration calibration in the presence of the interference. The standards are added to portions of the sample, thereby letting any interferent present in the sample also affect the standard.

The technique is illustrated in Figure ap.1. The solid line passing through the origin represents a typical calibration line for a set of aqueous standards. For a water blank, zero absorbance is defined, and as the concentration of analyte increases, a linear increase in absorbance is observed.

Let us now take a sample and split into three portions. Nothing is added to the first portion; different amounts of standard are added to portions 2 and 3. Finally, all portions are diluted to the same volume so that the final concentrations of the original sample constituents are the same in each case. Only

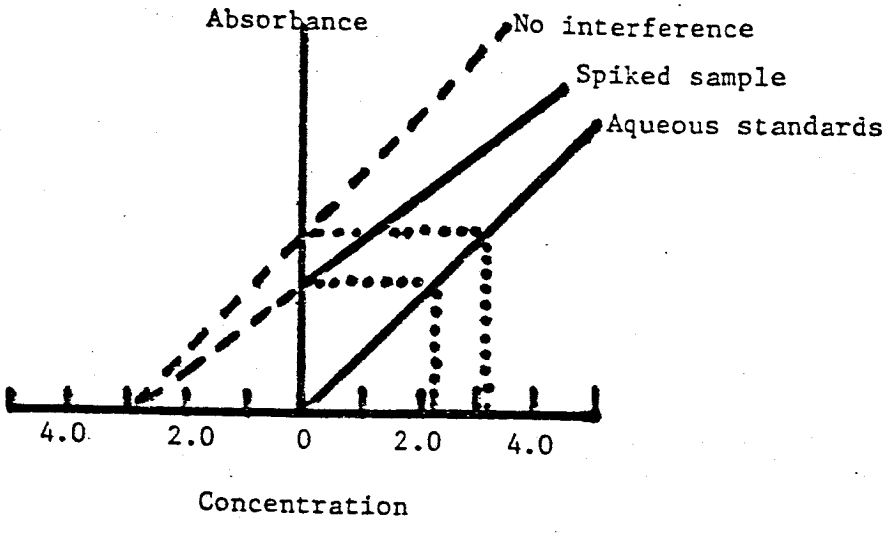

Fig. âp.1- Method of Standard Additions (from Beaty, 1978).

the amount of added analyte differs by a known amount. If no interference were present in this sample, a plot of measured absorbance versus the concentration of "added" standard would be parallel to the aqeuous standard calibration, and offset by an absorbance value resulting from the analyte present in the unspiked sample. If some material is present in the sample causing either a matrix, chemical, or ionozation interference, the number of ground state atoms producing atomic absorption will be reduced. The absorbance from the analyte in the unspiked sample will, therefore, be reduced by this interference. However, the absorbance increase from added standard will also be reduced by the same proportional amount, since the concentration of interferent is the same in each solution. Therefore, a straight

line will result, but because of the interference, its slope will be different from that observed for the aqueous standards.

In this situation, if the absorbance of the unspiked sample were to be compared directly to the aqueous calibration, negative error would result. If, however, the slope determined by the standard additions to our sample is used as the calibration slope, an accurate determination of the sample concentration can still be made. By continuing the concentration calibration on the abscissa backward from zero and extrapolating the calibration line backward until it intercepts the concentration axis, the concentration responsible for the absorbance of the unspiked sample is indicated. An accurate determination has been made by calibrating in the presence of the interference.

The method of standard additions is an extremely valuable tool in atomic absorption. The presence of an interference can be confirmed by observing the slope of the spiked sample calibration and determining whether or not it is parallel to the aqueous standard line. If it is not, an interference is present. Secondly, if an interference is present, the method of standard additions "may" allow an accurate determination of the unknown concentration by using the standard additions slope for the calibration. Caution should be used with the technique, however, as it can fail to give correct answers if the interferring species does not become chemically associated with the added standard to the same extent as the original analyte. Further, all concentrations must be adjusted to the linear working range in order to assure an accurate extrapolation.