BIOASSAY AND DISTRIBUTION OF THIAMINE IN THE SEA

A

DISSERTATION

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Kottayam Viswanathan Natarajan

B. S., University of Travancore, Trivandrum, India
M. S., Banaras Hindu University, Banaras, India

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APPROVED:

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Chairman

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Department Head

DATE _____

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Vice President for Research and
Advanced Study

ABSTRACT

A marine yeast, Cryptococcus albidus, has been used for the bioassay of thiamine in sea water and is found to be sensitive in the range of 10 to 300 pg/ml thiamine. The distribution of thiamine has been followed in the North Pacific Ocean, the Gulf of Alaska and the Arabian Sea.

Thiamine concentrations in surface water were found to range from 0-490 ng/liter and is generally high in coastal regions and low in the open ocean. The vertical distribution showed a decrease in concentrations with depth down to 50 to 75 m whereas below this depth the values were generally low or undetectable. It follows the temperature distribution with high concentrations in the thermally stratified layer above the thermocline. The seasonal distribution investigated in one area showed high concentrations during the months of April and September, decreasing drastically in November. A close relationship between primary productivity and thiamine was noticed with high values of thiamine in areas of high productivity. Uptake studies with ^{14}C-labeled thiamine suggested the presence of thiamine autotrophs in one region and auxotrophs in another. The turnover time of thiamine observed in one region was around four to seven hours.

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TABLE OF CONTENTS

	page
ABSTRACT	iii
ACKNOWLEDGMENTS	iv
INTRODUCTION	1
Vitamin requirements	2
Vitamin concentrations	3
Thiamine requirements of marine micro-organisms	4
Algae	4
Bacteria	5
Fungi	5
Thiamine concentrations in the sea	5
Sea water	5
Sediments	6
MATERIALS AND METHODS	8
Bioassay organism	8
Isolation and culture	8
Identification	11
Physiology and vitamin requirements of *Cryptococcus albidus*	11
Thiamine requirement of *Cryptococcus albidus*	11
Chemicals	12
Pretreatment of glassware	12
Collection of samples	12

page

Thiamine stock solutions 13
Preparation of thiamine-free water 13
Preparation of samples 14
Bioassay standards 15
Preparation of the inoculum. 16
Incubation 17
Calculation of results 17
Uptake studies with ^{14}C-labeled thiamine 17
Particulate nitrogen analysis. 18
RESULTS 20
Standardization of the bioassay. 20
The standard curve 24
Distribution of thiamine in the sea. 25
Prince William Sound. 26
Gulf of Alaska, Montague-Middleton Island 26
Gulf of Alaska, Valdez-Kodiak 27
Gulf of Alaska, Cordova-Yakutat-Juneau. 27
Gulf of Alaska, Seattle-Juneau. 28
Gulf of Alaska, Juneau-Cape Spencer 29
Southeast Alaska, Port Angeles-Juneau 29
North Pacific Ocean, San Diego-Kodiak Island. . . 29
North Pacific Ocean, Vancouver-Hawaii 30
Arabian Sea 30

page

Seasonal cycle 31

Uptake studies with ^{14}C-labeled thiamine 32

Prince William Sound. 32

Southeast Alaska. 32

Gulf of Alaska, Juneau-Cape Spencer 33

DISCUSSION. 36

Cycle of thiamine in the sea 36

Comparison between thiamine and vitamin B_{12}. 38

Origin of thiamine 40

Concluding remarks 43

SUMMARY 48

REFERENCES CITED. 50

APPENDIX A. 55

APPENDIX B. 66

LIST OF ILLUSTRATIONS

FIGURES

following page

1. Growth curves in synthetic sea water medium with different concentrations of thiamine 21

2. Typical standard curves in charcoal-treated natural sea water 24

3. A typical standard curve with the estimated regression line and the standard error of estimate. 24

4. The relation between particulate nitrogen and thiamine in Prince William Sound 26

5. Vertical distribution of thiamine and temperature in two open ocean stations. 28

6. Vertical distribution of thiamine and temperature in two coastal stations. 28

7. The relation between particulate nitrogen and thiamine in Southeast Alaska. 28

8. The relation between primary productivity and thiamine in the Arabian Sea 31

9. The relation between ^{14}C-thiamine uptake and ^{14}C-carbonate uptake in Prince William Sound. 32

10. The relation between ^{14}C-thiamine uptake and ^{14}C-carbonate uptake in Southeast Alaska. 32

11. The relation between ^{14}C-thiamine uptake and primary productivity in the Gulf of Alaska. 33

TABLES following page

1. Synthetic sea water medium 10

2. Procedure for the preparation of bioassay standards. 16

3. Utilization of carbon and nitrogen sources by Cryptococcus albidus. 20

4. Uptake of thiamine moieties by Cryptococcus albidus 21

5. Effect of enrichments in natural sea water. 21

6. Effect of salinity on thiamine bioassay 22

7. Effect of inoculum starvation on response to added thiamine 22

8. Effect of inoculum made from different slants 23

9. Effect of autoclaving on destruction of thiamine 23

10. Effect of autoclaving on destruction of thiamine 23

11. Effect of autoclaving on destruction of thiamine 23

12. Effect of charcoal treatment on the removal of thiamine from natural sea water. 23

13. Statistical analyses of results of 3 samples. 24

14. Variations in thiamine concentrations calculated from internal and external standards. 25

15. Cruise tracks of stations sampled in 1963, 1964 and 1965. 25

following page

16. Diurnal variations in thiamine in surface samples 29

17. Seasonal cycle of thiamine in the Gulf of Alaska, Juneau-Cape Spencer. 31

18. Uptake experiments in Prince William Sound. 32

19. Uptake experiments in Southeast Alaska 32

20. Uptake experiments in the Gulf of Alaska 33

21. Uptake of labeled thiamine by Cryptococcus albidus 34

22. Effect of storage of sea water at 22°C. 42

23. Thiamine activity in sea water 46

MAPS

1. Gulf of Alaska 54

2. Prince William Sound 54

3. Gulf of Alaska 54

4. Pacific Ocean. 54

5. Arabian Sea. 54

INTRODUCTION

Vitamins are presumed to be one of the major categories of biologically active compounds of ecological importance in phytoplankton succession in the sea. Provasoli and Pinter (1953) considered that the practical aim of predicting algal succession and blooms may well be achieved through a comprehensive knowledge of vitamin cycles as well as mineral cycles. Provasoli and Pinter (1960) also suggested that the chemical environment may be defined by studying the nutritional needs of "indicator" species. Lucas (1947, 1949, 1955, 1958, 1961) proposed a theory of non-predatory relationships in ecology wherein there is a general tendency for all cells to release metabolites into the surrounding medium. Lucas (1961) suggested the term "ectocrines" for those known and potent metabolites which occur within the cells, such as B_{12}, and which may be expected to play similar roles within the community. These substances, broadly speaking, are measurable in terms of parts per million or even less. Aside from speculations about the influence of external metabolites, we have very little data to prove or disprove the hypotheses which have been put forward.

A beginning already has been made with reference to the study of vitamins in sea water. The problem has been investigated along two different lines: 1. by investigating the exogenous requirements of marine micro-organisms for different vitamins and 2. by analyzing sea water samples for these vitamins.

Vitamin requirements

Since the advent of pure culture studies, the techniques have been applied to isolate marine algae, fungi, yeasts, and bacteria in pure cultures in order to study their vitamin requirements under laboratory conditions. It has become increasingly clear that laboratory findings substantiate the importance of vitamins in the growth of many marine micro-organisms (Provasoli, 1958, 1961, 1963; Provasoli and Pinter, 1953, 1960; Pinter and Provasoli, 1958; Lewin and Lewin, 1960; Harvey, 1955; Guillard, 1963; Droop, 1953, 1957, 1958, 1959; Daisley, 1957; McLaughlin and Zahl, 1959; Fries, 1959, 1961; Van Baalen, 1961; Burkholder, 1959, 1959a, 1963; Burkholder and Burkholder, 1958; Ahearn and Roth, 1962; Vishniac, 1961; Adair and Vishniac, 1958). The main objection to this approach is whether laboratory findings are enough proof for transposing these requirements to ecological environments. In most laboratory experiments single species cultures are used, whereas in nature there exists a close relationship between different types of organisms. According to Lucas (1961) "some of the laboratory results will prove to be illusory or irrelevant and others less important in vivo than in vitro." Lucas (1961) also postulated that some organisms appear to be susceptible and must have evolved significant deficiency patterns which certain neighbors can supply, doubtless attaining increased efficiency elsewhere in their own metabolism at the cost of an increased dependence.

It has generally been found that marine algae require only

three vitamins-B_{12}, thiamine, and biotin (Provasoli and Pinter, 1953; Provasoli, 1963) although they live in an environment presumably containing a variety of vitamins and other growth factors. Species of bacteria, yeasts, and other fungi have been found to require nicotinic acid, pantothenic acid, niacin, para-aminobenzoic acid, inositol, pyridoxine, and riboflavin in addition to B_{12}, thiamine, and biotin (Ahearn and Roth, 1962; Burkholder, 1963).

Vitamin concentrations

The other approach to the problem of vitamins in sea water is to analyze sea water samples for vitamins and correlate the presence or absence of vitamins with phytoplankton succession and blooms or with other measures of productivity. The distribution patterns of vitamin B_{12} have been worked out by many investigators (Cowey, 1956; Visniac and Riley, 1961; Daisley and Fisher, 1958; Kashiwada et al., 1957, 1957a, 1959; Menzel and Spaeth, 1962). Published values for B_{12} concentration vary from 0.01 ng/liter to 16 ng/liter according to position, depth, and season. Vishniac and Riley (1961) and Menzel and Spaeth (1962), have demonstrated a seasonal variation in vitamin B_{12} closely paralleling that of inorganic nutrients and plankton blooms.

The secondary attention given to thiamine is surprising even though it has been known for some time that this vitamin is an essential growth factor for many marine micro-organisms. A brief

review of the work done so far on the requirements of thiamine by marine micro-organisms and the suggested sources for this vitamin in sea water will be helpful in understanding the importance of thiamine in the marine environment.

Thiamine requirements of marine micro-organisms

Algae. Several reports concerning the thiamine requirements of algae have appeared in the literature (Droop, 1957, 1958, 1959, 1962; Provasoli, 1958, 1963; Pinter and Provasoli, 1963; Anderson, 1945; Lwoff, 1947; Lwoff and Lederer, 1935; Provasoli, McLaughlin, and Droop, 1957; McLaughlin, 1958; Lewin and Lewin, 1960). Thiamine auxotrophy exists in all major classes of marine algae (Appendix A, Table 1). About 64% of the algae examined required an exogenous source of thiamine (Appendix A, Table 2). The pattern of thiamine requirement varies with different species of algae, some requiring a part of the thiamine molecule, namely thiazole, others requiring the other part of the molecule, pyrimidine, and still others requiring the intact molecule (Appendix A, Table 3). Some algae also have been found to have multiple requirements for one or two vitamins besides thiamine. Even symbiotic algae isolated from marine coral reef invertebrates were found to respond by increased growth to the addition of exogenous sources of B_{12}, thiamine, and biotin (McLaughlin and Zahl, 1959). It thus can be seen that the availability of thiamine in the external environment will influence the types of algal communities present.

Bacteria. Burkholder (1963) examined 1,272 cultures isolated from sea water and sediments of tropical, temperate and sub-arctic regions and found that 501 cultures required thiamine. Numerous thiamine requiring cultures showed the ability to respond to the two different moieties of thiamine, thiazole and pyrimidine. MacLeod et al., (1954) reported that thiamine was required by two bacteria out of five vitamin requiring species isolated from sea water and from the surface and gut of fish and clams.

Fungi. A widespread requirement for thiamine also is apparent in marine fungi. Vishniac (1961) reported that thiamine is required by 39 non-filamentous marine Phycomycetes. Gustafsson and Fries (1956) examined ten species of marine fungi and found that five required thiamine. Sguros et al. (1962) and Sguros and Simms (1963) recorded the thiamine requirement of a deuteromycetous fungus and of an ascomycetous fungus (Appendix A, Table 4). Vitamin requirements are found to be characteristic of yeasts in general. Ahearn and Roth (1962) examined the vitamin requirements of 285 marine yeasts and found that 164 of them were auxotrophic to thiamine (Appendix A, Table 5).

Thiamine concentrations in the sea

Sea water. The only investigation dealing with the presence of thiamine in sea water is that of Vishniac and Riley (1961) who analyzed water samples from Long Island Sound and found barely detectable amounts of thiamine in the range of 0 to 65 ng/liter.

Sediments. Burkholder (1959a) reported thiamine activity in enriched marine sediments collected from City Island and Flushing Bay, New York, in the range of 0.06 to 0.16 µg/ml. Burkholder and Burkholder (1958) also examined sediment samples collected from Bahia Fosforescente and found that thiamine activity varied between 10 and 195 ng/gm of dry matter. In a core sample examined from the same region, thiamine activity was found to vary from 63 ng/gm dry matter at the surface to 0 at the bottom of the core, 83.3 cm.

From the above review of the literature on the requirements and suggested sources of thiamine in the marine environment, some preliminary conclusions can be drawn. Many marine micro-organisms including algae, fungi, yeasts, and bacteria require thiamine for growth. There are indications that sufficient thiamine is present in sea water for the growth of marine micro-organisms, although some of these organisms may be independent of any vitamin requirements. There are also indications that some organisms have the capacity of excreting thiamine into the surrounding medium during their growth phase or liberating thiamine by death and decay. It is surprising that there is a complete lack of information on the distribution of thiamine in sea water except for the work of Vishniac and Riley (1961), although the possibility exists that measurable quantities of thiamine may be present.

The purpose of this investigation is to examine the distribution patterns of thiamine in sea water from coastal and oceanic regions and to correlate the presence or absence of thiamine with primary

productivity as far as possible. Major emphasis has been placed on the surface and vertical distribution of thiamine in several areas with special emphasis on seasonal cycles. Attempts have also been made to investigate the uptake of thiamine by the use of radioactive thiamine.

MATERIALS AND METHODS

Bioassay organism

Bioassay techniques are comparatively new to oceanography and as such, this tool has not been much used. The major difficulty in the bioassay of thiamine in sea water has been the lack of a proper assay organism which can be grown easily in a sea water medium, although attempts have been made to use bacteria (Burkholder, 1959), a non-filamentous fungus (Vishniac, 1961) and a dinoflagellate (Provasoli and Gold, 1959, 1962). Some of the factors involved in the bioassay of thiamine in natural sea water are the presence of very low amounts of the substance, sensitivity of the method and the specificity of the organisms concerned. Except for the fungus used by Vishniac (1961), the other organisms have one or more drawbacks preventing their use in a large scale bioassay method (Appendix A, Table 6).

For the purpose of this study, a new bioassay technique was developed using a marine yeast, Cryptococcus albidus (Saito) Skinner. The main advantages of this organism were found to be its simple nutrient requirements, good growth rates, high sensitivity of the assay and stability of the strain.

Isolation and culture

The yeast, Cryptococcus albidus (Saito) Skinner, used in this

investigation was isolated from the Oregon coast during Cruise 6307-C of the R/V ACONA (Oregon State University) in July, 1963. The yeast was isolated from sea water 25 miles off the coast from a depth of 500 m using a newly devised bacteriological sampler previously tested on Cruise 2 of the R/V ATLANTIS II (Woods Hole Oceanographic Institution). The sampler consisted of a disposable sterile plastic syringe mounted in a metal frame with a trigger mechanism to operate the plunger in the syringe. The sampler is sent down on the wire with the nozzle of the syringe pointing upward and the plunger in the raised position. At sampling depth the messenger weight releases the trigger mechanism and the plunger is pulled down by rubber bands with the resultant sampling of a measured quantity of water in the syringe. As soon as the syringe with the water sample is on board, the mouth of the syringe is closed with a sterile cap and the sample processed immediately thereafter.

Two types of plating techniques were tested, the conventional spreading technique using 100 x 15 mm disposable petri dishes wherein a small quantity of sea water sample was spread with a bent glass rod, and a new filter paper technique using Millipore plastic disposable petri dishes 48 x 8.5 mm deep (outside 54 x 12 mm high) with wedge-fit covers to prevent leakage and accidental opening even under rough unfavourable ship operations. Due to the low quantities of yeasts usually found in marine surroundings, it was necessary to filter large volumes of sea water for isolating procedures. This was accomplished by using a Millipore Swinny hypodermic

adapter which can be fitted to the plastic syringe of the new sampler as soon as it is pulled out of the water. This Swinny adapter can be easily attached to the Luer fittings of the sampler syringe and also can be easily sterilized by immersing in alcohol. The stainless steel body, the photoetched support screen for 13 mm Millipore filters and the teflon gaskets were tested previously for toxic action and found suitable. The Swinny syringe filter set-up with 13 mm Millipore HA gridded paper was found to be very convenient and safe to handle in a moving ship with the added advantage of the grids in the paper in enumerating the number of colonies.

Soon after the end of filtration the filter paper was removed and placed on top of the agar in the petri plate. The adapters, needles and forceps were immersed for four to six hours or more in 95% ethyl alcohol and flame sterilized before re-use. This method involving the plastic syringe sampler and Swinny adapter make the operation á single step procedure with reduced danger of contamination. The conventional plating technique with large 100 mm petri dishes was found to be inconvenient mainly due to the high percentage of contamination and breakage.

A synthetic sea water medium of the following composition was used to isolate the yeast flora (Table 1). This medium was found to be selective for marine yeasts during previous investigations.

The petri plates were incubated at room temperature for five to seven days and the yeast colonies counted. Further isolation

Table 1. - Synthetic sea water medium.

NaCl	23.272 g
$MgSO_4 \cdot 7H_2O$	9.823 g
KCl	0.595 g
$CaCl_2 \cdot 2H_2O$	1.172 g
K_2HPO_4	0.139 g
$(NH_4)_2SO_4$	0.472 g
Glucose	10.000 g
Trace metals[a]	1 ml
Fe-EDTA[b]	1 ml
Vitamin mixture[c]	1 ml
Glass distilled water	1 liter

[a]Trace metals stock solution: $Na_2MoO_4 \cdot 2H_2O$, 25.2 mg; H_3BO_3, 114 mg; $MnSO_4 \cdot H_2O$, 12.3 mg; $ZnSO_4 \cdot 7H_2O$, 2.2 mg; $CoSO_4 \cdot 7H_2O$, 0.48 mg; Na_2EDTA, 100 mg; glass distilled water, 100 ml.

[b]Fe-EDTA stock solution: Dissolve 10.4 g of KOH in 186 ml of glass distilled water and add 16 g of EDTA. This solution then is mixed with an iron solution made by dissolving 13.7 g of $FeSO_4 \cdot 7H_2O$ in 364 ml of water. Air is bubbled overnight through this mixture to oxidize the iron to the ferric form. One ml of this final solution contains 5 mg of Fe and 13 mg of K.

[c]Vitamin mixture: Thiamine, 20 mg; niacin, 10 mg; pantothenic acid, 10 mg; riboflavin, 500 µg; pyridoxine HCl, 4 mg; para-aminobenzoic acid, 1 mg; biotin, 50 µg; choline dihydrogen citrate, 50 mg; inositol, 100 mg; orotic acid, 26 mg; vitamin B_{12}, 5 µg; folic acid, 250 µg; glass distilled water, 100 ml.

was carried out by removing single colonies and transfering them first to freshly prepared agar petri plates and then to agar slants of the same chemical composition.

Identification

The identification of the yeast was done using the conventional techniques used by zymologists with slight modifications using sea water media (Wickerham, 1951; Lodder and Kreger-van Rij, 1952).

Physiology and vitamin requirements of *Cryptococcus albidus*

Experiments were conducted on the nutritional requirements of the yeast to define best conditions for growth. All experiments were conducted with reference to the basal synthetic sea water medium described above. Different carbon and nitrogen sources were tested for best growth.

Vitamin requirements of the marine yeast were investigated in the synthetic sea water medium. A series of 11 vitamins were tested, both singly and in different combinations, with the inclusion of duplicate controls containing vitamin-free medium and a medium containing the full complement of the vitamins, to ascertain vitamin requirements, vitamin independence and vitamin interactions.

Thiamine requirement of *Cryptococcus albidus*

The yeast was found to require only thiamine in the synthetic

sea water medium. The two different moieties of thiamine, namely the thiazole moiety and the pyrimidine moiety, were tested for partial requirements in the synthetic sea water medium.

Chemicals

Most of the chemicals used during this investigation were reagent grade obtained from the following places: Difco Laboratories, Fisher Scientific, Nutritional Biochemicals Corporation and California Corporation for Biochemical Research. Thiazole was obtained from Merk & Company and Hoffmann-La Roche Inc.

Pretreatment of glassware

Glassware used in all experiments was soaked in hot Haemo-Sol detergent solution for 24 hr, washed in a Heinicke washing machine, distilled water rinsed many times, then filled with distilled water and autoclaved for 15 min at 15 psi, emptied and dried in a hot air oven.

Collection of samples

Surface sea water samples were collected with a four gallon plastic bucket from the side of the ship where there is least pollution of oil or other debris from the ship. The bucket was first rinsed with a sample of the sea water and a further sample was collected. An aliquot of the sea water was immediately filtered through a Millipore filter assembly using Hurlbut 984H glass filters.

The filtered sample was transfered to a plastic bottle and stored frozen. The filtration unit was always washed prior to filtering the final sample by sucking through and discarding a quantity of the same water. Whenever necessary a small quantity of unfiltered water sample also was transfered directly from the plastic bucket and frozen in a plastic bottle. Samples from vertical stations were collected with Nansen bottles and processed in the same manner. The plastic bottles used were of 125 ml capacity and were previously washed, rinsed in distilled water and then rinsed in acid. Samples were kept frozen until the time of assay.

Thiamine stock solutions

Thiamine stock solutions were made in glass distilled water. A stock solution of 10 ng/ml was initially made in bulk and aliquots of ca. 2 ml were transfered to glass ampules, sealed and stored frozen. The ampules were thoroughly cleaned before being used. This way of keeping the thiamine stocks was found to be very convenient and safe and avoided repeated freezing and thawing of the stock solutions. The thiamine stocks were renewed every three months.

Preparation of thiamine-free water

The bioassay standards were usually prepared in aged natural sea water. Sea water stored in plastic carboys for 6 to 12 months at room temperature was made thiamine-free by a slight modification of the method used by Ryther and Guillard (1962). Ten grams of Norit-A decolorizing carbon was pretreated by shaking for ten minutes with

500 ml of a 5% w/v solution of reagent grade sodium chloride in distilled water. The carbon was recovered on a Whatman No. 1 filter paper by using a Buchner funnel and was again treated with another 500 ml of sodium chloride solution. The process was repeated once again. Finally the treated carbon was shaken for ten minutes with 1 liter of distilled water. The carbon was finally recovered on a Whatman No. 1 paper and transfered to a clean beaker containing 1 liter of sea water to be treated. After shaking for one hour the treated water was finally filtered twice to remove all the carbon using HA Millipore filters. This method was found to be very effective in removing thiamine from sea water.

Preparation of samples

The frozen sea water samples were brought back to the laboratory and then assayed. The samples were thawed and 40 ml portions were enriched by the addition of the following stock solutions:

Ingredients	Stock solution	Added to 40 ml
Glucose		0.4 g
$(NH_4)_2SO_4$	472 mg/5 ml of distilled water	0.2 ml
Trace metals	Diluted original stock 10 times	0.4 ml
Fe-EDTA	Diluted original stock 10 times	0.4 ml

Ingredients	Stock solution	Added to 40 ml
K_2HPO_4*	139 mg/10 ml distilled water	0.4 ml

*Added after autoclaving, 0.1 ml/tube of 10 ml sample.

In making up the enrichment the addition of distilled water stock solutions reduced the salinity slightly but this was not taken into consideration.

After enrichment the samples were transfered to Bellco culture tubes (18 x 150 mm) with Morton stainless steel culture tube closures, 9.8 ml to two tubes and 9.7 ml to another two tubes, the first pair of tubes acting as sample assays and the second pair as internal standards. The internal standards were given 0.1 ml of thiamine stock solution (10 ng/ml) to give a final concentration of 0.1 ng/ml of thiamine. The internal standards were always included with each set of samples to check for inhibitors in the sea water as well as for checking the assay response. All tubes were autoclaved for 15 min at 15 psi. After autoclaving the tubes were cooled to room temperature, 0.1 ml of sterile phosphate stock solution was added and finally they were inoculated with 0.1 ml of a specially prepared inoculum.

Bioassay standards

Thiamine-free aged sea water was used in making standards. The sea water was enriched and standards with the following thiamine concentrations were made: 0, 0.025, 0.05, 0.1, 0.2, 0.3 ng/ml of

thiamine. All these standards were made in duplicate using the following procedure (Table 2). Two tubes of enriched sea water without thiamine were included in each batch of assay samples to act as sterility controls. These tubes were not given the usual amount of yeast inoculum.

Preparation of the inoculum

The stock cultures were maintained at room temperature in thiamine enriched basal medium and transfered every week to new slants. During the preparation of the inoculum a small quantity of the material growing on the slants was transfered to a culture tube containing 10 ml of sterile basal medium to which no thiamine has been added. This is shaken well and incubated for 24 hr. After 24 hr starvation in a thiamine deficient medium, two drops of this material again was transfered to another tube containing thiamine deficient medium, starved for another 24 hr; this was used as inoculum for the experiments. A third transfer rarely grew when tried. It has also been found that the first transfer could be used as an inoculum provided it was starved for 48 hr in the same tube.

During the experiment the general procedure followed was to equilibrate the assay samples to room temperature after autoclaving and then the tubes were given 0.1 ml of sterile phosphate stock solution and 0.1 ml of the inoculum. The growth in the thiamine deficient medium usually was very thin. After the inoculation procedure, which was carried out in a sterile transfer room, the

Table 2. - Procedure for the preparation of bioassay standards.

	ng/ml Thiamine					
Standards	0	0.025	0.05	0.1	0.2	0.3
	ml/tube					
Sea water, enriched	9.80	9.55	9.30	8.80	7.80	6.80
Substandard*	0.00	0.25	0.50	1.00	2.00	3.00
K_2HPO_4**	0.10	0.10	0.10	0.10	0.10	0.10
Inoculum**	0.10	0.10	0.10	0.10	0.10	0.10
Total volume	10.0	10.0	10.0	10.0	10.0	10.0

*Substandard prepared as follows: For making one set of duplicate standards, 2 ml of a thiamine stock solution (10 ng/ml made in distilled water and frozen) was added to 18 ml of enriched natural sea water to give 1 ng/ml of thiamine and this was used to make the actual standards in sea water. This was especially convenient in reducing the amount of pipetting errors and avoiding further reduction of salinity.

**Added after autoclaving.

samples were shaken and incubated.

Incubation

The assay samples and standards were mounted on wooden blocks and placed in a specially constructed rack in a New Brunswick Gyrotory shaker (Model G-10). The samples were shaken for about 110 hr. All the experiments were carried out in a constant temperature room maintained at ca. 22°C.

Calculation of results

At the end of the incubation period the tubes were taken out of the shaker, completely mixed again with a tube-buzzer and allowed to stand for about 30 sec. The samples then were read in a Beckman model DU spectrophotometer. The optical density was measured at 450 mμ using 1 cm corex cells. The instrument was zero-adjusted with uninoculated natural sea water. The optical density values for duplicate tubes were averaged in all treatments. The standards were read at the same time and the mean optical density values plotted against concentration of thiamine to make a standard curve. Thiamine concentration in the samples analyzed was read from the standard curve.

Uptake studies with ^{14}C-labeled thiamine

The radioactive thiamine (thiazole-2-^{14}C) was obtained from Nuclear-Chicago Corporation. The procedure followed for the uptake of radioactive thiamine studies is outlined below. Water samples

were collected from different depths in the euphotic zone with the use of a non-toxic sampler. The water samples were transfered to either 1 liter or 125 ml Pyrex bottles after rinsing the bottles with water from the same depths. The incubation bottles, one light and one dark, were given 0.1 to 1.0 μc of labeled thiamine, shaken well and incubated in a water bath cooled by running sea water and with flourescent light tubes for 12 to 24 hr. After the end of the incubation the samples were taken from the incubator, shaken and filtered using HA Millipore filters of 47 mm diameter. In the case of ^{14}C-Thiamine, the filtration was carried out using two HA Millipore filters placed one below the other. The activity of the second filter was always subtracted from the first filter to account for filter adsorption noticed in the case of the labeled thiamine. The filters were dried in a dessicator and the activity was counted. Two different radioisotope counters were used: a Tracerlab automatic Omni/Guard system with gas flow detector and a Tracerlab "1000" scaler with a GM tube detector. Wherever possible standard ^{14}C-primary productivity measurements also were conducted using the same methodology.

Particulate nitrogen analysis

Samples of sea water were collected from surface to 50 m with the use of a Van Dorn or other non-toxic sampler. Two liters of the samples were filtered using a Millipore glass filter holder with Hurlbut 984H glass filter papers. After filtration the filter papers were stored in a dessicator and analyzed in the laboratory.

The particulate analyses for nitrogen were done in a Coleman automatic nitrogen analyzer using the micro-Dumas method.

RESULTS

Standardization of the bioassay

Preliminary investigations on the nutrition of the marine yeast Cryptococcus albidus showed that it has simple nutrient requirements. The effects of different carbon and nitrogen sources on the growth of yeast were tested in the synthetic sea water medium and the results are shown in Table 3. Glucose was found to be a convenient and readily used carbon source. Nitrogen assimilation studies carried out with four different nitrogen sources, potassium nitrate, ammonium nitrate, ammonium sulfate and urea showed that ammonium sulfate and urea were better sources of nitrogen. It was decided to use ammonium sulfate as the basic source of nitrogen. Concentrations of 0.1, 1.0, 10, and 100 µg/ml of ammonium sulfate were tested; 100 µg/ml of nitrogen given as ammonium sulfate gave optimum growth. For all future experiments glucose at 10 g/liter and ammonium sulfate at 472 mg/liter were given as standard carbon and nitrogen sources.

Effect of temperature on the growth of the yeast was not investigated except at 22°C and 32°C. The growth was markedly reduced at 32°C when compared with the growth at 22°C. The temperature therefore was kept at ca. 22°C in all future experiments.

Vitamin requirement studies conducted under the above condition in a synthetic sea water medium showed that thiamine was the only

Table 3. - Utilization of carbon and nitrogen sources by *Cryptococcus albidus*.

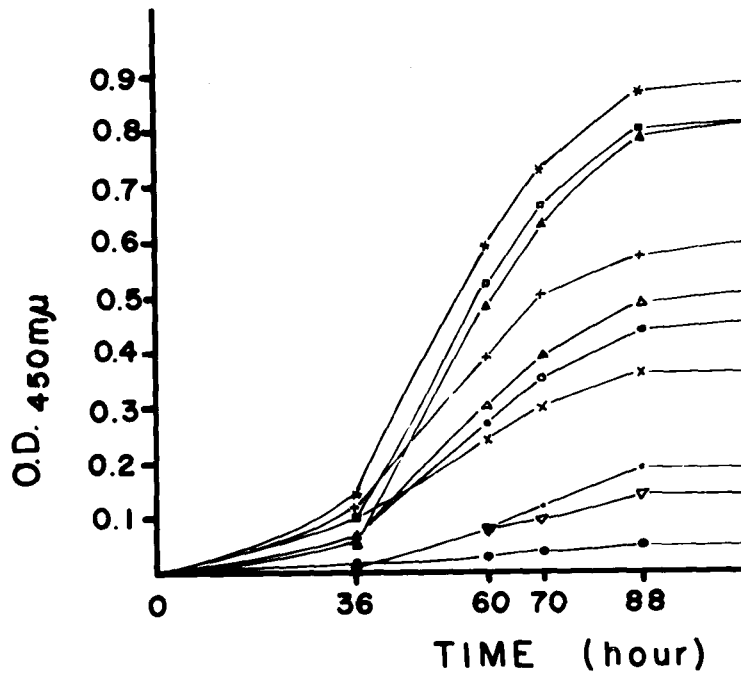
Source	Growth*
Glycerol	-
Pentoses	
l-arabinose	++
d-xylose	++
d-ribose	-
Hexoses	
Glucose	++
Galactose	+
d-mannose	++
Fructose	++
Rhamnose	++
Disaccharides	
Trehalose	+
Melibiose	-
Cellobiose	++
Lactose	-
Maltose	++
Sucrose	++
Trisaccharides	
Melizitose	++
Raffinose	+
Polysaccharide	
Starch	-
Nitrogen sources	
KNO_3	+
NH_4NO_3	+
$(NH_4)_2SO_4$	++
Urea	++

* - Negative
\+ Fair
++ Good

vitamin required by the yeast. The growth curves obtained under different thiamine concentrations are shown in Fig. 1. The results showed clearly that growth of the organism was markedly influenced by up to 1 ng/ml of thiamine. The time course of growth showed that growth was almost completed in about five days. The lag phase extended up to 36 hr and the exponential phase was completed at about 88 hr. The partial requirements for the different moieties then were investigated and the results are shown in Table 4. In the synthetic sea water medium the yeast was found to use the thiazole moiety and not the pyrimidine moiety. The thiamine requirement of the yeast thus can be satisfied by the intact molecule or can be replaced by the thiazole moiety.

Experiments were conducted in order to grow the yeast in natural sea water. Sea water collected off Homer, Alaska, was used during these experiments. Trial enrichment cultures showed that glucose can be used as the carbon source and ammonium sulfate as the nitrogen source. The sea water, however, was found to lack some other nutrients, possibly phosphate, trace metals or iron. It was found that for the best growth of the yeast the natural sea water has to be enriched with the above nutrients in addition to glucose and ammonium sulfate. The results of the enrichment experiment are shown in Table 5. Although it was found that trace metals were not required in the Homer water, it was suspected that their limitation is possible in sea water. The addition of trace metals in the enrichment was not inhibitory to the growth of the organism.

Figure 1. - Growth curves in synthetic sea water medium with different concentrations of thiamine.


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
O.D. 450mμ
0
36
60
70
88
TIME (hour)

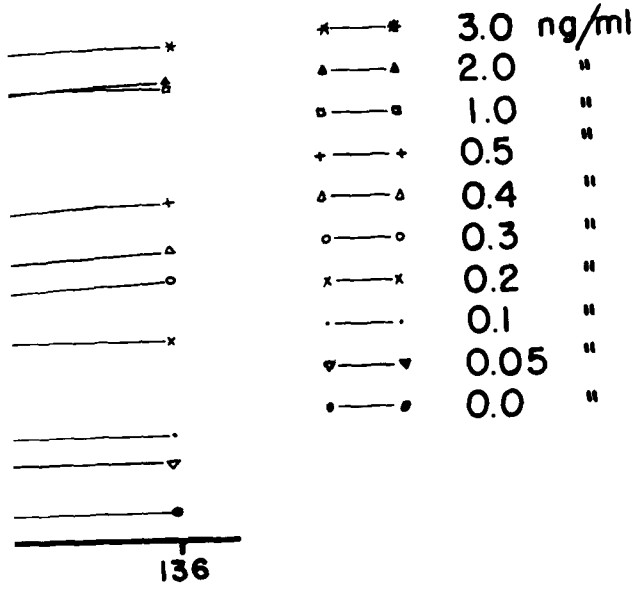

3.0 ng/ml
2.0 "
1.0 "
0.5 "
0.4 "
0.3 "
0.2 "
0.1 "
0.05 "
0.0 "
136

Table 4. - Uptake of thiamine moieties by Cryptococcus albidus.

Treatment	Mean OD_{450}	Dose
Thiazole	1.050	1 µg/ml
Pyrimidine HCl	0.040	1 µg/ml
Thiazole + Pyrimidine HCl	1.000	1 µg of each/ml
Thiamine	0.640	0.3 ng/ml
Control - no thiamine	0.050	none

4-methyl-5-β-hydroxyethyl thiazole, obtained from Merk & Co.; 2-methyl-4-amino-5-aminomethyl pyrimidine di hydrochloride, obtained from Nutritional Biochemicals Corporation. Incubation 121 hrs. All treatments in duplicate.

Table 5. - Effect of enrichments in natural sea water.

Treatment	Mean OD_{450}
Non-enriched	0.000
Glucose, $(NH_4)_2SO_4$	0.065
Glucose, $(NH_4)_2SO_4$, K_2HPO_4	0.062
Glucose, $(NH_4)_2SO_4$, K_2HPO_4, Fe-EDTA	0.475
Glucose, $(NH_4)_2SO_4$, K_2HPO_4, Trace metals	0.083
Glucose, $(NH_4)_2SO_4$, K_2HPO_4, Trace metals, Fe-EDTA	0.441
Glucose, $(NH_4)_2SO_4$, Trace metals	0.091
Glucose, $(NH_4)_2SO_4$, Fe-EDTA	0.383

Sea water collected off Homer, Alaska. Enrichments were made by the addition of the following: Glucose, 10 g/liter; $(NH_4)_2SO_4$, 472 mg/liter; K_2HPO_4, 139 mg/liter added after autoclaving; Fe-EDTA, 1 ml of stock solution/liter; Trace metals, 1 ml of stock solution/liter; Thiamine, 0.2 ng/liter. Incubation 111 hrs. All treatments in duplicate.

The effect of salinity on growth was investigated in the synthetic sea water medium. The results are shown in Table 6. It was found that the growth response of the yeast was significantly affected above 44°/$_{oo}$ and below 25°/$_{oo}$. Between 25°/$_{oo}$ and 44°/$_{oo}$ the growth response of the organism to added thiamine at two concentrations was similar. It is evident from the results that bioassay under the conditions of the experiment may not be applicable to sea water below 25°/$_{oo}$ and above 44°/$_{oo}$ without using internal standards. The standards always were made in natural sea water of 30°/$_{oo}$ to 32°/$_{oo}$ salinity, which is nearly equal to the salinity of the samples analyzed during this investigation.

To minimize carry-over of thiamine the yeast cells had to be starved in a thiamine-free medium. The procedure followed to reduce carry-over of thiamine consisted of transfering the cells to a thiamine-free medium every 24 hr. The results of starvation of inoculum on the dose response are shown in Table 7. The results show that to reduce carry-over 48 hr starvation in a thiamine-free medium is sufficient. The cells starved for 17 days were also found to give a good response. There was not much difference between cells starved for 48 hr in the first or second transfer. The growth was very poor in the cells transfered the third time into a thiamine-free medium. The inoculum starved for 48 hr in the same tube of thiamine-free medium was used in all bioassay procedures.

The stock cultures of the yeasts were maintained in a synthetic sea water medium solidified with 2% agar and fortified with 5 ng/ml

Table 6. - Effect of salinity on thiamine bioassay.

Salinity (‰)	0.4 ng/ml thiamine OD_{450}	0.2 ng/ml thiamine OD_{450}
5	0.120	0.100
10	0.175	0.160
20	0.275	0.220
25	0.320	0.245
30	0.335	0.245
35	0.360	0.270
44	0.340	0.250
60	0.305	0.235
88	0.265	0.220

Synthetic sea water medium used to make up the different salinities.

Incubation 116 hrs. All treatments in duplicate.

Table 7. - Effect of inoculum starvation on response to added thiamine.

	Days of starvation					
	2	5	6	8	11	17
Transfer no.	Mean OD_{450}					
1	0.345	0.360	0.330	0.335	0.365	0.365
2	0.315	0.335	0.330	0.330	0.320	0.375
3				0.335	0.315	0.335

Synthetic sea water medium used with 0.2 ng/ml thiamine. Inocula starved in the synthetic sea water medium without added thiamine. Incubation 112 hrs. All treatments in duplicate.

of thiamine. Experiments made to test whether the amount of thiamine given to the slants limits the sensitivity of the assay showed that the amount of thiamine in the parent slants did not influence the future assay response of the organism (Table 8). The results showed that yeasts maintained in slants with 1 ng/ml and 5 ng/ml of thiamine gave indistinguishable results.

The effects of autoclaving on the destruction of thiamine were investigated. It was found that the duration of autoclaving can be extended up to 25 min at 15 psi without much loss of activity in natural sea water (Table 9). In natural sea water enriched with 0.1 ng/ml of thiamine there was no loss of activity whether autoclaving is done for 5 or 15 min (Table 10). Although autoclaving natural sea water with added thiamine did not destroy the thiamine activity, thiamine stock solutions made in distilled water and autoclaved separately were found to lose much of their activity. The same type of destruction was also noticed if stock solutions made in 0.01N HCl were autoclaved separately (Table 11), although here the loss of activity was smaller. The apparent stability of thiamine in a salt solution as indicated by its reaction to heat and autoclaving may have ecological importance. The effect may not be due entirely to pH since the pH of the distilled water stock solution as well as of the synthetic sea water medium used was around 6.5.

The effect of charcoal treatment on the removal of thiamine from natural sea water is shown in Table 12. It can be seen that

Table 8. - Effect of inoculum made from different slants.

Thiamine in assay medium (ng/ml)	Inoculum from 1 ng/ml slant	Inoculum from 5 ng/ml slant
	Mean OD_{450}	Mean OD_{450}
0	0.088	0.093
0.2	0.560	0.570
0.4	0.900	0.920

Synthetic sea water medium used. Incubation 112 hrs. All treatments in duplicate.

Table 9. - Effect of autoclaving on destruction of thiamine.

Duration of autoclaving (minutes)	Mean OD_{450}
5	0.164
10	0.165
15	0.167
20	0.166
25	0.172

Sea water collected off Kodiak Island to which has been added 0.1 ng/ml thiamine. All treatments in duplicate.

Table 10. - Effect of autoclaving on destruction of thiamine.

Sample no.	Duration of autoclaving (minutes)	Thiamine in sample (ng/ml)	Thiamine in internal standard with 0.1 ng/ml added thiamine. (ng/ml)
1	5	0.049	0.141
1	15	0.054	0.150
2	5	0.118	0.210
2	15	0.122	0.223

Sea water collected from Gulf of Alaska. All treatments in duplicate.

Table 11. - Effect of autoclaving on destruction of thiamine.

Treatment	Recovery of thiamine (%)
DW thiamine added to SSW medium and autoclaved	100
DW thiamine autoclaved separately and added to SSW	23
HCl thiamine added to SSW medium and autoclaved	100
HCl thiamine autoclaved separately and added to SSW	64

DW thiamine prepared in glass distilled water. HCl thiamine prepared in 0.01 N HCl. SSW medium is synthetic sea water medium. Thiamine added in all experiments, 0.1 ng/ml. Autoclaving for 15 min at 15 psi. pH of distilled water 6.0 and 6.55 before and after autoclaving respectively. pH of HCl thiamine 1.95 and 1.95 before and after autoclaving respectively. pH of synthetic sea water medium 6.82 and 6.15 before and after autoclaving respectively. All treatments in duplicate.

Table 12. - Effect of charcoal treatment on the removal of thiamine from natural sea water.

Thiamine in assay medium (ng/ml)	Non-treated Mean OD_{450}	Treated Mean OD_{450}
0	0.513	0.031
0.1	0.623	0.139
0.2	0.735	0.258

The natural sea water collected off Kodiak Island. Incubation 120 hrs.

All treatments in duplicate.

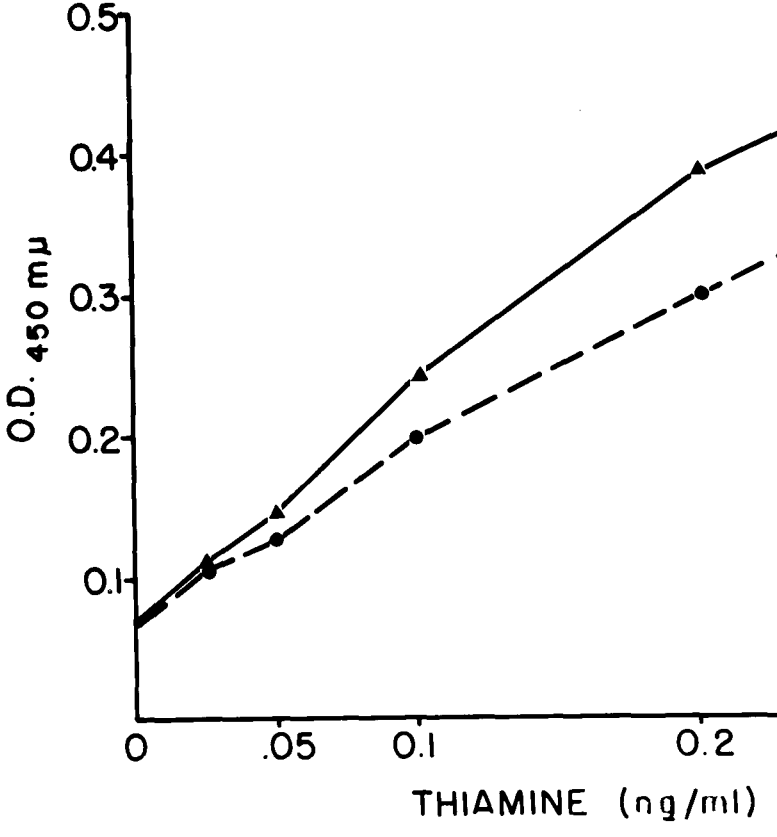
thiamine was removed completely by the charcoal treatment employed. The charcoal treatment also removed thiamine added to the natural sea water (Fig. 2). All the bioassay standards are, therefore, made with charcoal-treated, aged sea water.

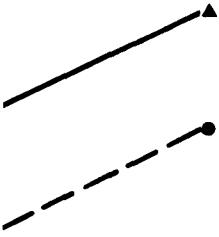
The standard curve

Typical standard curves are shown in Fig. 2 and 3. The yeast employed in this investigation gave a growth response in the dose range of 0 to 500 pg/ml. The linear portion of the curve generally was between 10 and 300 pg/ml. Above and below this range the dose response curve showed departures from linearity. Standard curves obtained from time to time showed variability which is unavoidable in biological assays. Variability of standard curves also has been noticed by many authors (Antia, 1963; Vishniac, 1961; Ryther and Guillard, 1962). The difficulty due to the variability of standard curves has been avoided by running a standard curve with each batch of sea water samples assayed. The standards run with each batch of samples were in the range of 0-300 pg/ml and in the case of samples falling above this range they have been diluted with thiamine-free natural sea water and assayed again.

The statistical analysis of three samples to test the variability of the results showed that the coefficient of variation falls in the range of 6 to 12% which is normal in bioassay methods (Table 13). The standard error of estimate of one standard curve is plotted in Fig. 3 to provide an indication

Figure 2. - Typical standard curves in charcoal-treated natural sea water.


0.5
0.4
0.3
0.2
0.1
O.D. 450 mμ
0
.05
0.1
0.2
THIAMINE (ng/ml)



—— SEA WATER PLUS THIAMINE (10ng/ml) CHARCOAL TREATED

—— SEA WATER CHARCOAL TREATED

0.3

Figure 3. - A typical standard curve with the estimated regression line and the standard error of estimate.

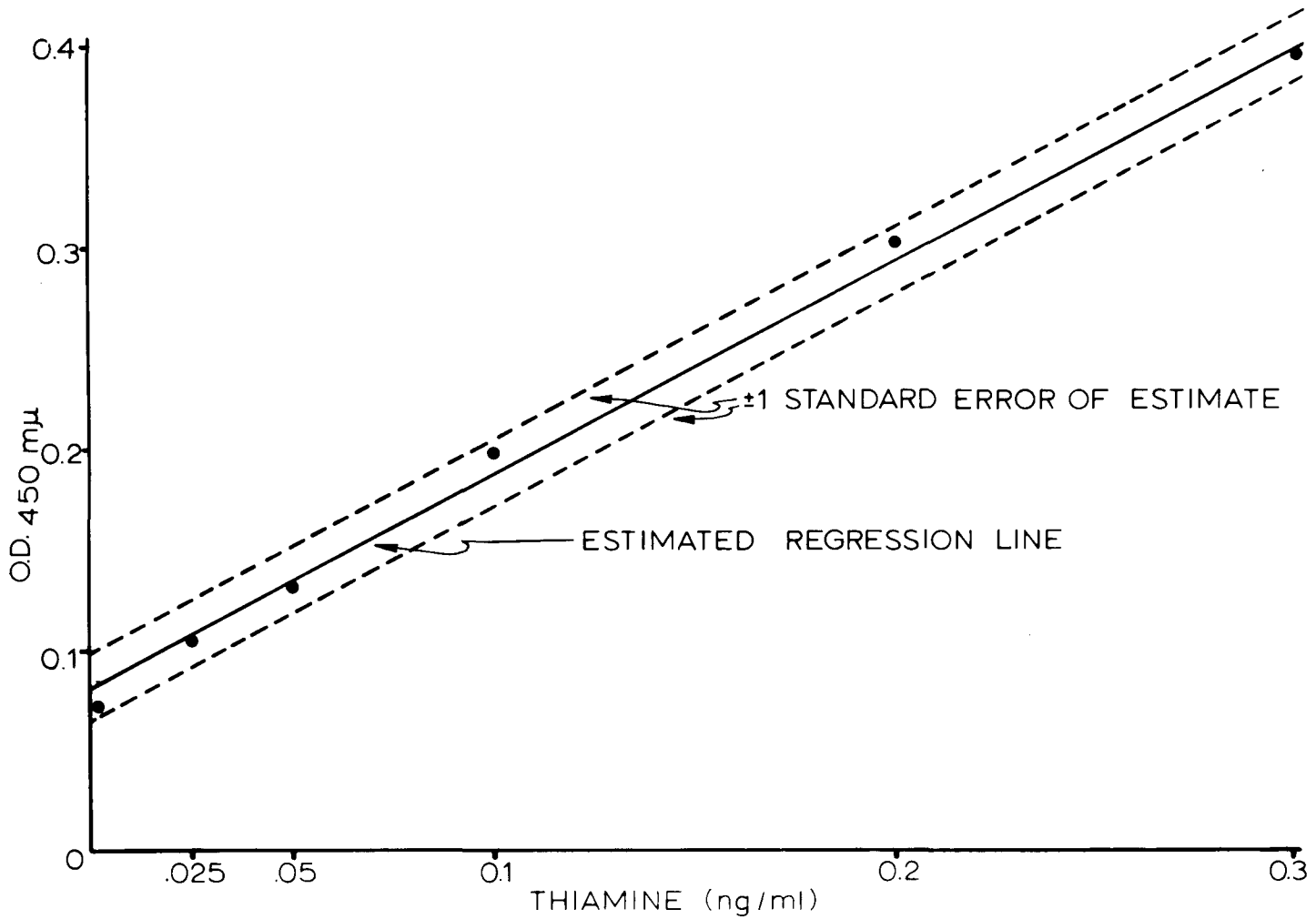

0.4
0.3
0.2
0.1
0
O.D. 450 mμ
±1 STANDARD ERROR OF ESTIMATE
ESTIMATED REGRESSION LINE
.025
.05
0.1
0.2
0.3
THIAMINE (ng/ml)

Table 13. - Statistical analyses of results of 3 samples.

R/V ACONA Station no. (Cruise 7)	Depth (m)	Determinations	Thiamine (pg/ml)
117	0	1st	16.1
		2nd	14.2
		3rd	14.2
117	10	1st	9.0
		2nd	8.5
		3rd	9.6
117	30	1st	17.9
		2nd	14.2
		3rd	15.3

Mean	Standard deviation	Coefficient of variation (%)
14.83	1.1	7.4
9.03	0.55	6.1
15.8	1.9	12.0

of the general reliability of estimates.

All the results reported here have been calculated with reference to the external standards included with each batch of assay samples. The internal standards included with each sample analyzed showed satisfactory recovery. Typical results are shown in Table 14. Departures from 100% recovery may be due to the salinity differences between samples analyzed and that of the external standard used to calculate the results. Similar effects also have been noticed by Ryther and Guillard (1962) and Daisley (1958). The reduced recovery of the internal standards also may indicate the presence of inhibitors to the yeast. In some deep water samples the growth of the yeast was slightly less than that in the thiamine-free sea water. The reason for this phenomenon may be the presence of inhibitors or such deep water samples actually may be more thiamine-free than the best thiamine-free water prepared in the laboratory.

Distribution of thiamine in the sea

The results of analyses of sea water samples are given in this section. Table 15 shows the cruises taken during 1963, 1964 and 1965 with the number of stations sampled. Maps 1 - 5 show the stations and the surface concentrations of thiamine during the months indicated. Appendix B, Tables 1 - 13 show the results. For convenience the results are discussed separately for each area. The results of individual experiments conducted in those areas also are considered.

Table 14. - Variations in thiamine concentrations calculated from internal and external standards.

Sample no.	Depth (m)	Thiamine calculated from external standard (ng/liter)	Thiamine calculated from internal standard (ng/liter)	Recovery (%)
1	0	250.7	248.8	99.2
2	10	124.2	176.7	142.2
3	20	131.2	124.4	94.8
4	30	72.6	43.7	60.2
5	50	56.2	55.8	99.3
6	75	44.5	47.5	106.7

Samples analyzed from Station 8 of Cruise 1 of R/V ACONA. Internal standards given 0.1 ng/ml thiamine.

Table 15. - Cruise tracks of stations sampled in 1963, 1964 and 1965.

Vessel	Cruise no.	Location	Date	No. of stations	Map no.
USC&GSS HODGSON	1	Juneau-Cordova	4/29/64 - 5/02/64	45	1,3
USC&GSS HODGSON	2	Prince William Sound	7/20/64 - 7/24/64 8/08/64 - 8/14/64	32	2
USC&GSS HODGSON	3	Montague Is.-Middleton Is.	7/29/64	10	1
USC&GSS HODGSON	4	Cordova-Juneau	8/31/64 - 9/02/64	48	1,3
USC&GSS SURVEYOR	1	Valdez-Kodiak	5/05/64 - 5/08/64	17	1,2
R/V AGASSIZ	1	San Diego-Kodiak	8/07/64 - 8/19/64	15	1,4
R/V ANTON BRUUN	4A	Arabian Sea	10/12/63 -10/24/63 10/28/63 -11/08/63	20	5
R/V ACONA	1	Seattle-Juneau	9/22/64 -10/08/64	53	3,4
R/V ACONA	3	Juneau-Cape Spencer	11/10/64 -11/19/64	17	3
R/V ACONA	7	Vancouver-Hawaii	1/18/65 - 2/03/65	36	4
R/V ACONA	7	Port Angeles-Juneau	3/18/65 - 3/22/65	12	3

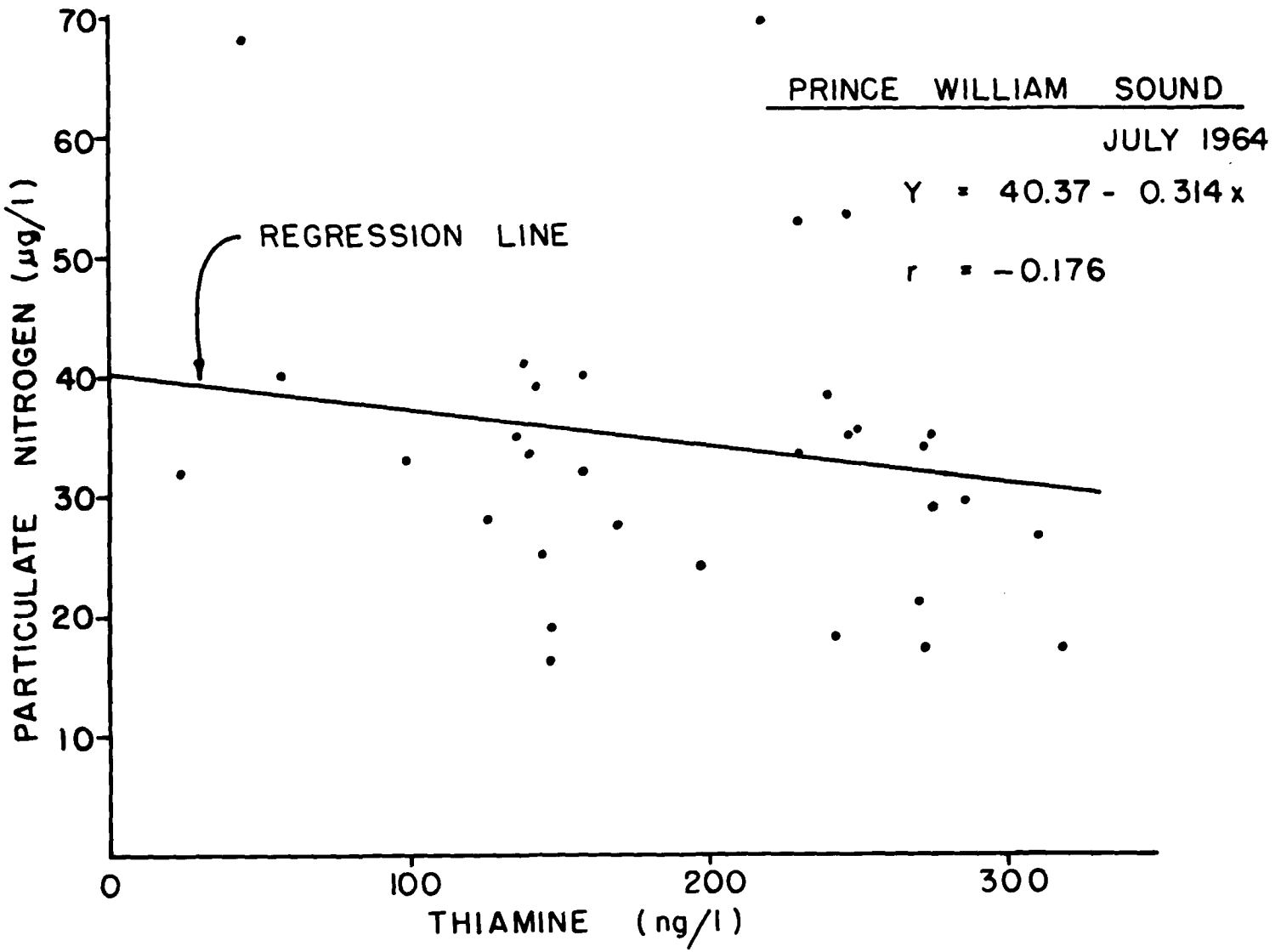
Prince William Sound. - In July, 1964, 32 samples were obtained for the analysis of thiamine. The surface concentrations are shown in Map 2 and in Appendix B, Table 1. The range of concentration was 144 to 310 ng/liter with a mean of 184 ng/liter. The near uniform pattern of the distribution may be due to the enclosed nature of the Sound, most of the sampling areas being near the small clustered islands. In the region near Valdez thiamine was undetectable. The concentration was found to decrease steadily as Valdez Arm was approached with an increasing glacial silt content. The presence of glacial silt in this area made the water quite turbid and may probably reduce light penetration thereby limiting productivity.

Two vertical series of samples were taken down to 500 m and the results are shown in Appendix B, Table 2. The concentration of thiamine was found to decrease with depth down to 25 m with undetectable amounts below this depth. Thiamine was found to be restricted to the thermally stratified layer above the thermocline.

Particulate nitrogen analyses were done during this sampling program and the results are plotted against thiamine concentration in Fig. 4. The regression line calculated by the least squares method is also shown. The correlation between these variables was poor.

Gulf of Alaska, Montague-Middleton Island. - In July, 1964, 10 surface samples were obtained for thiamine assay in this region. The concentration of thiamine found was in the range of 5 to 46 ng/liter with a mean of 25 ng/liter (Appendix B, Table 3). When

Figure 4. - The relation between particulate nitrogen and thiamine in Prince William Sound.


PRINCE WILLIAM SOUND
JULY 1964
Y = 40.37 - 0.314x
r = -0.176
REGRESSION LINE
PARTICULATE NITROGEN (µg/l)
70
60
50
40
30
20
10
0
100
200
300
THIAMINE (ng/l)

compared to the range of thiamine values present in Prince William Sound during the same month, the values found in this region were very low. The surface concentrations of thiamine are plotted in Map 1.

Gulf of Alaska, Valdez - Kodiak. - In May, 1964, 17 stations were sampled in this region. The range of surface concentration of thiamine found was 0 to 141 ng/liter with a mean of 55 ng/liter (Appendix B, Table 4). At one station a vertical series of samples down to 255 m were analyzed. Thiamine was found only down to 30 m (Appendix B, Table 5). The surface concentrations of thiamine are plotted in Maps 1 and 2.

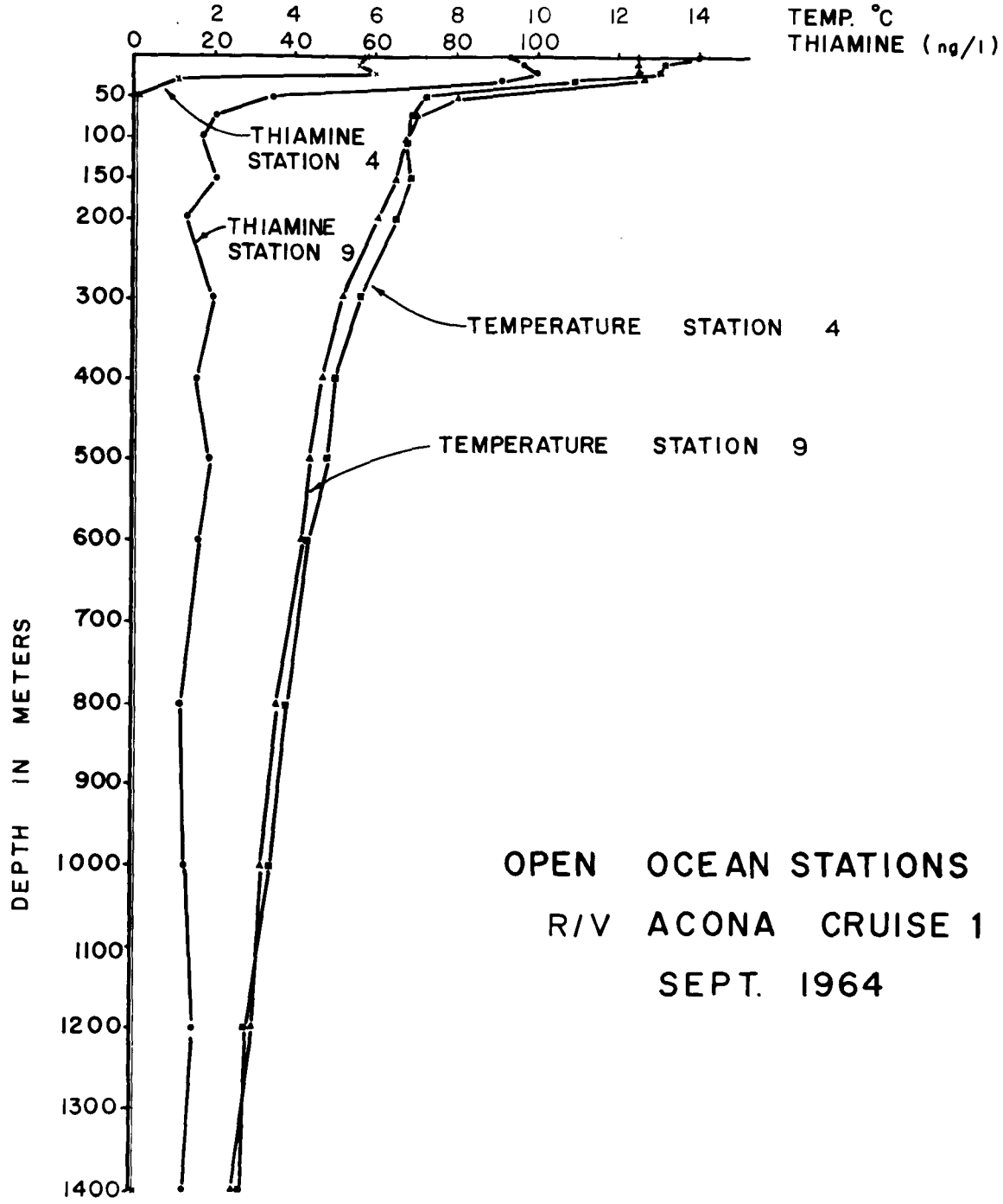
Gulf of Alaska, Cordova - Yakutat - Juneau. - Two surface sampling programs were conducted along this transect, one in April, 1964, and the other in September, 1964. The surface distribution of thiamine is shown in Maps 1 and 3 and in Appendix B, Tables 6 and 7. A distinct pattern of distribution was shown during both the sampling programs. In April the concentration was very high in coastal areas near Cordova (288 ng/liter) and Juneau (490 ng/liter) and decreased in areas away from these regions. The range of concentration found during this month was 0 to 490 ng/liter. The presence of high levels of thiamine was correlated with plankton productivity as estimated crudely by examining the filter papers used in the filtration of the water samples. The distribution of thiamine showed no correlation with water depth of the stations.

In September the same pattern of distribution was noticed with thiamine values ranging from 30 to 238 ng/liter.

Gulf of Alaska, Seattle - Juneau. - During the months of September and October, 1964, samples from 53 stations, both coastal and open ocean, were analyzed. The surface concentrations are shown in Maps 3 and 4. The complete results are given in Appendix B, Table 8. The range of surface concentrations found was 0 to 441 ng/liter. When compared to the coastal stations, the concentrations found in the open ocean stations were generally lower although in one station it was as high as 251 ng/liter. In coastal regions in and near Glacier Bay thiamine was undetectable, whereas the samples near the Juneau area showed high concentrations up to 441 ng/liter.

Some typical vertical distributions of thiamine are shown in Fig. 5 and 6. In most stations thiamine decreased with depth down to about 30 m. The concentrations found below 30 m generally were low and were found to maintain a uniform level. Samples from below 1000 m showed concentrations ranging from 0 to 56 ng/liter. In many stations the distribution of thiamine was found to be related with temperature.

Surface samples from all the stations were analyzed for particulate nitrogen and the results are shown in Fig. 7 where surface thiamine values are plotted against particulate nitrogen along with the calculated regression line. The correlation was found to be good between these two variables, thereby showing that they are in some way related to each other.

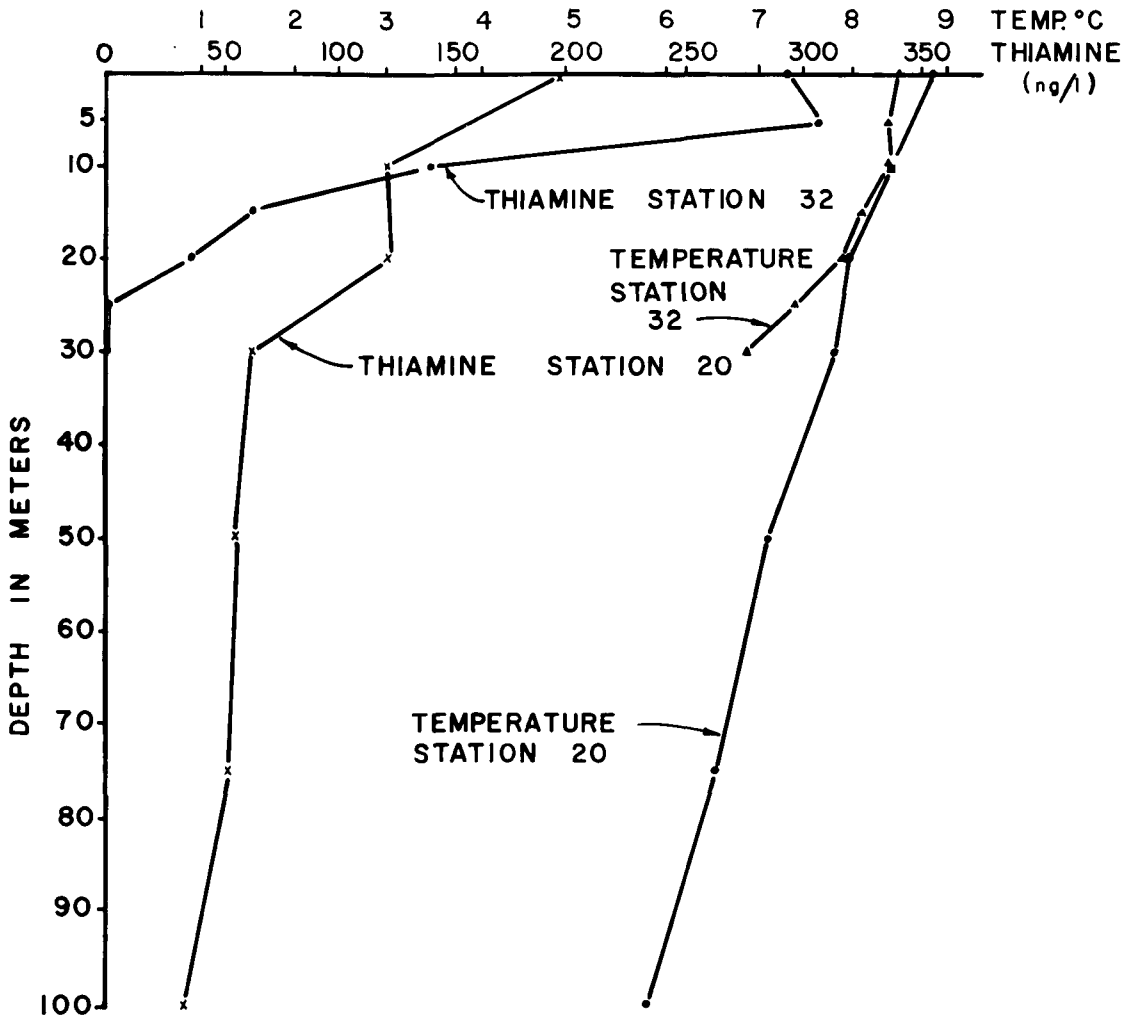


OPEN OCEAN STATIONS

R/V ACONA CRUISE 1

SEPT. 1964

Figure 6. - Vertical distribution of thiamine and temperature in two coastal stations.

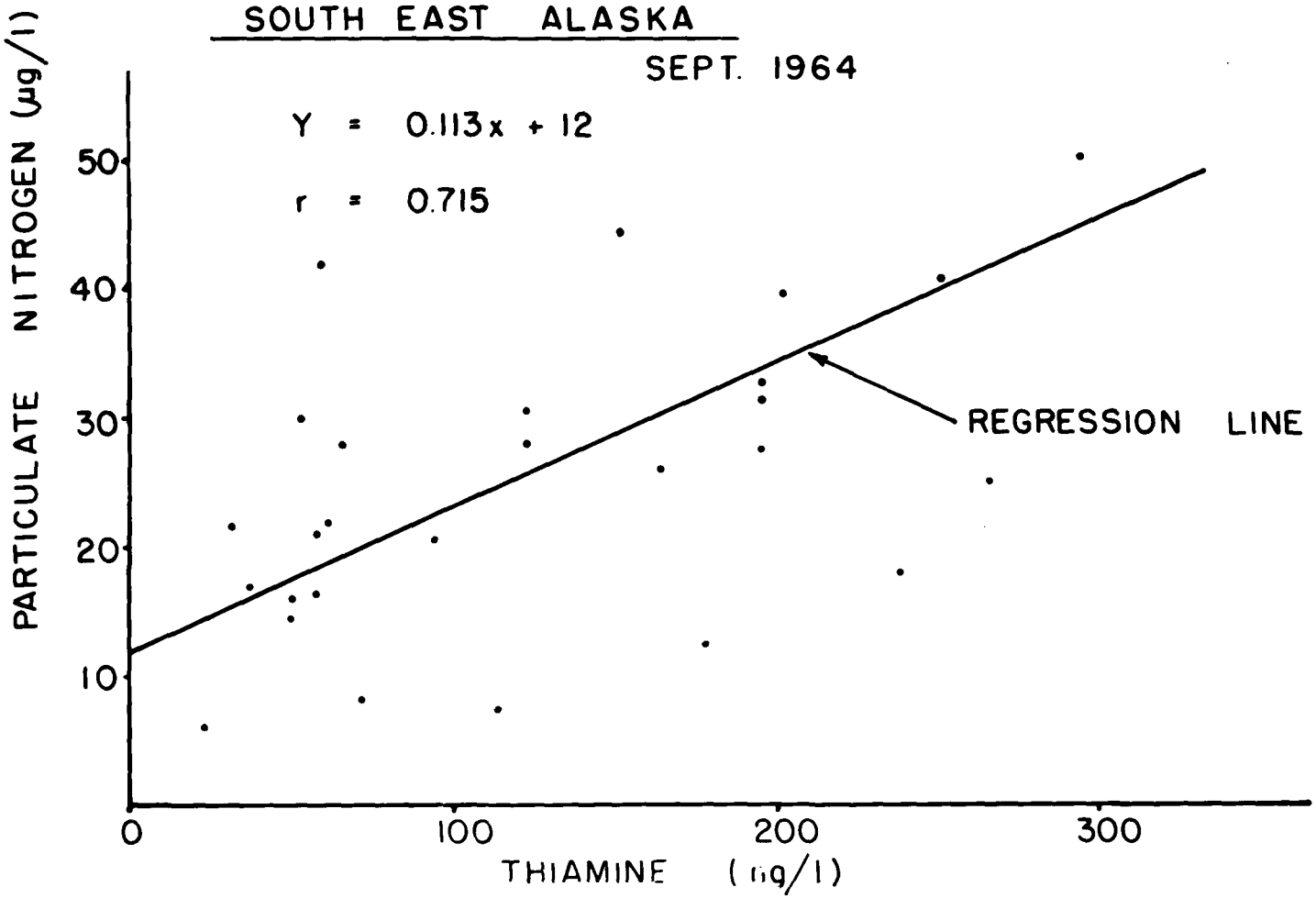


COASTAL STATIONS

R/V ACONA CRUISE 1

SEPT. 1964

Figure 7. - The relation between particulate nitrogen and thiamine in Southeast Alaska.


SOUTH EAST ALASKA
SEPT. 1964
Y = 0.113x + 12
r = 0.715
REGRESSION LINE
PARTICULATE NITROGEN (µg/l)
50
40
30
20
10
0
100
200
300
THIAMINE (ng/l)

Gulf of Alaska, Juneau - Cape Spencer. - In November, 1964, 17 stations were sampled in this region including some of the stations sampled previously in April and September. The surface distribution of thiamine is shown in Map 3 and the complete results in Appendix B, Table 9. The concentration of thiamine generally was lower during this month than during the previous samplings with a range of 0 to 70 ng/liter and a mean of 18 ng/liter. At many stations thiamine was undetectable.

The vertical distribution showed a decrease in concentration with depth down to 75 m, although the values were lower than that previously noticed.

At one station diurnal sampling was done extending over a period of 24 hr and the results are shown in Table 16. There was found to be no diurnal fluctuations in the distribution of thiamine.

Southeast Alaska, Port Angeles - Juneau. - In March, 1965, 12 stations were sampled. The results are shown in Appendix B, Table 10. The concentration of thiamine was found to be low during this month.

North Pacific Ocean, San Diego - Kodiak Island. - In August, 1964, 15 stations were sampled. The surface concentrations of thiamine are shown in Maps 1 and 4. The results of the complete analyses are shown in Appendix B, Table 11. Undetectable levels of thiamine were found in most of this region. At stations sampled near Kodiak Island concentrations ranging from 17 to 147 ng/liter

Table 16. - Diurnal variations in thiamine in surface samples.

Station no. R/V ACONA (Cruise 3)	Sampling time	Thiamine (ng/liter)
3-52	16:30 hr	16.0
3-52	20:30 hr	16.7
3-52	00:30 hr	14.4
3-52	04:30 hr	16.1
3-52	08:30 hr	16.7
3-52	12:30 hr	15.0

were noticed, the concentration increasing as Kodiak Island is approached.

Samples from five vertical stations showed the absence of thiamine up to 4800 m although the sampling depths were irregular. Some of the samples analyzed were not filtered.

North Pacific Ocean, Vancouver - Hawaii. - During January and February of 1965, samples from 36 stations were analyzed. The surface distribution of thiamine is shown in Map 4 and the complete data are given in Appendix B, Table 12. The range of concentrations found was 0 to 304 ng/liter. Samples showed increasing concentrations of thiamine as the Hawaiian Islands are approached, reaching the highest concentration of 304 ng/liter at one station about 200 miles northeast of Oahu. Except in a few stations near Hawaii the values found were low.

The samples from vertical stations showed an erratic pattern, the causes of which are not known. There was no regularity in the depth-wise distribution as shown during previous investigations. Most of the deep water samples showed the absence of thiamine though in a few cases 11-25 ng/liter were noticed at a depth of 4000 m.

Arabian Sea. - During the International Indian Ocean Expedition samples from 20 stations on Cruise 4A of the R/V ANTON BRUUN were analyzed. These samples were frozen unfiltered and the possibility of liberation of bound cellular thiamine exists during freezing and thawing, even though they were filtered before bioassay. The positions

of the sampling stations are shown in Map 5 and the results are shown in Appendix B, Table 13. The lowest and the highest concentrations of thiamine found between 0 and 28 m were 48 and 274 ng/liter respectively.

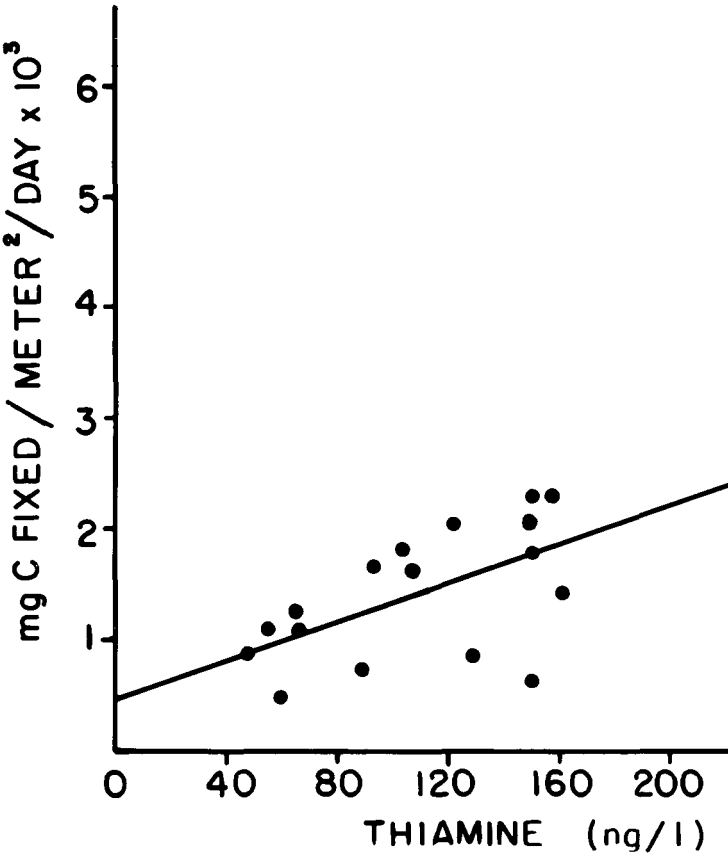
At one vertical station, samples analyzed showed a decrease in concentration with depth although at 30 and 80 m the values were relatively higher.

Fig. 8 shows the relationship between thiamine and primary productivity measured by ^{14}C-uptake ($mgC/m^2/day$). A straight line has been fitted to the data by the use of the method of least squares which shows a positive correlation between thiamine and primary productivity with a correlation coefficient of 0.56.

Seasonal cycle

The seasonal distribution of thiamine was investigated at nine stations located in the Juneau - Cape Spencer region. Surface samples were taken during the months of April, September, October and November. Although the sampling intervals were irregular, the data showed indications of a seasonal cycle (Table 17). During the months of April, September and October the concentration of thiamine was found to be generally high, whereas in November it was very low. In some of the open ocean stations A, B, C, D, (Map 3) the maximum was found to be in April and September, reaching low values in October and November, whereas the coastal stations continued to be high in thiamine in October. Thus thiamine appears to follow a seasonal

Figure 8. - The relation between primary productivity and thiamine in the Arabian Sea.


mg C FIXED / METER² / DAY x 10³
1
2
3
4
5
6
0
40
80
120
160
200
THIAMINE (ng/l)

ARABIAN SEA

OCT - NOV. '63

$Y = 444 + 8.936\,x$

$r = 0.56$

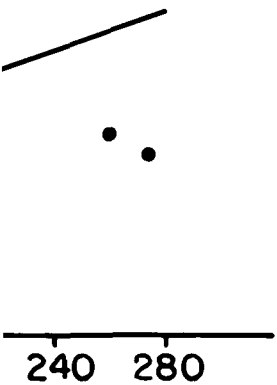


Table 17. - Seasonal cycle of thiamine in the Gulf of Alaska, Juneau-Cape Spencer.

Station Code	April 29	September 1-2	October 4-6	November 10-13
		Thiamine (ng/liter)		
A	43	140	50	0
B	71	96	37	0
C	19	89	37	0
D	103	177	0	13
E	178	121	91	28
F	348	148	195	
G	288	178	122	40
H	490	131	293	
I	318		441	26

pattern positively correlated with the expected seasonal pattern of productivity for a northern region, that is with a minimum occuring in the winter months. Sufficient data are not yet available to draw in details of the pattern.

Uptake studies with ^{14}C-labeled thiamine

Uptake experiments were done during this investigation using ^{14}C-labeled thiamine concurrently with ^{14}C-studies of primary productivity. Experiments conducted in three areas are discussed below.

Prince William Sound. - The results of three experiments done in July, 1964, are shown in Table 18 and Fig. 9 with the regression line. Thiamine uptake and primary productivity were found to be negatively correlated with a correlation coefficient of -0.9995. This may be an indication of the presence of thiamine autotrophs in this region. Light and dark bottle uptakes of thiamine were similar, suggesting that thiamine uptake is a non-photosynthetic process.

Southeast Alaska. - The results of experiments done at six stations from R/V ACONA (University of Alaska) Cruise 1 are shown in Table 19 and Fig. 10 with the regression line. A good correlation is found to exist between thiamine uptake and photosynthetic carbon uptake in surface waters with a correlation coefficient of 0.956. The uptake of thiamine closely paralleled the carbon uptake with depth

Table 18. - Uptake experiments in Prince William Sound.

Experiment no.	Lat. N	Long. W	Depth (m)	Light or dark	Incubation (hr)	^{14}C-carbonate[a] uptake (count/min)	^{14}C-thiamine[b] enrichment (%)	^{14}C-thiamine[c] uptake (count/min)	^{14}C-thiamine uptake (ng/liter/hr)
1	59°58.5'	147°41.7'	0	L	24	2345	99.7	110	0.21
			0	D	24	103	99.7	99	0.19
2	60°17.2'	148°08.3'	0	L	24	1568	99.3	148	0.28
			0	D	24	132	99.3	154	0.29
3	60°57.4'	147°37.3'	0	L	22	3914	100.0	42	0.69
			0	D	22	137	100.0	39	0.64

[a] ^{14}C-carbonate treatments in 125 ml bottles with 5 μc of $H^{14}CO_3$.

[b] 1 μc (13 μg) thiamine added to each bottle.

[c] Count/min obtained after subtracting the activity of the second filter paper.

Figure 9. - The relation between ^{14}C-thiamine uptake and ^{14}C-carbonate uptake in Prince William Sound.

PRINCE WILLIAM SOUND

AUG. 1964

$Y = 4829 - 22.2x$

$r = -0.999$

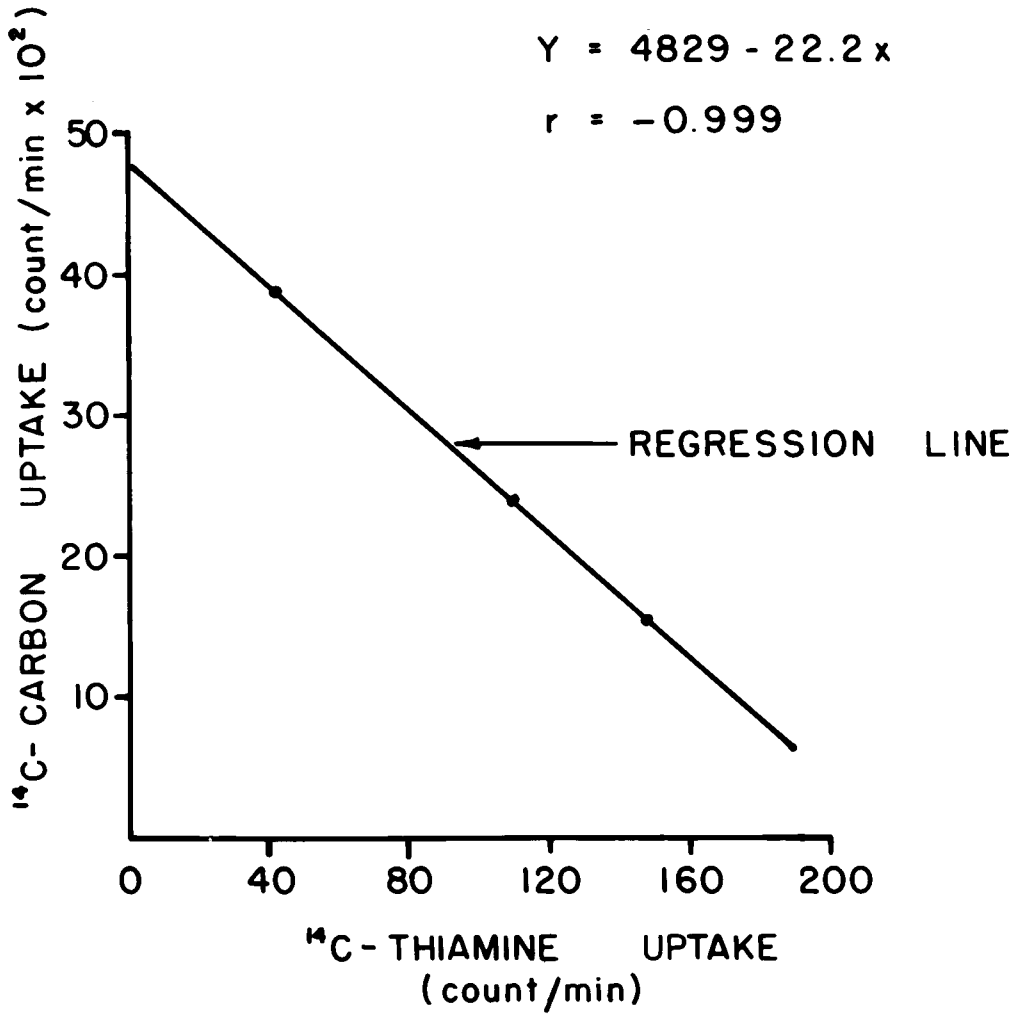


Table 19. - Uptake experiments in Southeast Alaska.

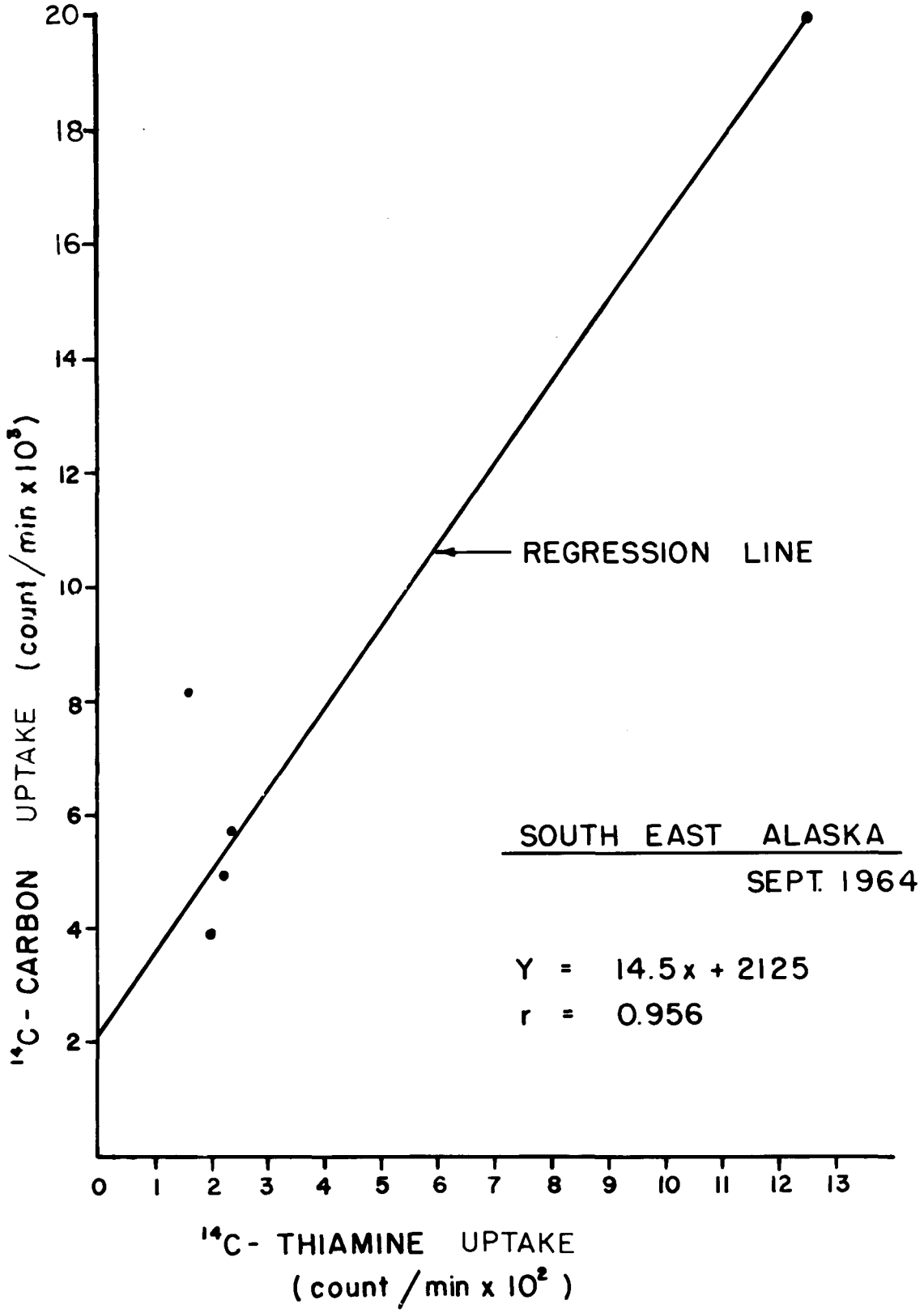
R/V ACONA Station no. (Cruise 1)	Depth (m)	Light or dark	Incubation (hr)	^{14}C-carbonate[a] uptake (count/min)	^{14}C-thiamine[b] enrichment (%)	^{14}C-thiamine[c] uptake (count/min)	^{14}C-thiamine uptake (ng/liter/hr)
12	0	L	22	4908	91.6	223	0.45
	0	D	22	200	91.6	215	0.44
	10	L	22	3594	92.7	92	0.19
	10	D	22	95	92.7	97	0.20
	25	L	22	1243	95.2	64	0.13
	25	D	22	77	95.2	62	0.13
19	0	L	18	3926	99.5	200	0.50
	0	D	18	165	99.5	178	0.44
28	0	L	18	8143	97.6	160	0.40
	0	D	18	156	97.6	137	0.34
30	0	L	24	5701	97.2	236	0.44
	0	D	24	192	97.2	307	0.58
32	0	L	24	20027	97.8	1249	2.34
	0	D	24	624	97.8	979	1.83
	10	L	24	8604	97.7	558	1.05
	10	D	24	318	97.7	549	1.03
	25	L	24	989	100.0	156	0.29
	25	D	24	81	100.0	111	0.21

[a] ^{14}C-carbonate treatments in 125 ml incubation bottles with 5 µc of $H^{14}CO_3$.

[b] ^{14}C-thiamine treatments done in one liter bottles. 1 µc (13 µg) thiamine added to each bottle.

[c] Count/min obtained after subtracting the activity of the second filter paper.

Figure 10. - The relation between ^{14}C-thiamine uptake and ^{14}C-carbonate uptake in Southeast Alaska.


20
18
16
14
12
10
8
6
4
2
0 1 2 3 4 5 6 7 8 9 10 11 12 13
^{14}C - CARBON UPTAKE (count/min x 10^3)
^{14}C - THIAMINE UPTAKE
(count / min x 10^2)
REGRESSION LINE
SOUTH EAST ALASKA
SEPT. 1964
Y = 14.5x + 2125
r = 0.956

also. In the Juneau area the bloom of phytoplankton noticed in a 5 m plankton tow showed the presence of the following diatoms: Coscinodiscus sp., Nitzschia seriata, Tabellaria sp., Rhizosolenia sp., Ceratium sp., Chaetoceros sp., and the silicoflagellate Distephanus sp., predominated by many species of Chaetoceros. During this particular period surface thiamine concentrations were high in the Juneau area (441 ng/liter). Even under these conditions the results show the presence of phytoplankton auxotrophic to thiamine.

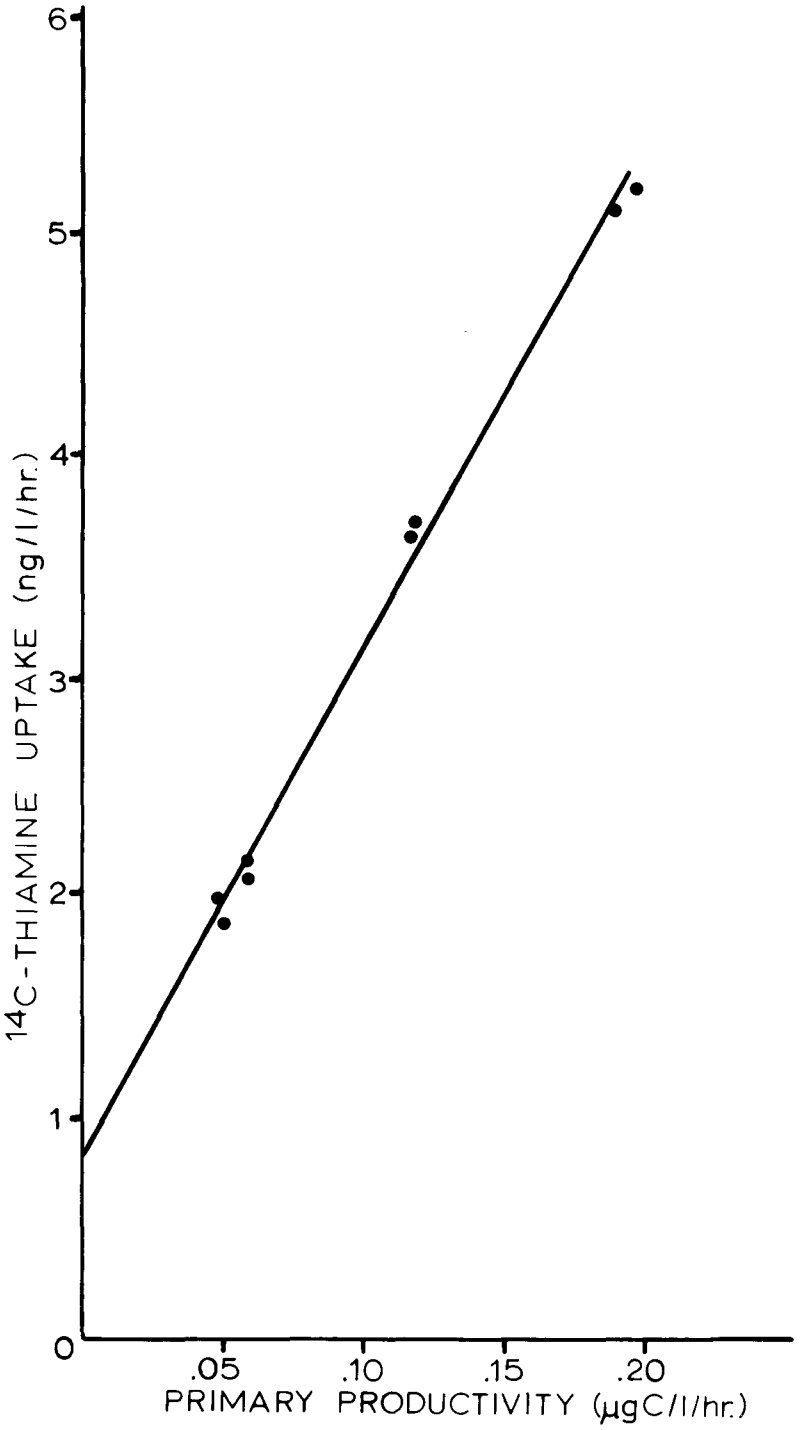
Gulf of Alaska, Juneau - Cape Spencer. - The results of experiments done in this region are given in Table 20 and Fig. 11. The data from light bottle experiments of thiamine and photosynthetic carbon uptakes are plotted against each other. The relationship between these two variables seems to be linear. One interesting feature of these data is that the dark bottle uptakes at two stations gave values virtually identical to the Y intercept of the graph suggesting that photosynthetic uptake of thiamine is indeed being measured. The dark bottle uptake may, thus, represent uptake by non-photosynthetic organisms. The turnover time of thiamine, calculated from the concentration present prior to the addition of radioactive thiamine, falls between 4 and 7 hr, sometimes going up to 19 hr.

Certain reservations have to be made in interpreting these data. The experiments were done during the low productivity time of the year. The amount of thiamine added, 130 µg/liter (10 µc) is very

Table 20. - Uptake experiments in the Gulf of Alaska.

R/V ACONA Station no. (Cruise 3)	Depth (m)	Light or Dark	Bioassay thiamine (ng/liter)	µgC/liter/hr	ngB_1/liter/hr	Turnover time (hr)
53	0	Light	25.5	0.19	5.11	5.0
53	0	Dark	25.5		1.39	18.4
53	10	Light	22.2	0.20	5.20	4.3
53	25	Light	21.6	0.12	3.65	5.9
53	50	Light		0.12	3.69	
35	0	Light	40.3	0.06	2.08	19.4
35	10	Light	11.3	0.05	1.98	5.7
35	25	Light	14.5	0.05	1.89	7.7
58	0	Light	13.1	0.06	2.16	6.1
58	0	Dark	13.1		1.15	11.4

Figure 11. - The relation between ^{14}C-thiamine uptake and primary productivity in the Gulf of Alaska.


6
5
4
3
2
1
0
^{14}C-THIAMINE UPTAKE (ng/l/hr.)
.05
.10
.15
.20
PRIMARY PRODUCTIVITY (µgC/l/hr.)

large when compared to the concentrations existing in the sea and this possibly may inhibit cellular synthesis of thiamine in favour of availability from the surrounding water. Therefore, the uptake measured may be maximal under these conditions. The turnover time may also be fastest possible. The other feature noticed during this particular experiment is that the dark bottle uptakes of thiamine were significantly lower than the light bottle uptakes which has not been noticed before.

A rough estimate can be made from these experiments on the amount of thiamine taken up per unit carbon utilized. The figure obtained from the slope of the straight line in Fig. 11, indicates that 0.023 µg thiamine are assimilated per µg of carbon.

In interpreting the thiamine uptake data in the Juneau area some doubts arose as to whether the uptake was an active uptake or rather an apparent passive uptake. Evidence has been accumulating with respect to cell wall accumulation of vitamin B_{12} (Sasaki and Kitahara, 1963; Kitahara and Sasaki, 1963). It has been found by the above authors that Lactobacillus delbrueckii, a B_{12} requiring organism, accumulates a far excess of this vitamin up to one hundred-fold of normal growth requirement when placed in a B_{12}-rich medium. Though this phenomenon is shown only by active growing cells, suspicion arose as to whether or not the cell walls have the property of adsorbing the thiamine molecule. An experiment conducted to test this gave the results shown in Table 21. The results show that thiamine uptake is an active process. Though the dead cells do take up thiamine

Table 21. - Uptake of labeled thiamine by Cryptococcus albidus.

Treatment	Count/min
Thiamine deficient cells	5734
Thiamine deficient cells killed by formalin	296
Thiamine sufficient cells	4382
Thiamine sufficient cells killed by formalin	264

Thiamine sufficient cells grown in synthetic sea water medium with 0.1 ng/ml thiamine and deficient cells grown in thiamine-free medium. Both grown for 72 hrs. Incubated cells with 0.5 μc of ^{14}C-labeled thiamine/100 ml of synthetic sea water medium for 22 hrs at 22°C. After incubation, cells filtered using HA Millipore filter paper and counted.

the quantity adsorbed is only five to six per cent. The cells grown in a thiamine enriched medium take up the thiamine molecule at a slower rate than cells which have been grown in a thiamine-free medium.

DISCUSSION

Cycle of thiamine in the sea

The presence of thiamine seems to be positively correlated with primary productivity in general. The results of ^{14}C-primary productivity measurements in the Arabian Sea and the particulate nitrogen analyses in the North Pacific Ocean showed that thiamine is closely related with primary productivity. In the Gulf of Alaska surface thiamine concentration increased in regions where there was a high phytoplankton growth (crudely estimated by visual examination of the filter papers). The distribution pattern in general showed low thiamine values can be expected in areas of low productivity and high values in areas of high productivity. For example high concentrations (441 ng/liter) were noticed in the Juneau area at a time of year when there was a diatom bloom, and in samples taken near the Hawaiian Islands high thiamine values (310 ng/liter) were associated with a region known to show high productivity. The only exception to this close relationship was found in the Prince William Sound area where it was found, both in particulate nitrogen analyses and uptake studies, that the correlation is negative. This region gave results contrary to most regions sampled and must be considered separately in evaluating the results.

The restricted distribution of thiamine in the thermally stratified layer above the thermocline shown in many vertical stations may probably be due to its production in the euphotic zone and the absence of mixing with the deeper waters. This again indicates the

close association between thiamine and the productive parts in the sea, namely the euphotic zone.

Labeled thiamine uptake studies showed in all areas except in Prince William Sound heavy uptakes in regions of high productivity and low uptakes in low productive areas. It was also seen that thiamine uptake closely paralleled ^{14}C-carbonate uptake with depth. This directly involves the phytoplankton as the chief consumer of added thiamine in the experiments.

The seasonal studies on the distribution of thiamine investigated in a few areas also showed the close relationship existing between thiamine and primary productivity. High thiamine values were observed during months of high productivity (April and September) and low concentrations during months of low productivity (November).

The close relationship noticed between thiamine and primary productivity is difficult to interpret. Whether the high phytoplankton production is due to high thiamine or the high thiamine is a resultant of phytoplankton production, cannot be well substantiated with the data on hand.

Calculations made from uptake experiments in November showed that the turnover time of thiamine tends to fall between 4 and 7 hr (exceptionally to 19 hr), indicating that it is being cycled at a rapid rate. This may be due to the property of the thiamine molecule which is known to be highly unstable at the alkaline pH of sea water. With this high turnover time thiamine destruction is reduced to a minimum and can be constantly made available to the organisms. The

restricted distribution of thiamine in the euphotic zone can also be explained by the rapid turnover in that it is being produced and utilized simultaneously, a point which is corroborated by the uptake experiments. One objection to the calculations made here is that one diurnal sampling conducted in the same region and season did not show any flutuations in the concentration during a span of 24 hr. It is quite possible this may be again due to the continuous production and utilization which always keeps a constant residual amount in the surrounding. A reservation to be made here is that this explanation may be applicable only to one particular season and the region studied.

Comparison between thiamine and vitamin B_{12}

It is interesting to compare the distribution patterns of thiamine shown during this investigation and those known for B_{12}. In the work done so far on the distribution of B_{12}, high concentrations have been observed in coastal areas (Provasoli, 1963; Kashiwada et al., 1957; Droop, 1955; Lewin, 1954; Cowey, 1956), which is compatible with the observations made in the case of thiamine during this work.

The vertical distribution of B_{12} showed an entirely different pattern than that shown by thiamine. Maximum concentrations of B_{12} generally are found at a depth of 500 m (Menzel and Spaeth, 1962; Kashiwada et al., 1959). Menzel and Spaeth (1962) noticed that the concentrations of B_{12} above 100 m were low ranging from 0.01 to 0.1 ng/liter, reaching a maximum of 0.22 ng/liter at 500 m in the Sargasso

Sea. Daisley and Fisher (1958) found in the Bay of Biscay low B_{12} concentrations in the upper illuminated zone and in the greatest depths, whereas at intermediate depths (190-2,110 m) the values were generally higher, up to 5 ng/liter in two instances. The reason for the marked variability with depth and high concentrations at intermediate layers can be due to the light sensitive property of B_{12} (Kashiwada et al., 1959). It is possible that B_{12} is destroyed in the surface layers by light.

The seasonal distribution of thiamine has not been worked out completely but it showed similarities with the seasonal patterns of B_{12}. Vishniac and Riley (1961) noticed in Long Island Sound that the surface cobalamin concentrations go up to about 16 ng/liter during the winter, fall markedly with the late winter diatom bloom and rise again in the summer with the increasing temperature. They also found that the pattern of cobalamin concentration nearly paralleled those of PO_4-P and NO_3-N concentrations. A seasonal cobalamin cycle increasing during the spring bloom with a decrease immediately after also has been noticed in the Sargasso Sea (Menzel and Spaeth, 1962). In the Butt of Lewis, northern North Sea and Norwegian Deeps, Cowey (1956) found a high winter maximum of about 2 ng/liter of cobalamin and a summer minimum of 0.2 ng/liter.

The different patterns of vertical distribution as shown by thiamine with surface maximum and B_{12} with maximum concentrations in deeper layers at about 500 m, may have important ecological significance. It is tempting to suggest the possibility that thiamine

auxotrophy is predominant in the upper euphotic zone and B_{12} auxotrophy in deeper layers below the euphotic zone. Whether there is a selection going on in marine micro-organisms with reference to depth and vitamin nutrition or whether the differential distribution is merely the resultant of circulation patterns is a matter of speculation.

Origin of thiamine

The problem of origin of thiamine in the sea has not been attacked directly during this investigation. The origin of coastal thiamine has been attributed to land drainage. Vishniac and Riley (1961) found barely detectable amounts of thiamine, 0 to 20 ng/liter, in the body of Long Island Sound. They also found a decline in the concentration at the surface as one moves offshore, from 63 ng/liter within the breakwater of the Indian River to 40 ng/liter at Charles Island, the concentration further decreasing to about 20 ng/liter at station 5A. The decline in concentration was attributed to the destruction of thiamine in more alkaline conditions in the open ocean areas or due to the thiamine being derived from land drainage. Though during the present investigation a similar pattern of decline in concentration was found as one moves offshore, thiamine destruction due to more alkaline conditions cannot be well substantiated as thiamine also was found to be present in many areas away from land. The suggestion that thiamine is derived from land drainage seems to be a possibility with a few exceptions. High concentrations always

were found in coastal areas of high productivity. In a few exceptional cases, as in Glacier Bay, no detectable thiamine was found, even though the area was sampled three times. The same is the case for regions near Valdez where there was no thiamine present. The reason for the absence of thiamine in these two areas may be due to the high glacial silt content of the water. The presence of black silt in the water near Valdez makes the water quite turbid, reducing light penetration with consequent low productivity. Glacial silt may be an effective adsorbent for thiamine. If true, this phenomenon could also account for the low thiamine levels noticed in these regions.

There is also the possibility that land drainage brings in not thiamine but other essential nutrients which otherwise limit productivity in these areas. The nutrients derived from land drainage increase the primary productivity which may in turn result in the production of thiamine either by phytoplankton activity or by bacterial activity.

Marine bacteria have been known to produce physiologically significant amounts of thiamine in laboratory cultures. It is also known that actual vitamin exchanges take place simultaneously between different kinds of micro-organisms (Burkholder, 1963). Algae are notorious for liberating all kinds of extracellular substances into the surrounding medium, for example, organic acids, polypeptides, carbohydrates, antibiotics, toxins and enzymes (Fogg, 1962). Lewin (1958) reported the liberation of thiamine in the

culture medium of Coccomyxa. Red and brown algae have been found to contain 0.4 to 0.83 µg of thiamine per gram of algae (Teeri and Bieber, 1958). All these sources including bacteria, planktonic algae and benthic algae have to be taken into account as good sources for thiamine, especially in coastal areas.

There has been so far no direct evidence for the regeneration of thiamine in marine environments except the indications mentioned above. In one experiment, conducted in the laboratory, storage of enriched sea water did show the production of thiamine by bacterial action (Table 22). In the enriched samples a marked increase in thiamine with time was seen which may be attributed to the presence of a large number of bacteria noticed at the end of 14 days. When compared to the enriched samples the thiamine concentration in the unenriched samples showed little increase or a slight decrease with time together with less bacterial growth. In the filtered samples only the majority of the phytoplankton were removed and the samples were not sterile. Daisley (1959) noticed a similar phenomenon, a marked increase in B_{12}, in samples with no preservative treatments and in samples without refrigeration for two weeks.

The origin of thiamine from marine sediments (Burkholder, 1958) has not been well substantiated in this study. The presence of thiamine has no correlation with the water depth, which excludes the possibility of migration of thiamine from the bottom mud due to bacterial activity.

The vertical distribution of B_{12} indicates that the B_{12} maximum at 500 m may be due to the production of this vitamin in deeper layers

Table 22. - Effect of storage of sea water at 22°C.

Treatment	0 days thiamine (ng/liter)	14 days thiamine (ng/liter)	155 days thiamine (ng/liter)
Unfiltered enriched	111.4	291.1	334.5
Filtered enriched	90.2	350.5	302.2
Unfiltered unenriched	60.8	26.7	24.1
Filtered unenriched	98.8	140.2	117.8

All treatments initially contained 100 ng/liter added thiamine. Enriched samples were given 10 g glucose, 472 mg $(NH_4)_2SO_4$, 1 ml trace metals stock solution, and 1 ml of Fe-EDTA stock solution per liter of sea water. Stored in plastic bottles.

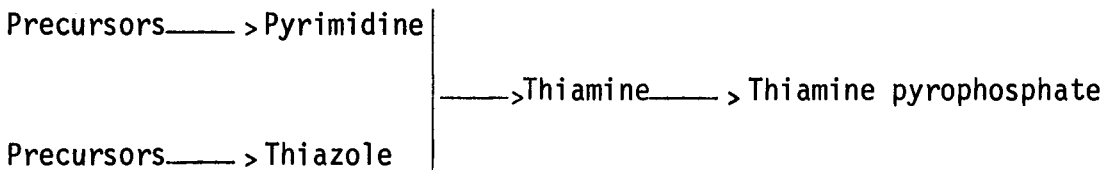
mostly due to bacterial activity or it may be of animal origin. Vitamin B_{12} produced in the deeper layers may be brought to the surface by eddy diffusion or upwelling and is used up in the surface layers as fast as it is brought up or part of it is lost by light destruction. In the case of thiamine, there are enough evidences to show that it is being produced in the surface layers and is kept available in the layer above the thermocline. Whether thiamine which migrates into the deeper layers by mixing is destroyed by high salinity and pH has yet to be verified.

The low concentrations of thiamine found generally in the open ocean may be due to several factors. General low productivity in the open ocean regions studied may be the main reason, as thiamine is of biological origin. It may also be due to the destruction by the high pH found in open ocean waters though there are inconclusive evidences to the contrary showing that thiamine is stable in salt solutions. The possibility of the movement of coastal thiamine into the open ocean, depending on the circulation patterns of particular regions, has to be investigated. This may explain the reason for the low concentrations as the thiamine brought into the open ocean is more thinly spread.

Concluding remarks

The bioassay technique employed during this investigation using Cryptococcus albidus has inherent limitations. The use of the term thiamine activity is more appropriate here to emphasize the fact that

the quantities measured are either the activity of the thiamine molecule or of the thiazole moiety alone or the combined activity of both, as the organism can utilize the thiazole moiety as well as the intact thiamine molecule but not the pyrimidine moiety. It is known that biosynthesis of thiamine takes place by the coupling of preformed portions of pyrimidine and thiazole moieties as indicated below (Guirard and Snell, 1962).



It is, thus, possible to divide the organisms into the following categories according to the nature of the biosynthetic step missing in the pathways of thiamine biosynthesis: (1) organisms requiring thiazole or intact thiamine, (2) organisms requiring pyrimidine or intact thiamine, (3) organisms requiring thiazole and pyrimidine or intact thiamine and (4) organisms requiring intact thiamine molecule (Guirard and Snell, 1962).

Thiamine is known to be unstable at alkaline pH (Robbins and Kavanagh, 1942) and readily splits into its thiazole and pyrimidine components when aqueous solutions are heated at pH above five. Lability is further enhanced at higher pH or by treatment with sulfite. In highly alkaline solutions, thiamine is oxidized by ferricyanide into thiochrome and this property is used for the chemical assay of thiamine by extracting the thiochrome with isobutanol and measuring the fluores-

cence. Maier and Metzler (1957) and Metzler and Maier (1962) reported that thiamine reacts with hydroxyl ions to form a pseudo-base and a thiol form in which the thiazole ring has been opened. Thiamine displays a transient yellow color in strongly alkaline solutions which is more stable in methanolic solutions than in aqueous solutions. They reported also that thiamine undergoes a remarkable series of chemical reactions in basic solutions involving nine different forms. The nature of the functions of these different forms is unknown and it is suspected that they may be only laboratory curiosities. The yellow form and the tricyclic form which is formed from the yellow form are recognized to be important in biochemical functions. The yellow form can be rapidly converted into thiamine hydrochloride upon the addition of hydrochloric acid. Thus, it appears that the apparent destruction of thiamine in alkaline solutions is only of a transient nature and the molecule can revert back into the intact form with a reduction of pH.

It is, thus, possible that thiamine may exist in different forms at the sea water pH of 8 to 8.3. If the molecule is unstable in sea water and exists in the form of pyrimidine and thiazole, the bioassay employed has the advantage that it can detect at least one form of thiamine, namely the thiazole moiety. This may be the reason for the low thiamine values reported in a previous investigation (Vishniac and Riley, 1961). Whether this lack of specificity of the yeast has resulted in the measurement of one part of thiamine molecule alone, during the whole investigation, is questionable. This can be verified only by using organisms with different specificities to each of the three molecules.

To test this possibility, two samples of sea water were analyzed with three species of yeast with different specificities and the results are shown in Table 23.

The result seems to indicate that the thiamine molecule exists in sea water either as the intact molecule or that thiamine is split into its two moieties but is not completely destroyed. The results are inconclusive because of lack of data on the growth response of two of the species of yeasts used in this experiment.

The concentrations of thiamine reported during this investigation are many orders of magnitude higher than the concentrations of B_{12} so far known. The range of concentrations found for B_{12} varies between 0.2 and 2 ng/liter according to position, depth and season (Hood, 1963). There are indications that B_{12} would never in practice be limiting in the sea (Droop, 1957; Menzel and Spaeth, 1962). There are very few experiments done to prove or disprove whether B_{12} is really limiting in the natural sea water environment. Menzel and Ryther (1961) showed that in the Sargasso Sea enrichment of raw sea water with vitamins never gave indications of causing increased rates of ^{14}C-carbonate uptake. The whole problem of whether B_{12} is really limiting in ecological surroundings is still being debated. Calculations made by Burkholder and Burkholder (1958) in sediment samples indicated that B_{12} is in excess of half maximum growth requirements in mud by 14,000 fold, biotin by 350 fold and thiamine only about 4.5 times, apparently showing that thiamine is nearly limiting for organisms growing in marine muds. They also found that, for five different mud samples, the ratio of B_{12}:biotin:thiamine was 40:1:10. The present

Table 23. - Thiamine activity in sea water.

	Thiamine activity (ng/liter)			
	Sample 1	Percentage	Sample 2	Percentage
Cryptococcus albidus	111.6	100	209.5	100
Cryptococcus sp.	80.8	72	148.5	71
Rhodotorula sp.	78.5	70	111.6	53

Thiamine activity calculated from internal standards with 100 ng/liter thiamine. Cryptococcus albidus requires thiazole or thiamine. Cryptococcus sp. isolated from the Gulf of Alaska, requires thiazole or thiamine but grows better with thiazole and pyrimidine. Rhodotorula sp. requires pyrimidine or thiamine, also isolated from the Gulf of Alaska.

investigations show clearly that thiamine is found in high concentrations compared with the amounts of B_{12} so far reported. This cannot be very well confirmed unless the data for B_{12} are acquired for the areas investigated.

The presence of thiamine autotrophs in Prince William Sound in July and thiamine auxotrophs in Juneau area in September, makes any conclusions on thiamine limitation difficult. Definite conclusions can be reached only by more intensive investigations. One way of attacking the problem is by enriching natural sea water with thiamine and measuring ^{14}C-carbonate uptake which unfortunately has not been done during this investigation.

SUMMARY

A marine yeast, Cryptococcus albidus, has been used for the bioassay of thiamine in sea water. The method is found to be sensitive in the range of 10 to 300 pg/ml thiamine. The thiamine concentration in sea water has been measured over a period of several months during the years 1963, 1964 and 1965.

Thiamine concentrations in surface water were found to be generally high in coastal regions and low in the open ocean. The vertical distribution showed a decrease in concentrations with depth down to 50 to 75 m whereas below this depth the values were generally low or undetectable. It follows the temperature distribution with high concentrations in the thermally stratified layer above the thermocline. The seasonal distribution investigated in one area showed high concentrations during the months of April and September, decreasing drastically in November. A close relationship between primary productivity and thiamine was noticed with high values of thiamine in areas of high productivity. Uptake studies with ^{14}C-labeled thiamine suggested the presence of thiamine autotrophs in one region and auxotrophs in another. The turnover time of thiamine observed in one region was around four to seven hours.

Evidence for thiamine production by bacteria and for the stability of thiamine in marine environments are discussed. The distribution patterns of thiamine and vitamin B_{12} are compared. The important difference between the vertical distribution of thiamine with a surface

maximum and B_{12} with maximum concentrations at 500 m or below are indicated to be ecologically significant.

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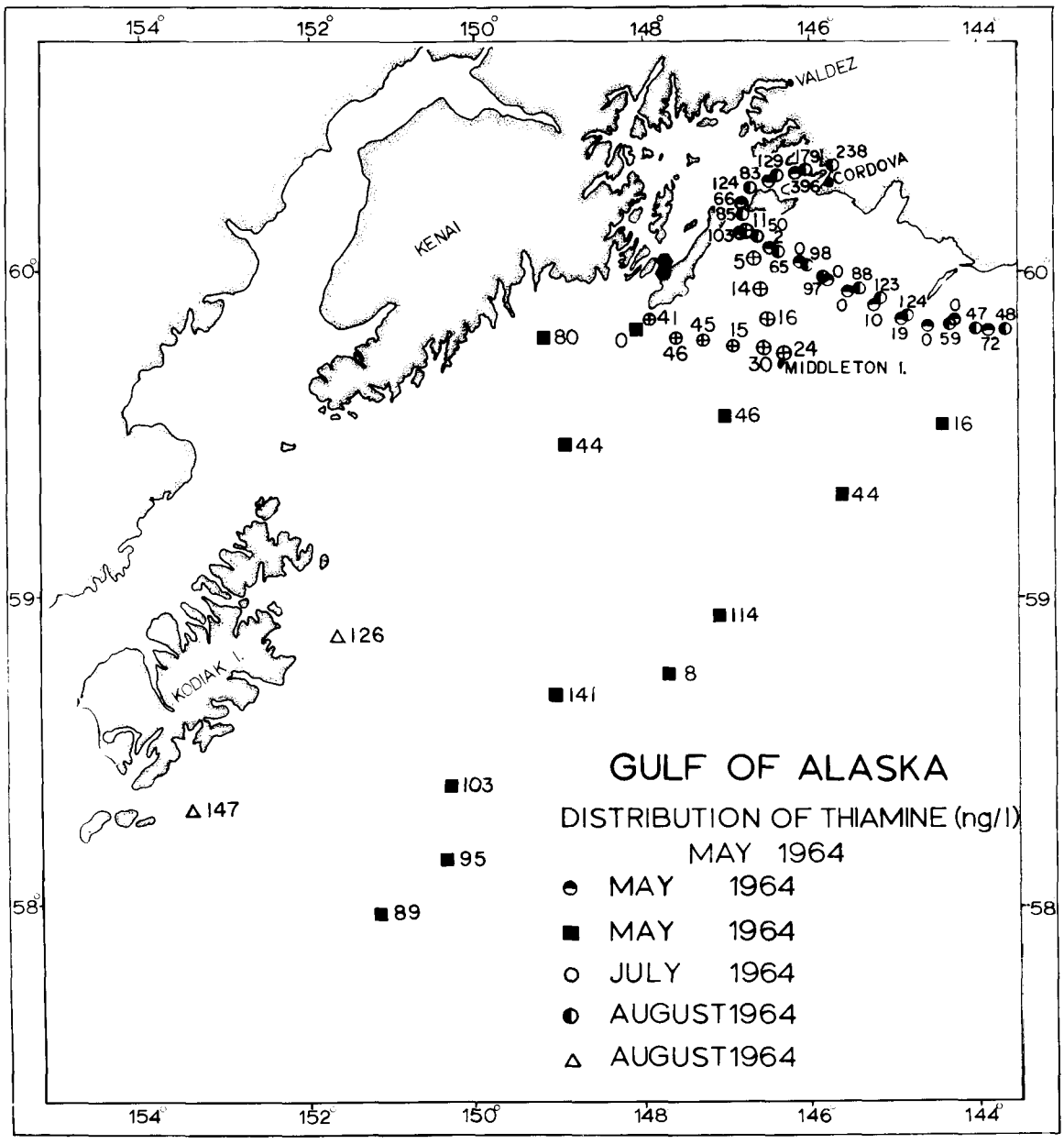
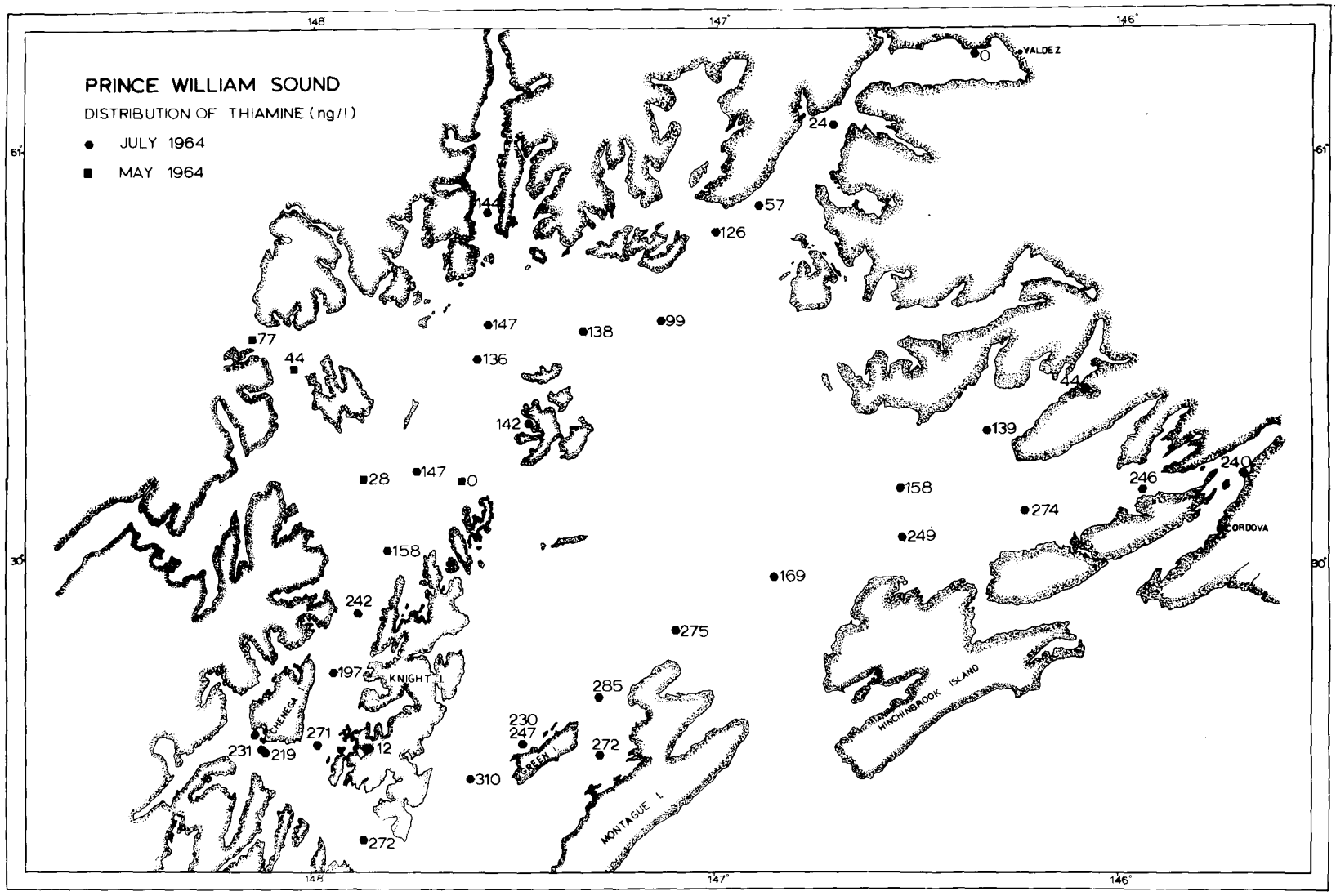
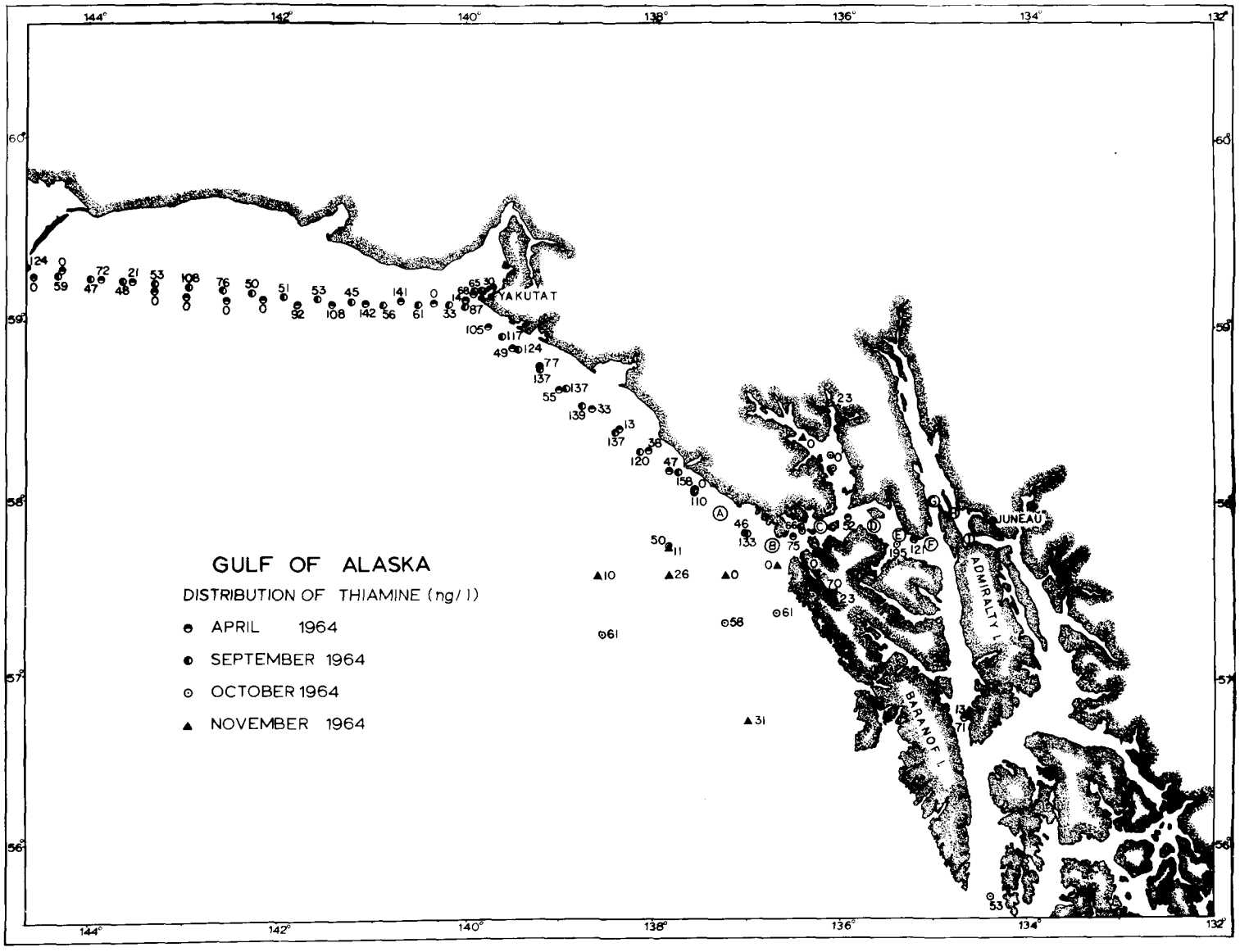
Map 1. - Gulf of Alaska


154°
152°
150°
148°
146°
144°
60°
59°
58°
VALDEZ
CORDOVA
KENAI
MIDDLETON I.
KODIAK I.
GULF OF ALASKA
DISTRIBUTION OF THIAMINE (ng/l)
MAY 1964
MAY 1964
MAY 1964
JULY 1964
AUGUST 1964
AUGUST 1964

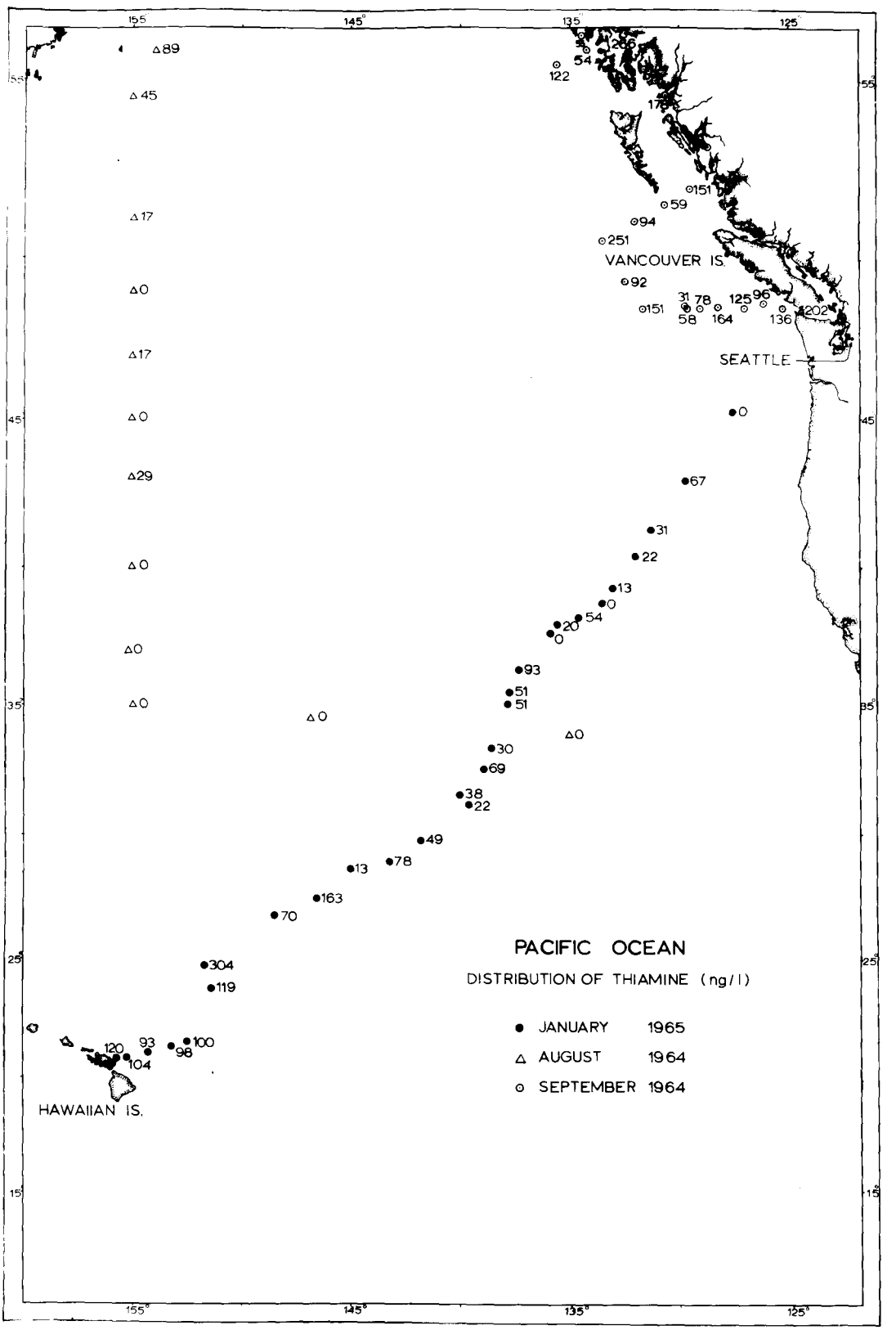
Map 2. - Prince William Sound


PRINCE WILLIAM SOUND
DISTRIBUTION OF THIAMINE (ng/l)
JULY 1964
MAY 1964
148°
147°
146°
61°
60°
VALDEZ
0
24
144
57
126
147
138
99
77
44
136
142
139
28
147
0
158
274
246
240
249
CORDOVA
169
242
275
197
KNIGHT I.
285
HINCHINBROOK ISLAND
230
247
271
231
219
12
272
310
MONTAGUE I.
272

Map 3. - Gulf of Alaska


GULF OF ALASKA
DISTRIBUTION OF THIAMINE (ng/l)
APRIL 1964
SEPTEMBER 1964
OCTOBER 1964
NOVEMBER 1964
YAKUTAT
JUNEAU
ADMIRALTY I.
BARANOF I.
144°
142°
140°
138°
136°
134°
132°
60°
59°
58°
57°
56°

Map 4. - Pacific Ocean


155°
145°
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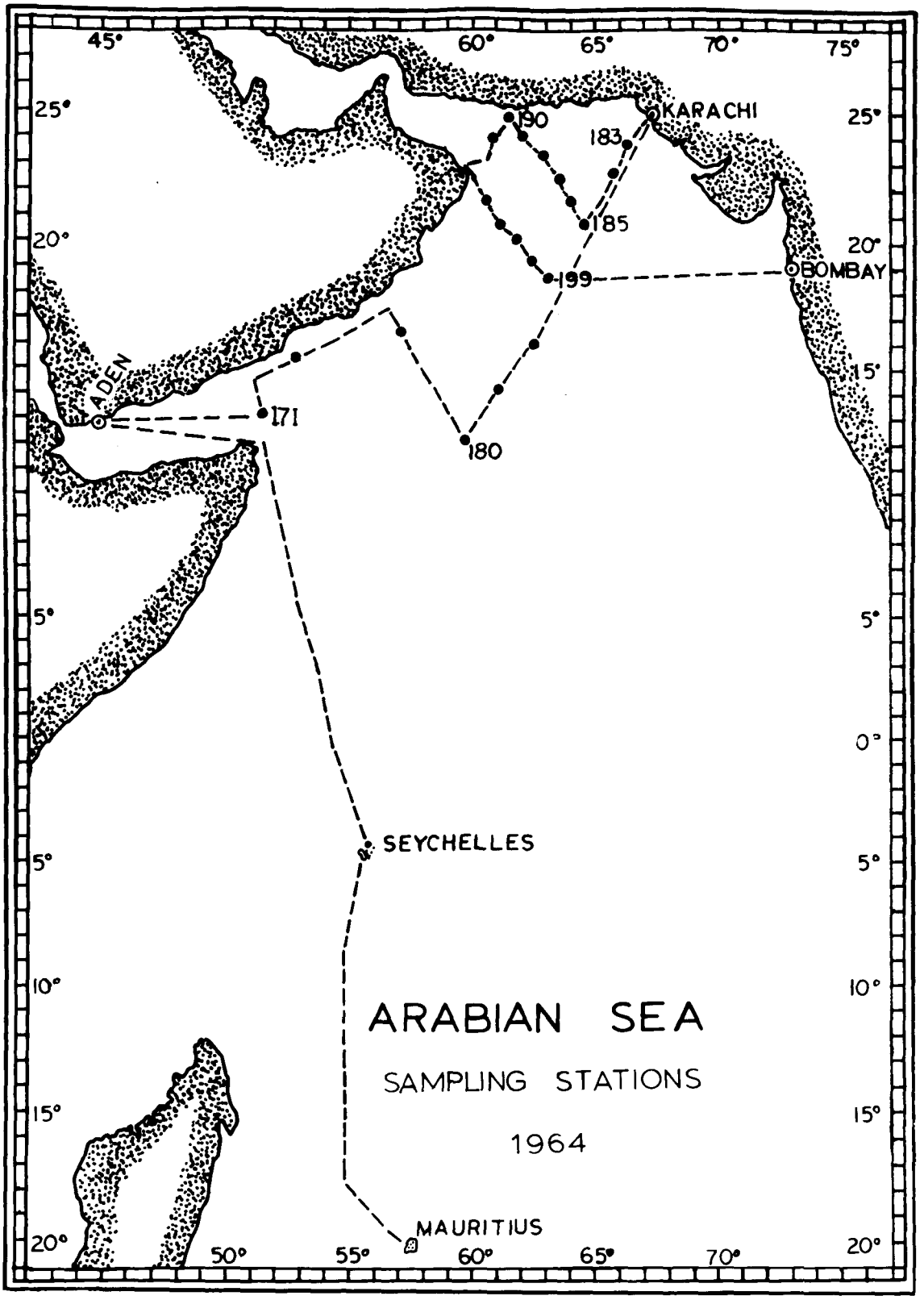
125°
55°
45°
35°
25°
15°
Δ89
Δ45
Δ17
Δ0
Δ17
Δ0
Δ29
Δ0
Δ0
Δ0
Δ0
Δ0
Δ0
122
54
266
17
151
59
94
251
VANCOUVER IS.
92
151
31
58
78
164
125
96
136
202
SEATTLE
0
67
31
22
13
0
54
20
0
93
51
51
30
69
38
22
49
78
13
163
70
304
119
120
93
104
98
100
HAWAIIAN IS.
PACIFIC OCEAN
DISTRIBUTION OF THIAMINE (ng/l)
● JANUARY 1965
Δ AUGUST 1964
o SEPTEMBER 1964

Map 5. - Arabian Sea


45°
60°
65°
70°
75°
25°
20°
15°
5°
0°
10°
KARACHI
BOMBAY
ADEN
190
183
185
199
171
180
SEYCHELLES
MAURITIUS
50°
55°
ARABIAN SEA
SAMPLING STATIONS
1964

APPENDIX A

APPENDIX A - TABLE 1

Algae requiring thiamine

(Modified after Provasoli, 1963)

CHLOROPHYCEAE

Brachiomonas submarina
Pyramimonas inconstans
Polytoma ocellatum
Stephanosphaera pluvialis
Prototheca zopfii
Polytomella caeca
Chlamydomonas moewusii (mutant)

BACILLARIOPHYCEAE

Amphipleura rutilans
Amphora coffaeiformis
Nitzschia closterium
Amphiprora paludosa var. duplex
Amphora coffaeiformis

CHRYSOPHYCEAE

Pleurochrysis scherffelii
Hymenomonas elongata
Isochrysis galbana
Microglena arenicola
Monochrysis lutheri
Prymnesium parvum
Coccolithus huxleyi
Ochrosphaera neapolitana
Syracosphaera sp.
Hymenomonas sp.
Pavolva gyrans
Syracosphaera elongata

CRYPTOPHYCEAE

Hemiselmis virescens
Rhodomonas sp.
Rhodomonas lens
Chilomonas paramecium

DINOPHYCEAE

Amphidinium klebsii
A. rhynchocephalum
Gynmodinium breve
Oxyrrhis marina

EUGLENOPHYCEAE

Euglena gracilis
E. pisciformis
E. viridis
Astasia quartana
A. chattonii

APPENDIX A - TABLE 2

Thiamine requirement in major classes of algae

(Modified after Provasoli, 1963)

Class	no. species examined	no. species requiring vitamins	B_1	B_1+B_{12}	B_1+B_{12} + Biotin	B_1 + Biotin	Per cent requiring B_1
Chlorophyceae	68	44	8	26			77
Euglenineae	9	9	1	6			78
Cryptophyceae	11	11	1	7			73
Dinophyceae	17	16			4	1	31
Chrysophyceae	27	26	10	9	2	1	85
Bacillariophyceae	40	19	4	4			42
Cyanophyceae	11	2					0
Rhodophyceae	5	5					0
Total	188	132	24	52	6	2	

APPENDIX A - TABLE 3

Thiamine requirement in algae

(After Droop, 1962)

Species	Thiazole	Pyrimidine	Reference
Chlorophyta			
Polytoma ocellatum	+	-	Lwoff (1947)
Brachiomonas submarina	+	-	Droop (1961)
Stephanosphaera pluvialis	+	-	Droop (1961)
Prototheca zopfii	+	+	Anderson (1945)
Polytomella caeca	+	+	Lwoff (1947)
Chlamydomonas moewusii (mutant)	-	+	Lewin (1952)
Cryptophyta			
Hemiselmis virescens	+	-	Droop (1958)
Chilomonas paramecium	+	+	Lwoff (1947)

APPENDIX A - TABLE 3 (continued)

Species	Thiazole	Pyrimidine	Reference
Pyrrophyta			
Oxyrrhis marina	+	-	Droop (1958)
Chrysophyta			
Monochrysis lutheri	-	+	Droop (1958)
Hymenomonas elongata	-	+	Droop (1958)
Prymnesium parvum	-	+	Droop (1958)
Microglena arenicola	-	+	Droop (1958)
Euglenophyta			
Euglena gracilis	-	+	Lwoff (1947)
E. pisciformis	+	+	Lwoff (1947)
E. viridis	+?	-?	Lwoff (1947)
Astasia quartana	+?	-?	Lwoff (1947)
A. chattonii	+?	-?	Lwoff (1947)

APPENDIX A - TABLE 4

Thiamine requirement in marine fungi

Species	no. of species examined	no. of species requiring thiamine	Reference
Phycomycetes	39	39	Vishniac (1961)
Ascomycetes			
Halosphaeria mediosetigera	1	1	*Sguros et al.*, (1963)
Lulworthia opaca	1	1	Gustafsson and Fries (1956)
Lulworthia salina	1	0	Gustafsson and Fries (1956)
Trematosphaeria orae-maris	1	1	Gustafsson and Fries (1956)
T. thalassica	1	1	Gustafsson and Fries (1956)
Pleospora purpurascens	1	1	Gustafsson and Fries (1956)
Sphaerulina orae-maris	1	1	Gustafsson and Fries (1956)

APPENDIX A -

Species	no. of species examined
S. longirostris	1
Remispora maritima	1
Deuteromycetes	
Culcitalna achraspora	1
Helicoma maritimum	1
Diplodia orae-maris	1

TABLE 4 (continued)

no. of species requiring thiamine	Reference
0	Gustafsson and Fries (1956)
0	Gustafsson and Fries (1956)
1	Sguros et al., (1962)
0	Gustafsson and Fries (1956)
0	Gustafsson and Fries (1956)

APPENDIX A - TABLE 5

Thiamine requirement in marine yeasts

(Ahearn and Roth, 1962)

Species	Number of isolates	Thiamine requirement	
		Essential	Partial
Asporogenous			
Candida parapsilosis	25	15	3
Candida tropicalis	12		1
Candida guilliermondii	11	2	1
Candida intermedia	3	2	
Candida curvata	2	1	
Candida tenuis	1	1	
Rhodotorula glutinis var. dairiensis	8	7	1
Rhodotorula aurantiaca	4	3	1
Rhodotorula mucilaginosa	52	48	4
Rhodotorula pilimanae	17	14	3
Rhodotorula minuta	24	24	

APPENDIX A - TABLE 5 (continued)

Species	Number of isolates	Thiamine requirement	
		Essential	Partial
Cryptococcus laurentii	8	4	3
Cryptococcus albidus	6	5	1
Cryptococcus neoformans var. uniguttulatus	2		1
Torulopsis famata	6	2	1
Torulopsis sp.	3	1	
Trichosporon cutaneum	15	8	1
Sporogenous			
Debaryomyces kloeckeri	9	3	2
Hanseniaspora valbyensis	1		1

APPENDIX A - TABLE 6

Summary of thiamine assay organisms in sea water

Organism	Sensitivity	Incubation	Measurement	Reference
Gyrodinium cohnii	0.1-4 ng/ml	7 days	Cell count and OD	Provasoli and Gold (1959, 1962)
Unidentified bacterium	4 ng-1 µg/ml	3 days	Agar plate	Burkholder,(1959)
Unidentified non-filamentous fungus	.025-0.5 ng/ml	7 days	OD	Vishniac,(1961)

APPENDIX B

APPENDIX B - TABLE 1

PRINCE WILLIAM SOUND

USCGSS HODGSON CRUISE 2 JULY 20-24,1964

SURFACE SAMPLES

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
60 36.8	145 41.7	0013	11.5		239.8
60 35.5	145 56.6	0209	12.5		245.5
60 33.8	146 13.9	0135	14.5		273.9
60 32.0	146 32.0	0119	14.0		248.9
60 29.0	146 51.3	0384	15.5		169.4
60 25.0	147 05.6	0166	15.5		275.1
60 20.1	147 16.9	0128	14.5		285.3
60 14.8	147 16.9	0024	13.5		271.7
60 16.7	147 28.4	0024	13.5		229.6
60 16.7	147 28.4	0024	13.0		246.7
60 14.0	147 36.1	0172	13.5		310.3
60 09.4	147 51.7	0285	15.0		271.7
60 16.1	148 07.0	0236	10.5		230.8
60 16.3	147 58.8	0486	15.5		270.5
60 26.3	147 52.8	0366	15.5		242.1
60 36.7	147 44.2	0732	15.5		146.6
60 47.3	147 33.7	0507	16.0		146.6
60 55.5	147 34.0	0309	14.5		143.5
60 47.7	147 08.1	0411	12.0		098.8
60 54.2	146 55.9	0338	12.5		125.8

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
61 02.0	146 42.7	0320	11.0		023.9
61 07.2	146 21.7	0201	.		000.0
60 56.1	146 53.6	0310	12.0		056.8
60 47.0	147 19.6	0422	12.0		138.1
60 40.2	147 27.5	0027	12.0		142.1
60 31.3	147 48.5	0567	12.0		157.8
60 21.8	147 56.4	0192	13.0		196.5
60 35.6	146 32.4	0130	12.5		157.8
60 39.8	146 19.9	0129	12.0		138.9
60 42.8	146 05.0	0015	.		044.2

APPENDIX B - TABLE 2

PRINCE WILLIAM SOUND

USCGSS HODGSON CRUISE 2 JULY 20-24,1964

VERTICAL SAMPLES

LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
60 44.8	147 35.2	0000			136.2
		25			087.3
		75			000.0
		100			000.0
		150			000.0
		200			000.0
		450			000.0
		500			000.0
60 15.9	148 06.8	0000			219.4
		25			130.2
		50			000.0
		75			000.0
		100			000.0
		150			000.0
		200			000.0

APPENDIX B - TABLE 3

GULF OF ALASKA

MONTAGUE I. - MIDDLETON I.

USCGSS HODGSON CRUISE 3 JULY 29,1964

SURFACE SAMPLES

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
60 13.5	146 46.4	0238	13.5		011.0
60 03.1	146 39.3	0110	13.5		005.0
59 52.2	146 34.3	0064	13.0		014.0
59 41.5	146 29.5	0090	13.0		016.0
59 28.6	146 17.9	0011	13.0		024.0
59 31.2	146 32.2	0095	13.0		030.0
59 32.3	146 53.9	0126	13.0		015.0
59 33.6	147 15.2	0201	13.0		045.0
59 34.8	147 36.3	0121	13.5		046.0
59 41.1	147 54.0	0065	13.5		041.0

APPENDIX B - TABLE 4

GULF OF ALASKA

VALDEZ - KODIAK

USCGSS SURVEYOR CRUISE 1 MAY 5-8,1964

SURFACE SAMPLES

LAT. (N)	LONG. (W)	TEMP. (C)	SAL. (0/00)	THIAMINE FILT. (NG/L)	THIAMINE UNFILT. (NG/L)
60 46.3	148 08.9	04.5		077.0	074.3
60 40.4	148 02.6	05.1		044.0	038.1
60 36.1	147 52.2	04.8		027.6	041.1
60 36.0	147 37.5	04.5		000.0	000.0
60 16.1	147 51.7	04.8		011.6	025.0
59 03.0	144 23.0	05.1		016.4	008.2
58 36.8	145 37.0	04.7		044.4	056.8
59 07.0	147 00.0	04.7		045.6	055.0
59 38.0	148 04.0	04.9		000.0	000.0
59 36.0	149 10.0	05.1		080.3	082.5
57 52.4	147 04.0	05.1		113.6	134.0
57 30.0	147 40.0	05.1		007.6	012.1
57 22.0	149 00.0	04.8		140.8	134.0
56 46.0	150 16.0	04.8		103.0	097.0
56 17.0	150 19.0	04.9		095.4	069.7
55 55.0	151 10.0	04.4		089.4	004.5

APPENDIX B - TABLE 5

GULF OF ALASKA

VALDEZ - KODIAK

USCGSS SURVEYOR CRUISE 1 MAY 5-8,1964

VERTICAL SAMPLES

LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP (C)	SAL. (0/00)	THIAMINE FILT. (NG/L)	THIAMINE UNFILT. (NG/L)
58 55.6	148 55.2	0000	05.0	32.04	044.2	054.2
		10	04.8	32.08	050.0	051.5
		20	04.8	32.08	048.4	010.6
		30	04.8	32.08	038.5	033.3
		50	04.8	32.24	000.0	000.0
		75	04.9	32.54	000.0	000.0
		100	04.9	32.84	000.0	000.0
		125	05.1	33.36	000.0	000.0
		150	05.1	33.64	000.0	000.0
		170	05.0	33.75	000.0	000.0
		190	04.7	33.84	000.0	000.0
		245	04.3	33.94	000.0	000.0

APPENDIX B - TABLE 6

GULF OF ALASKA

CORDOVA - JUNEAU

USCGSS HODGSON CRUISE 4 AUGUST 31-SEPTEMBER 2,1964

SURFACE SAMPLES

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
60 37.5	145 41.2	0011	11.5		238.3
60 35.3	146 01.5	0170	12.5		179.3
60 33.5	146 23.8	0113	13.5		129.2
60 29.0	146 41.8	0198	12.0		124.1
60 20.0	146 47.0	0258	12.5		085.3
60 11.8	146 37.3	0179	13.5		050.2
60 06.8	146 22.0	0055	13.0		065.2
60 01.2	146 01.2	0073	13.4		097.8
59 57.0	145 49.1	0082	13.4		096.6
59 52.6	145 23.6	0091	14.0		087.8
59 49.0	145 07.5	0192	14.0		122.9
59 43.2	144 40.7	0048	13.5		124.1
59 39.8	144 18.3	0135	14.0		059.1
59 39.0	143 57.5	0115	13.5		046.7
59 38.0	143 37.5	0128	13.5		047.9
59 37.0	143 17.0	0265	13.5		053.2
59 36.0	142 56.0	0366	13.5		108.1

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
59 35.0	142 35.0	0521	13.5		076.2
59 34.0	142 15.5	0240	13.0		049.6
59 33.0	141 55.0	0165	13.5		051.4
59 32.0	141 34.0	0137	14.0		053.2
59 31.0	141 12.0	0146	12.8		044.9
59 29.9	140 52.5	0293	13.0		055.5
59 29.8	140 31.5	0256	12.3		060.9
59 29.9	140 09.8	0201	10.5		032.6
59 34.2	139 55.2	0064	10.5		065.2
59 34.3	139 49.5	0079	12.8		029.6
59 29.2	140 00.5	0155	12.0		086.9
59 19.6	139 36.0	0161	14.0		116.5
59 15.8	139 26.5	0155	14.0		123.8
59 09.4	139 13.3	0082	13.0		137.1
59 02.5	138 56.0	0095	13.5		137.1
58 57.3	138 45.5	0117	13.5		178.7
58 48.4	138 23.6	0073	13.5		137.1
58 41.9	138 07.5	0091	13.0		119.6
58 35.2	137 45.0	0097	13.0		158.2
58 28.5	137 33.8	0174	12.5		110.0
58 21.5	137 17.0	0183	12.5		139.9
58 14.4	137 00.8	0123	12.5		132.9
58 10.2	136 43.4	0095	10.5		095.5

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
58 13.4	136 30.7	0229	08.5		074.9
58 18.0	136 16.1	0238	09.0		089.4
58 19.6	135 56.4	0040	08.0		052.4
58 16.0	135 34.2	0128	10.0		176.9
58 12.0	135 14.1	0283	12.0		121.2
58 13.3	134 59.0	0627	12.0		147.9
58 24.3	134 59.0	0406	12.0		177.8
58 19.5	134 46.5	0077	11.5		130.5

APPENDIX B - TABLE 7

GULF OF ALASKA

JUNEAU - CORDOVA

USCGSS HODGSON CRUISE 1 APRIL 29-MAY 2,1964

SURFACE SAMPLES

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
58 13.2	134 35.7	0048	.	28.64	318.0
58 21.3	134 49.8	0062	06.0	20.93	490.4
58 21.7	134 59.2	0439	05.5	35.60	288.0
58 10.2	135 01.0	0201	06.5	27.95	348.0
58 13.5	135 21.7	0263	06.0	24.99	178.0
58 17.1	135 42.2	0143	05.4	30.79	103.0
58 19.8	136 04.0	0119	06.0	30.41	019.0
58 16.1	136 25.8	0219	06.0	28.25	066.0
58 09.8	136 42.4	0097	05.7	30.80	070.7
58 13.8	137 00.5	0135	06.0	29.43	046.2
58 20.0	137 15.5	0192	06.0	29.52	042.4
58 28.4	137 33.0	0174	06.0	32.56	000.0
58 35.3	137 49.7	0101	06.1	18.53	047.2
58 42.0	138 03.0	0097	06.3	30.89	037.7
58 49.2	138 21.5	0071	06.5	23.60	013.2
58 55.7	138 38.7	0133	06.5	28.74	033.0
59 02.3	138 59.9	0093	06.0	30.11	054.7
59 09.9	139 11.7	0077	05.5	31.87	077.3
59 15.9	139 29.8	0154	05.2	28.64	049.3

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
59 22.8	139 45.3	0150	06.0	30.50	105.2
59 34.4	139 55.4	0037	06.8	35.30	068.0
59 31.5	139 59.5	0049	06.5	30.01	014.0
59 30.8	140 19.8	0198	06.5	32.07	000.0
59 31.2	140 41.0	0265	06.5	23.41	141.4
59 31.2	141 03.2	0227	06.7	29.72	141.5
59 31.1	141 24.6	0134	07.0	31.29	108.0
59 31.5	141 46.6	0157	07.0	31.68	092.2
59 31.8	142 08.4	0179	06.4	32.17	000.0
59 32.0	142 31.6	0611	06.0	30.80	000.0
59 32.8	142 56.9	0640	06.0	32.66	000.0
59 34.5	143 18.0	0280	06.0	33.05	000.0
59 37.5	143 30.5	0234	05.5	30.01	021.0
59 38.8	143 51.5	0146	05.5	31.58	072.0
59 42.0	144 15.8	0155	05.5	31.29	000.0
59 39.8	144 34.0	0146	06.5	30.41	000.0
59 43.0	144 53.2	0106	06.0	31.97	019.1
59 48.0	145 13.0	0168	05.0	33.44	009.6
59 52.4	145 32.0	0086	05.0	30.41	000.0
59 55.8	145 47.8	0088	05.1	30.11	000.0

LAT. (N)	LONG. (W)	WATER DEPTH (M)	TEMP. (C)	SAL. (0/00)	THIAMINE (NG/L)
60 02.5	146 06.8	0064	06.0	26.27	000.0
60 07.6	146 27.4	0069	06.0	30.70	002.0
60 13.0	146 48.2	0198	04.9	29.52	103.0
60 24.6	146 48.1	0278	05.5	27.46	066.0
60 31.7	146 29.5	0110	05.0	31.19	083.0
60 34.5	146 08.5	0139	05.5	30.31	396.0

APPENDIX B - TABLE 8

GULF OF ALASKA

SEATTLE - JUNEAU

R/V ACONA CRUISE 1 SEPTEMBER 22-OCTOBER 8,1964

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
001	48 23.0	124 22.0	0000	10.17	30.93	05.01	202.2
			10	10.05	30.93	04.94	185.5
			19	09.65	31.19	04.20	156.7
			29	09.27	31.41	03.61	124.4
			48	07.95	33.02	02.73	015.6
002	48 29.1	125 11.8	0000	11.01	32.34	05.23	135.5
			10	09.56	32.66	03.91	084.4
			20	08.08	33.17	02.58	054.4
			30	07.84	33.25	02.28	052.2
			50	06.86	33.79	01.58	035.6
002A	48 38.1	126 07.0	0000				96.1
003	48 29.5	128 13.0	0000	15.48	32.05	05.35	164.4
			10	14.58	32.01	05.31	080.0
			20	14.57	32.04	05.46	202.2
			30	14.09	.	05.34	200.0
			50	08.30	32.36	05.71	058.9
003A	48 28.0	126 57.0	0000				125.3
004	48 30.0	129 41.0	0000	13.94	31.95	05.47	057.9
			10	13.09	31.95	05.46	055.6
			20	12.99	32.01	05.33	059.5
			30	10.88	32.42	05.62	010.8
			50	07.16	32.62	06.03	000.0
			74	06.93	32.76	05.87	000.0
			100	06.81	33.14	04.63	000.0
			147	06.88	33.87	03.17	000.0
			200	06.51	34.01	02.28	000.0
			297	05.62	33.90	01.47	000.0
			396	04.97	33.90	00.88	000.0
			484	04.78	34.19	00.50	000.0
			582	04.27	34.30	00.36	000.0
			784	03.78	34.32	00.36	000.0
			972	03.36	34.46	00.29	000.0
			1164	.	34.51	00.44	000.0
			1358	02.72	34.53	00.54	000.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
004A	48 27.0	129 05.0	0000				77.9
005	48 30.8	129 41.8	0000	14.46	32.42	05.49	031.2
			10	14.25	32.39	05.38	051.9
			20	14.08	32.46	05.49	031.2
			30	14.01	32.50	05.41	034.6
			50	08.51	32.64	06.16	000.0
			75	07.40	32.64	06.01	000.0
			100	06.71	32.87	05.58	000.0
			150	06.53	33.68	04.48	000.0
			199	06.41	33.91	03.77	000.0
			299	05.50	33.98	02.65	000.0
			398	04.91	34.06	00.91	000.0
			498	03.54	34.18	01.39	000.0
			598	04.13	34.27	00.44	000.0
			797	03.71	34.33	00.29	000.0
			996	03.38	34.40	00.28	000.0
			1395	02.52	34.50	00.64	000.0
006	48 29.0	131 39.8	0000	14.27	32.62	05.41	151.1
			10	14.27	32.51	05.43	141.1
			20	14.07	32.64	05.50	143.3
			30	.	32.55	05.42	166.7
			50	09.63	32.69	05.90	097.8
			74	07.40	32.80	06.05	027.8
007	49 17.5	132 30.0	0000	13.62	32.42	05.54	092.4
			10	13.64	32.50	05.46	091.0
			20	13.65	32.86	05.52	086.7
			50	09.57	32.59	05.91	064.8
			75	06.96	32.65	06.03	015.2
			100	06.41	32.82	06.03	012.4
			150	05.93	33.06	04.20	011.0
			200	05.67	33.92	03.29	019.1
			300	04.77	33.83	02.28	014.8
			400	04.35	34.02	01.47	013.8
			500	04.15	34.12	00.95	013.8
			600	03.86	34.32	00.64	011.9

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (O/OO)	OXY. (ML/L)	THIAMINE (NG/L)
008	50 30.0	133 29.0	0000	13.01	32.30	05.53	250.7
			10	12.79	32.23	05.52	154.6
			20	12.79	32.31	05.67	131.2
			30	08.94	32.53	05.68	072.6
			50	06.83	32.69	05.86	056.2
			75	06.49	32.82	05.32	044.5
			100	06.29	33.32	04.50	049.2
			150	06.04	33.83	03.21	035.1
			200	05.87	33.94	02.50	035.1
			298	04.82	34.37	01.68	046.9
			397	04.41	34.04	01.09	046.9
			496	04.27	34.15	00.69	042.2
			595	04.01	34.18	00.51	072.6
			793	03.53	34.28	00.36	044.5
			986	03.14	34.39	00.44	053.9
			1176	02.79	34.46	00.43	051.5
			1328	02.47	34.49	00.51	056.2
009	51 04.7	132 02.0	0000	12.50	32.34	05.54	093.9
			10	12.51	32.29	05.43	095.6
			20	12.53	32.31	05.60	099.9
			30	12.55	32.20	05.53	091.3
			50	.	32.55	04.52	034.2
			75	07.01	32.99	04.29	019.9
			100	06.81	33.40	03.53	017.3
			150	06.46	33.90	02.82	021.2
			200	06.08	33.95	02.21	013.0
			300	05.15	34.19	01.57	019.9
			400	04.68	34.12	01.12	015.6
			499	04.35	34.17	00.66	018.6
			598	04.17	34.26	00.46	016.4
			796	03.62	34.34	00.33	012.1
			995	03.19	34.46	00.36	012.5
			1194	02.92	34.36	00.42	015.1
			1392	02.52	.	00.53	013.4

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
010	51 32.3	130 40.0	0000	12.34	31.92	05.68	059.4
			10	12.16	31.88	05.53	059.4
			20	12.09	31.85	05.46	080.3
			30	11.79	31.96	05.29	081.8
			50	08.21	32.84	03.62	059.4
			75	06.79	33.60	02.98	034.0
			100	06.54	34.09	02.60	030.9
			150	06.27	34.04	01.84	021.6
			185	06.13	34.01	01.74	030.1
			278	05.84	34.14	01.43	021.6
			371	05.35	34.10	01.06	027.0
			557	04.71	34.18	00.53	034.7
			742	04.07	34.30	00.34	014.7
			928	03.71	34.45	00.30	027.8
			1113	03.16	34.47	00.44	026.2
011	51 57.0	129 36.0	0000	12.36	31.73	05.60	151.2
			10	12.29	31.70	05.47	084.5
			19	12.18	31.72	05.40	088.9
			29	10.99	32.05	04.50	062.3
			48	07.84	32.94	03.31	084.5
			72	06.97	33.29	03.01	062.3
			97	06.43	33.54	02.30	057.8
			145	05.95	33.77	01.78	051.1
011A	53 10.6	128 42.0	0000				60.8
011B	53 13.8	128 47.4	0000				059.6
011C	53 18.1	128 55.9	0000				087.6
012	53 16.7	129 07.3	0000	10.72	20.63	05.77	237.9
			10	11.14	30.29	05.60	204.6
			20	09.60	31.10	04.09	160.1
			30	06.20	31.38	03.48	104.5
			50	08.16	31.84	03.16	104.5
			75	07.36	32.48	02.65	102.3
			99	07.00	32.84	02.44	055.6
			149	06.73	33.11	02.49	011.1
			440	06.66	33.38	02.18	080.0
012A	53 20.5	129 14.4	0000				159.4
012B	53 26.0	129 25.6	0000				165.5

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
012C	53 31.4	129 34.6	0000				129.0
012D	53 35.3	129 40.7	0000				069.3
012E	53 39.3	129 45.0	0000				051.1
013	54 31.0	130 33.0	0000	10.59	28.11	05.38	178.3
			11	10.62	30.55	04.66	121.7
			22	10.03	31.17	04.15	088.7
			34	09.33	31.54	03.69	071.7
			55	08.12	32.17	03.60	032.1
			80	06.84	32.68	02.43	022.6
			105	06.24	33.25	02.19	025.5
013A	54 43.0	130 50.0	0000				144.8
013B	54 59.4	131 05.1	0000				136.3
013C	55 07.3	131 11.1	0000				086.4
013D	55 12.8	131 21.8	0000				164.2
013E	55 18.8	131 35.9	0000				147.2
014	55 39.0	132 15.8	0000	10.59	29.33	05.44	265.7
			10	10.47	29.45	05.29	175.9
			20	10.20	29.71	05.01	148.0
			30	09.01	30.46	04.20	125.0
			50	07.71	31.45	03.12	065.5
			75	07.08	32.25	02.60	047.3
			100	06.65	32.94	02.33	041.3
			149	05.86	33.49	01.93	032.8
015	56 24.2	133 11.1	0000	08.49	30.07	04.43	112.9
			10	08.25	30.79	04.14	107.9
			20	08.02	31.25	03.99	076.1
			30	.	31.61	03.88	093.9
			40	07.77	31.78	03.76	085.0
			50	07.70	31.73	03.69	058.4
			75	07.57	32.00	03.54	054.6
			100	07.50	32.03	03.48	039.3

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
016	55 44.0	134 14.0	0000	10.77	31.45	05.54	054.4
			10	09.07	31.42	05.60	073.1
			20	10.74	31.58	05.32	090.0
			30	10.04	31.94	04.79	080.6
			40	09.79	32.01	04.60	078.8
			50	08.86	32.35	04.29	050.6
			75	07.78	32.76	04.11	043.1
017	55 21.0	135 36.0	0000	11.43	32.32	05.60	121.9
			10	11.43	32.39	05.57	123.8
			20	11.43	32.36	05.60	125.6
			30	11.71	32.48	05.57	106.9
			50	06.93	32.85	05.99	060.0
			75	05.62	33.13	06.19	026.3
			100	05.18	32.96	06.05	035.6
018	56 07.8	134 24.9	0000	09.73	31.62	05.40	052.8
			10	09.96	31.72	05.17	061.5
			20	09.86	31.74	05.04	057.1
			30	09.19	31.84	04.87	041.8
			50	08.79	31.92	04.39	059.3
			75	07.72	32.35	03.59	040.7
			100	07.38	32.67	03.40	040.7
			150	05.96	33.42	02.65	023.1
			297	05.18	33.95	01.86	013.2
			386	04.96	33.90	01.63	006.6
			438	04.85	33.95	01.55	000.0
019	57 10.9	134 40.7	0000	07.68	30.33	04.98	071.3
			10	07.68	30.35	04.87	066.0
			20	07.68	30.54	04.92	070.0
			30	07.61	30.57	04.57	194.0
			50	07.06	31.52	03.76	046.2
			75	06.20	32.57	02.80	038.3
			100	05.75	33.03	02.38	025.1
			150	05.59	33.18	02.22	034.3
			200	05.26	33.45	01.95	000.0
020	58 08.0	135 03.5	0000	08.87	29.09	05.77	195.4
			10	08.38	30.13	05.18	118.8
			20	07.95	30.58	05.32	122.8
			30	05.72	30.77	04.28	062.0
			50	07.08	31.39	03.94	055.4
			75	06.55	31.80	03.32	051.5
			100	05.81	32.35	02.80	033.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
021	58 10.0	135 25.0	0000	08.19	29.75	05.44	194.9
			10	07.84	30.49	04.63	111.1
			25	07.31	30.94	04.28	023.4
			50	07.16	31.25	03.96	000.0
022	58 13.5	135 21.7	0010	08.77	28.91	05.80	091.2
			30	07.39	30.85	04.32	039.6
			50	.	31.93	03.91	017.6
			100	06.11	32.45	02.69	033.0
023	58 09.0	136 45.5	0000	08.85	31.74	04.87	036.7
			10	08.85	31.68	04.77	033.7
			25	08.81	31.84	04.81	041.1
			50	07.83	31.88	04.14	027.9
			75	07.27	32.22	03.76	019.1
024	58 21.0	137 19.0	0000	11.17	31.53	05.52	049.9
			10	11.19	31.48	05.50	042.5
			20	11.19	31.48	05.57	086.5
			30	11.25	31.49	05.43	088.0
			50	11.19	31.46	05.44	041.1
			75	09.18	31.00	04.36	032.3
025	58 10.0	137 50.0	0000	10.75	31.54	05.56	049.9
			10	10.75	31.54	05.52	052.8
			20	10.77	31.53	05.66	033.7
			30	10.78	31.53	05.53	039.6
			50	10.05	31.73	04.84	026.4
026	57 40.5	138 32.0	0000	10.58	31.90	05.57	060.6
			10	10.58	31.80	05.40	072.0
			20	10.58	31.83	05.46	067.5
			30	10.73	31.85	05.44	064.4
			50	08.33	32.43	04.53	025.0
			75	06.75	32.67	03.87	020.5
027	57 44.0	137 14.0	0000	10.57	31.44	05.63	058.4
			10	10.57	31.41	05.63	057.6
			20	10.56	31.38	05.63	055.3
			30	10.14	31.71	05.00	038.7
			50	08.75	32.23	04.28	027.3
			75	08.08	32.53	04.98	019.0
			100	06.66	32.84	04.28	016.0
			150	05.99	33.43	03.34	008.3

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
028	57 47.4	136 41.5	0010	10.92	31.37	05.84	065.0
			20	10.94	31.34	05.90	056.3
			30	10.76	31.64	05.57	055.6
			50	10.00	31.87	04.51	025.5
			75	08.11	32.29	04.07	014.1
			100	06.81	32.72	04.56	065.0
028A	58 15.0	136 25.0	0000				47.0
028B	58 36.3	136 06.0	0000				40.2
029	58 58.0	136 07.6	0000	02.36	24.18	05.46	022.8
			5	04.68	29.37	04.73	025.5
			10	04.91	30.16	04.57	027.5
			20	05.32	30.05	04.44	005.4
			30	05.87	30.61	04.41	013.4
			50	06.54	30.56	04.37	023.5
			100	06.73	30.77	04.26	024.1
030	58 40.5	136 06.7	0000	05.94	29.70	05.03	000.0
			10	06.43	30.11	04.61	000.0
			25	06.90	30.88	04.30	000.0
			50	06.99	30.85	04.29	000.0
			100	06.81	31.01	04.29	000.0
030A	58 20.0	136 02.1	0000				037.1
031	58 15.2	135 41.1	0000	07.66	30.63	04.73	000.0
			10	07.55	30.88	04.50	000.0
			20	07.17	31.19	04.20	000.0
031A	58 21.2	134 59.1	0000				122.1
032	58 21.3	134 49.8	0000	08.53	27.57	06.48	293.4
			5	08.42	28.13	05.94	305.4
			10	08.42	28.71	05.77	138.6
			15	08.07	29.56	04.98	062.2
			20	07.93	29.90	04.80	035.5
			25	07.43	30.56	04.35	000.0
			30	06.84	31.21	03.83	000.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
033	58 12.0	134 38.0	0000	08.84	27.24	07.54	441.2
			5	08.36	28.91	05.55	198.8
			10	08.17	29.32	05.09	118.8
			20	07.86	30.08	04.54	230.0
			30	06.84	30.78	04.13	130.6
			40	.	30.97	03.83	048.7
			50	06.72	31.21	03.65	019.5

APPENDIX B - TABLE 9

GULF OF ALASKA

JUNEAU - CAPE SPENCER

R/V ACONA CRUISE 3 NOVEMBER 10-19,1964

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
034	58 12.0	134 37.0	0000	06.02	29.36	06.40	025.8
			10	06.02	29.35	06.37	008.1
			20	06.32	29.60	05.83	015.3
			30	06.61	30.74	04.69	025.0
			40	06.65	30.66	04.36	021.0
035	58 25.0	134 57.5	0000	06.07	.	06.04	040.3
			10	06.14	29.48	05.87	011.3
			20	06.19	29.58	05.92	012.1
			30	06.34	29.78	05.96	017.0
			55	06.47	30.76	04.95	012.9
036	58 13.8	135 23.7	0000	06.23	29.80	06.44	028.2
			9	06.36	.	06.24	000.0
			18	06.61	30.19	05.95	000.0
			27	06.64	30.22	05.95	000.0
			36	06.75	30.41	05.51	000.0
			45	06.73	30.55	05.21	000.0
			68	06.83	31.01	04.67	000.0
037	58 17.3	135 41.7	0000	06.13	29.72	06.36	012.9
			10	06.25	29.88	06.04	024.2
			20	06.38	30.16	05.70	000.0
			29	06.34	30.52	.	008.1
			39	06.34	30.66	05.36	027.6
			49	06.39	30.84	05.11	012.1
			74	06.30	.	04.62	012.9
038	58 47.0	136 24.9	0000	04.72	28.87	06.82	000.0
			10	05.34	29.38	06.39	000.0
			20	06.36	30.15	05.62	000.0
			30	06.49	.	05.29	000.0
			40	06.41	30.82	05.54	000.0
			50	06.52	.	05.52	000.0
			75	06.71	.	05.18	000.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
039	58 16.9	136 13.2	0000	06.30	30.72	05.70	009.7
			10	06.32	30.77	05.62	000.0
			20	06.35	.	05.63	000.0
			30	06.41	30.86	05.52	000.0
			40	06.40	30.88	05.47	000.0
			50	06.49	30.97	05.43	015.8
			75	06.76	.	05.40	021.8
044	58 03.5	136 41.0	0000	07.96	31.53	06.56	000.0
			9	07.96	31.49	06.44	000.0
			19	08.10	31.59	06.37	000.0
			28	08.51	31.78	06.20	000.0
			38	08.52	31.79	06.21	000.0
			47	08.02	31.80	06.08	054.9
			70	07.55	31.87	05.20	000.0
045	58 00.0	137 14.0	0000	08.36	32.07	06.27	000.0
			10	08.37	32.07	06.20	000.0
			20	08.37	32.10	06.26	000.0
			30	08.46	32.09	06.16	000.0
			50	08.50	32.27	06.46	000.0
			75	08.44	32.29	06.30	000.0
			100	07.24	32.73	04.27	000.0
046	58 21.0	137 18.0	0000	07.97	31.46	06.65	000.0
			10	08.00	31.47	06.43	000.0
			20	08.01	31.48	06.53	000.0
			30	08.04	31.49	06.51	000.0
			50	08.05	31.54	06.29	000.0
			75	08.34	31.77	06.12	000.0
			99	08.36	31.86	06.04	000.0
047	58 10.0	137 50.0	0000	07.88	31.75	06.35	010.9
			10	07.88	31.75	06.15	012.5
			20	07.95	31.77	06.20	039.3
			30	08.40	31.88	06.25	020.1
			50	08.51	32.07	05.97	020.9
			75	08.34	32.12	05.78	029.3
048	58 00.0	137 50.1	0000	08.39	32.15	.	025.9
			10	08.41	32.12	06.14	000.0
			20	08.33	32.15	06.20	000.0
			30	08.22	32.15	06.34	000.0
			50	08.34	32.27	06.40	016.7
			75	07.85	32.51	04.53	059.4

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
049	58 00.0	138 35.0	0000	08.07	32.19	06.46	010.0
			10	08.08	32.18	06.47	009.2
			19	08.07	32.19	06.44	027.6
			28	08.07	32.14	06.44	015.9
			43	08.10	32.18	06.52	031.0
			78	07.03	32.77	03.94	000.0
			990	03.19	34.29	00.40	000.0
			1188	02.79	34.36	00.43	000.0
050	58 02.4	136 21.9	0000	07.06	29.32	06.63	000.0
			10	07.58	31.15	06.29	000.0
			20	07.56	31.29	06.37	015.9
			30	07.63	31.40	06.27	024.3
			50	07.62	31.52	06.06	013.4
			75	07.42	31.59	05.55	008.4
051	57 56.1	136 12.0	0000	06.37	24.43	06.64	070.0
			30	07.86	31.39	05.60	011.7
			50	08.04	31.38	05.08	021.1
			75	08.10	31.69	04.81	019.7
			100	07.81	31.89	04.50	014.6
052	57 51.0	136 04.0	0000	04.17	09.43	08.15	023.3
			10	08.19	30.79	05.15	016.0
			20	08.61	31.57	04.21	029.2
054	57 10.0	137 00.0	0000	08.32	31.97	06.56	030.6
			10	08.31	31.96	06.39	014.6
			20	08.32	31.96	06.46	016.8
			30	08.33	31.97	06.39	016.8
			49	08.28	32.15	06.14	013.1
			74	08.24	32.31	05.94	029.9
057	57 11.0	134 39.7	0000	06.77	30.84	06.04	013.1
			10	06.77	30.84	05.98	031.3
			20	06.78	30.90	06.06	005.8
			30	06.79	30.92	05.96	008.7
			50	06.76	30.96	05.98	000.0

APPENDIX B - TABLE 10

SOUTHEAST ALASKA

PORT ANGELES - JUNEAU

R/V ACONA CRUISE 7 MARCH 18-22,1965

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
106	49 49.2	124 52.3	0000				149.0
107	50 27.8	126 03.6	0000				055.1
108	53 16.3	129 08.0	0000				234.1
109	53 55.1	130 13.7	0000				058.8
110	54 45.7	130 59.6	0000				070.1
111	55 39.0	132 16.0	0000				097.7
112	56 22.0	133 11.0	0000				052.6
113	56 08.0	134 26.0	0000				017.5
			10				036.3
			20				022.5
			30				062.6
			50				023.8
			75				027.5
			100				022.5
			150				017.5
			200				000.0
			300				017.7
			400				058.4
114	57 11.0	134 40.0	0000				065.2
			10				037.8
			20				006.4
			50				017.2
			75				021.1
			100				015.2
			150				025.0
			200				015.2
115	58 02.7	134 52.0	0000				028.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
116	58 21.3	134 47.0	0000				036.4
			10				022.7
			20				003.6
			30				014.9
			50				019.0
117	58 12.0	134 38.0	0000				014.8
			10				009.0
			30				015.8

APPENDIX B - TABLE 11

NORTH PACIFIC OCEAN

SAN DIEGO - KODIAK

R/V AGASSIZ CRUISE URSA MAJOR AUGUST 7-19,1964

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
001	33 48.0	135 01.0	0001				000.0
003	34 30.0	146 48.0	0000				0.0
			10				000.0
			20				000.0
			50				000.0
			75				000.0
			100				000.0
			150				000.0
			200				000.0
			497				000.0
			797				000.0
			995				000.0
			1194				000.0
			1492				000.0
011	49 00.0	154 58.0	0010				000.0
			19				000.0
			45				000.0
			66				000.0
			128				000.0
			167				000.0
			666				000.0
			1021				000.0
			1301				000.0
004	35 00.0	155 00.0	0000				0.0
			97				000.0
			484				000.0
			1460				000.0
			3568				000.0
005	37 00.0	155 10.0	0001				000.0
007	40 00.9	155 00.5	0001				000.0
008	43 06.0	155 01.0	0001				028.8

STA.	LAT. (N)	LONG. (E)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
009	45 00.0	155 00.0	0150				000.0
			4800				000.0
010	47 01.2	155 00.0	0001				017.3
011	49 00.0	154 58.0	0000				0.0
			100				000.0
			500				000.0
			1000				000.0
			2000				000.0
012	51 09.0	154 52.2	0001				016.7
013	54 34.0	155 00.0	0001				044.9
014	55 48.0	153 56.0	0001				088.5
014B	56 37.0	153 24.0	0001				147.4
015	57 44.0	151 40.0	0001				125.6

APPENDIX B - TABLE 12

NORTH PACIFIC OCEAN

VANCOUVER - HAWAII

R/V ACONA CRUISE 7 JANUARY 18-FEBRUARY 3,1965

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
071	45 15.0	127 44.9	0000				0.0
			10				000.0
			20				000.0
			30				000.0
			50				000.0
			75				042.9
			100				000.0
			150				000.0
			200				000.0
072	43 00.0	129 53.0	0000				066.6
			10				050.0
			20				018.0
			30				008.3
			50				044.4
			75				000.0
			100				000.0
			150				000.0
			200				000.0
			300				000.0
			400				000.0
			500				000.0
			600				000.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
073	41 19.5	131 29.5	0000				031.2
			10				020.0
			20				000.0
			30				009.6
			50				066.2
			75				000.0
			100				000.0
			150				000.0
			200				000.0
			300				000.0
			400				000.0
			500				000.0
			600				000.0
			800				000.0
			2000				000.0
			3000				000.0
073B	40 24.0	132 13.0	0000				021.8
074	39 18.0	133 15.0	0000				013.4
			10				000.0
			20				000.0
			30				000.0
			50				000.0
			75				000.0
			100				000.0
			150				000.0
			200				000.0
			300				000.0
			400				000.0
			500				000.0
			600				000.0
			800				000.0
			1000				000.0
			1200				000.0
			1500				000.0
			2000				000.0
			3000				000.0
074A	38 46.0	133 44.0	0000				0.0
074B	38 14.0	134 50.0	0000				054.2

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
075	38 00.0	135 46.0	0000				20.4
			20				028.3
			30				031.7
			50				000.0
			75				028.3
			100				000.0
			150				000.0
			200				000.0
			300				031.7
			400				000.0
			500				032.8
			600				041.8
			800				037.3
			1000				054.3
			1200				029.4
			1500				000.0
			2000				000.0
			3000				023.8
075A	37 40.0	136 05.0	0000				0.0
075B	36 22.0	137 32.0	0000				092.7
075C	35 30.0	137 56.0	0000				051.3
075D	35 05.0	138 05.0	0000				51.3

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
076	33 25.0	138 50.0	0000				29.8
			10				031.0
			20				028.6
			30				013.1
			50				016.7
			75				025.0
			100				020.2
			150				019.0
			200				000.0
			250				000.0
			300				000.0
			400				010.7
			500				008.3
			600				014.3
			800				060.7
			1000				020.2
			1200				025.0
			1500				011.9
			2000				000.0
			3000				000.0
			4000				025.0
076A	32 36.0	139 10.0	0000				068.8
076B	31 14.0	139 52.0	0000				021.8
077	30 36.0	140 00.0	0000				037.7
			10				016.7
			20				014.0
			30				018.9
			50				031.9
			75				024.0
			100				030.5
			150				017.0
			200				010.2
			250				015.0
			300				015.2
			400				020.0
			500				014.0
			600				024.0
			800				016.7
			1000				010.0
			1200				020.0
			1500				011.6
			2000				025.9
			3000				018.1
			4000				021.8

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
078	29 48.0	142 04.0	0000				48.7
			10				010.0
			20				000.0
			30				062.2
			50				000.0
			75				074.3
			100				000.0
			150				029.7
			200				000.0
			250				000.0
			300				021.6
			400				000.0
			500				012.2
			600				000.0
			800				000.0
			1000				067.6
078A	28 56.0	143 31.0	0000				077.8
079	28 42.0	145 00.0	0000				013.1
			10				015.7
			20				022.0
			30				016.8
			50				022.5
			75				014.7
			100				016.2
			150				012.6
			200				024.1
			250				008.4
			300				050.3
			400				000.0
			500				013.6
			600				017.8
			800				013.6
			1000				015.2
			1200				017.8
			2000				008.3
			3000				024.1
			4000				010.5
079A	27 30.0	146 49.0	0000				163.0

STA.	LAT. (N)	LONG. (W)	SAMP. DEPTH (M)	TEMP. (C)	SAL. (0/00)	OXY. (ML/L)	THIAMINE (NG/L)
080	26 42.0	148 24.0	0010				070.3
			30				000.0
			50				006.0
			75				000.0
			100				087.9
			150				083.1
			200				019.3
			250				000.0
			300				000.0
			400				027.7
			500				000.0
			600				028.9
			800				031.4
			1000				042.1
			1200				025.3
			1500				000.0
			2000				175.7
080A	24 40.0	151 40.0	0000				303.5
080B	23 37.0	151 20.0	0000				119.0
080D	21 29.0	152 25.0	0000				100.2
080E	21 17.0	153 08.0	0000				098.3
080F	21 02.0	154 13.0	0000				092.9
080G	20 49.0	155 11.0	0000				104.0
080H	20 43.0	155 39.3	0000				119.7
080I	20 40.0	155 48.0	0000				054.2
080J	20 40.0	155 56.0	0000				042.8
080K	20 37.2	156 04.2	0000				038.5
080L	20 34.2	156 12.9	0000				068.8
080M	20 33.5	156 23.0	0000				042.8
080N	20 37.2	156 31.0	0000				052.7
080O	20 40.4	156 39.0	0000				095.7
080P	20 40.4	156 48.3	0000				073.3

APPENDIX B - TABLE 13

ARABIAN SEA

R/V ANTON BRUUN CRUISE 4A OCTOBER 12-NOVEMBER 8,1964

STA.	LAT. (N)	LONG. (E)	SAMP. DEPTH (M)	CARBON (MG/SQ M/DAY)	THIAMINE (NG/L)
171	13 11.0	51 28.0	0000	0832	047.5
173	15 27.0	52 50.0	0015	1624	107.0
176	16 29.0	57 09.0	0000	1254	065.0
180	12 15.0	59 42.0	0026	0464	059.5
181	14 09.0	61 07.0	0026	1060	066.7
182	15 58.0	62 33.0	0028	0601	150.0
183	23 43.0	66 21.0	0020	2289	158.0
184	22 33.0	65 56.0	0016	0862	129.0
185	20 39.0	64 41.0	0015	0725	089.0
186	21 31.0	64 06.0	0015	2054	150.0
187	22 23.0	63 32.0	0015	1403	161.0
188	23 19.0	62 50.0	0015	1816	104.0
189	24 00.0	62 04.0	0015	2035	122.0
190	24 48.0	61 37.0	0014	1647	092.6
191	23 57.0	60 58.0	0015	2279	150.0
195	21 31.0	60 41.0	0015	5284	256.0
196	20 44.0	61 15.0	0012	1620	274.0
197	20 02.0	62 00.0	0015	1802	260.0
199	18 31.0	63 08.0	0000	1091	054.8

STA.	LAT. (N)	LONG. (E)	SAMP. DEPTH (M)	CARBON (MG/SQ M/DAY)	THIAMINE (NG/L)
198	19 17.0	62 29.0	0001		321.8
			10		187.3
			20		162.4
			30		286.9
			40		130.5
			50		105.6
			60		105.6
			70		074.7
			80		258.0
			90		091.6
			100		112.6