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Calcification in a Solitary Coral, *Fungia scutaria* Lamarck in Relation to Environmental Factors

KIYOSHI YAMAZATO

TABLE OF CONTENTS

ABSTRACT .................................................................................................................. 59
I. INTRODUCTION .................................................................................................. 60
   1. Calcification in corals .............................................................................. 60
   2. Outline of the present work .................................................................. 62
II. MATERIALS AND METHODS .......................................................................... 63
   1. Experimental animals ........................................................................... 63
   2. Media and procedures used for incubation ........................................... 64
   3. Preparation and measurement of radiocalcium and radiophosphorus 65
   4. Chemical analyses for calcium, phosphorus, and nitrogen ............... 66
   5. Radioautography ..................................................................................... 67
III. RESULTS ......................................................................................................... 68
   1. The effect of some environmental factors on calcium uptake ........... 68
      a. Temperature ...................................................................................... 68
      b. Salinity ............................................................................................ 70
      c. Calcium concentration .................................................................... 74
      d. Phosphate concentration .................................................................. 78
   2. The effect of the removal of zooxanthellae on calcium uptake ...... 82
   3. The effect of dinitrophenol on calcium uptake ................................... 85
   4. The effect of light on uptake and release of phosphorus ................. 87
      a. Phosphorus uptake .......................................................................... 87
      b. Distribution of radiophosphorus in tissues ..................................... 95
      c. Phosphorus release .......................................................................... 98
   5. The effect of temperature on phosphorus uptake ............................ 102
   6. The effect of the removal of zooxanthellae on phosphorus uptake 105
IV. DISCUSSION .................................................................................................... 107
   1. Factors controlling calcification ............................................................. 107
      a. Temperature ..................................................................................... 107
      b. Salinity .......................................................................................... 109

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c. Calcium concentration ......................................................... 109

d. Phosphate concentration ..................................................... 110

e. Other factors ................................................................. 112

2. Phosphorus metabolism in relation to calcification ...................... 114

V. SUMMARY ......................................................................... 115

VI. ACKNOWLEDGEMENT ......................................................... 116

VII. LITERATURE CITED .......................................................... 117

EXPLANATION OF PLATE FIGURES ......................................... 121

PLATE ................................................................................. 122
ABSTRACT

With the ultimate purpose of clarifying the role of zooxanthellae in calcification in corals, a study was conducted (1) to analyze the effects of some environmental factors on the rate of calcium uptake by Fungia scutaria Lamarck and (2) to obtain a clear picture of the uptake and release of phosphorus by the same animal under various conditions.

The effect of the factors investigated on the rate of calcium uptake was strikingly different in the light and in the dark. In the dark, the rate increased linearly with increase in temperature (15° - 32°C) and also with increase in the ambient calcium concentration from 0 to 200 mg/l. A steady state was maintained for calcium concentrations from 200 to 600 mg/l. A drastic decrease occurred in solutions of 75 to 25% sea water, and a lesser decrease in 125% sea water. The rate decreased linearly with the logarithmic increase in the ambient phosphate concentration from $10^{-7}$ (natural sea water) to $10^{-2}$ M.

In the light, the rate of calcium uptake was maximum at 24°C, and in natural and 75% sea water. Deviations from either of these conditions resulted in decrease in the rate of calcium uptake. Uptake rates increased linearly with increase in the ambient calcium concentration from 0 to 400 mg/l and with a steeper slope from 400 to 600 mg/l. The effect of the ambient phosphate concentration on calcium uptake was not clear but there was a general trend of decrease in the uptake rate with increasing concentration of phosphate. The greatest difference in the rate of calcium uptake between the dark and light conditions was observed at the lower phosphate concentrations. Dinitrophenol completely inhibited calcium uptake at concentrations of $10^{-4}$M and higher both in the light and in the dark. At a concentration of $10^{-5}$M a complete inhibition was observed in the dark but not in the light. The removal of zooxanthellae from the animal caused a decrease in the rate of calcium uptake to 1/12 the control value in the light and to 1/8 in the dark. These ratios were unaltered in the polyps devoid of zooxanthellae kept in the same container with the normal polyps.

The rate of phosphorus uptake from sea water supplemented with phosphate (2.7 µg-at. P/l) was approximately two times greater in the light than in the dark. Net uptake occurred both in the light and in the dark at this phosphate concentration, while net release occurred in the light and in the dark in natural sea water (0.27 µg-at. P/l). In the light, the polyps devoid of zooxanthellae absorbed phosphorus at the rate 1/8 that of the normal polyps; in the dark this ratio was about 1/6. Some indication of interaction between the normal and zooxanthella-free polyps in the same container was observed. The effect of temperature on phosphorus uptake was about the same as that on calcium uptake.

Approximately the same quantity of organic phosphorus (particulate and dissolved) was released in the light as in the dark, while nine times more inorganic phosphorus was released in the dark than in the light. More organic phosphate labelled with P$^{32}$ was released by the normal polyps than by the zooxanthella-free polyps under both light and dark conditions.
1. INTRODUCTION

1. Calcification in corals

Coral reef is one of the most distinctive manifestations of life processes acting upon the inanimate world and hence inducing changes upon it. Consequently, the study of corals and coral reefs has attracted the interests of many investigators. Since Darwin's first attempt to classify reef structures and to show how atolls are formed, numerous attempts have been made to estimate the growth rates of corals by measuring the increments in weight, size or number of polyps of a colony during periods of months to years. Noteworthy among these early investigators are Agassiz (1890), Vaughan (1910), Mayor (1924), Edmondson (1929), Tamura and Hada (1932), Stephenson and Stephenson (1933), Boschma (1936), Ma (1937), Abe (1940), Motoda (1940), and Kawaguti (1941).

Because the bulk of the coral body is composed of a mineral skeleton, and over 98% of the ash residue consists of calcium carbonate (CaCO₃) (Vinnogradov, 1953), deposition of CaCO₃ is considered to be the major feature of coral growth. The first attempt to measure the rate of calcium uptake by corals was made by Kawaguti and Sakumoto (1948). Their study included chemical determination of calcium in sea water surrounding corals. More effective and exact methods of measuring the rate of calcium uptake involving the use of radiocalcium (Ca⁴⁵) (Goreau and Bowen, 1955) and radiocarbon (C¹⁴) (Goreau, 1961b) have made it possible to examine factors affecting calcification. Using this technique, as will be seen below, Goreau and his coworkers investigated various aspects of calcification in corals and other reef-builders.

Extrapolating the information available from the previous investigations, the processes of calcification may be divided into three steps: (1) the source and entrance of calcium and carbonate ions into the coral body, (2) the transport of these ions from the site of entrance to that of deposition, and (3) the deposition of calcium carbonate outside the calicoblastic layer.

It has been shown that the primary source of both calcium and carbonate ions is sea water, with only negligible quantities being supplied from food (Goreau and Bowen, 1955; Goreau, 1959). A secondary source of carbonate is available from metabolism of dissolved glucose or other organic substances which are absorbed by corals from sea water (Stephens, 1962). It has been suggested on the basis of electronmicroscopic observations of epithelial cells that the general body surface serves as the site of entrance of these ions (Goreau and Philpott, 1956). No information is available to show how the uptake of calcium and carbonate is regulated, except a suggestion (Goreau, 1959) that the rate of uptake from sea water is directly proportional
to the rate of calcium carbonate deposition in the skeleton. This suggestion seems to be justified in view of the fact that the calcium content of the coral tissue is only 88 per cent of that of sea water (Goreau and Bowen, 1955). It has therefore been postulated that the coral tissue serves as a simple medium for calcium transport, and is intimately associated with the regulation of both uptake and deposition.

The direction of calcium transport through tissues appears to be irreversible under normal conditions. This was shown by the finding (Goreau and Goreau, 1960b) that the rate of Ca\textsuperscript{45} release by the living polyps was lower than the rate of diffusion exchange by the dead corallites.

The transport of carbonate through tissues apparently involves much more complex pathways than calcium. It has been observed (Goreau, 1961a) that more than half of C\textsuperscript{14}-carbonate taken up by corals was recovered in the organic fraction of the tissue. By the concurrent exposure of corals to Ca\textsuperscript{45} and C\textsuperscript{14}-carbonate he observed that the quantity of exchangeable carbonate in the tissue was 2 to 15 times greater than that of exchangeable calcium, further substantiating the irreversibility of calcium transport.

Histological evidence indicates that calcium carbonate is deposited on the organic matrix secreted by the calicoblastic epidermis (Matthai, 1918; Hayashi, 1937; Goreau, 1956a, b). In his studies Goreau (1959) identified an acid-mucopolysaccharide-like substance in the organic matrix and subsequently proposed that calcium was adsorbed by ion exchange on the acid space lattice provided by the mucopolysaccharide in the organic matrix. In contrast, Wainwright (1962) detected chitin in the skeleton of Pocillopora and proposed that chitin fibers served as the template for calcium carbonate deposition.

Ample evidences have been accumulated to show the importance of zooxanthellae in regulating the rate of calcification. It has been postulated that zooxanthellae accelerate the rate of calcification in the light by removing carbon dioxide (Goreau, 1959; Goreau and Goreau, 1959). Other metabolic wastes of corals such as phosphates (Yonge and Nicholls, 1931a, b), nitrates, sulfates and ammonia (Kawaguti, 1953) are also utilized by zooxanthellae in photosynthesis. Light is then an important environmental factor indirectly affecting the rate of calcification by its effect on the photosynthesis of zooxanthellae. However the fact that corals with zooxanthellae calcified at higher rate than corals without zooxanthellae in the dark suggests that the role of zooxanthellae in calcification involves some other activity in addition to that associated with photosynthesis. Goreau (1959) suggested the possible production of some stimulant (vitamin or hormone-like factors) by zooxanthellae.

Some phosphate compounds, such as pyrophosphate, adenosine triphosphate, glycerophosphate and orthophosphate, have been shown to inhibit deposition of calcium carbonate on an inorganic template in artificial sea water.
(Simkiss, 1964a, b, c). Consequently, Simkiss proposed that the stimulating effect of zooxanthellae on calcification is related to the removal of these inhibitors which might be accumulated in the site of calcium carbonate deposition in corals. Although Simkiss attempted to explain, on the basis of his proposal, the difference in the rate of calcification in the dark between normal corals and corals devoid of zooxanthellae, no evidence has been reported to show that zooxanthellae take up phosphate in the dark at comparable rates. Nevertheless, it has been shown (Yonge and Nicholls, 1931a, b) that normal corals take up phosphate from the medium under natural conditions, while corals devoid of zooxanthellae excrete phosphate into the medium. The role of phosphatase, which was found in coral tissues (Goreau, 1953) has not been understood in relation to either calcification or phosphate metabolism.

2. Outline of the present work

As can be seen in the above review, one of the unique features of calcification in corals is the participation of zooxanthellae in the process. Although there is no question that the zooxanthellae are not absolutely essential to calcification (for there are many corals depositing a calcareous skeleton without having the symbiotic algae), the evidence is overwhelmingly in favor of their acting as a stimulating agent. It is therefore felt that by characterizing the role of zooxanthellae in calcification it might be possible to obtain a clearer picture on the important aspects of calcification in the hermatypic corals.

In order to achieve this purpose, experiments were conducted along two main lines: (1) analysis of the effects of various physical and chemical factors on the rate of calcium uptake in the light and in the dark by *Fungia scutaria*, a solitary hermatypic coral; and (2) investigation of phosphorus metabolism of the same animal with special emphasis upon the function of the zooxanthellae.

Because photosynthesis leads to carbon dioxide fixation and energy storage, the best way to analyze the role of zooxanthellae in calcification is to compare the reactions of normal polyps to various conditions with those of polyps from which zooxanthellae have been removed. It is impractical to obtain polyps free from zooxanthellae in sufficient numbers for such experiments. Consequently, as a first approximation it is assumed that for comparative purposes, normal polyps kept in the dark would furnish satisfactory information. This seems reasonable, because one can extrapolate from the works on other algae that in darkness respiration ensues and hence stored energy utilized.

The physical and chemical factors analyzed in the present work included light, temperature, salinity, and calcium and phosphate concentration of the surrounding media. In addition the effect of dinitrophenol, an uncoupler of phosphorylation, was also investigated. The latter agent was used to obtain
some information on the energy requirement of calcification. In order to strengthen the findings of these experiments, an experiment was also carried out employing polyps free from zooxanthellae.

With respect to phosphorus uptake and release by F. scutaria a major concern of the present work was to clarify the question of whether or not zooxanthellae in situ can remove phosphate, a potential inhibitor of calcification, from the host tissue in the dark. Consequently, experiments were conducted to examine phosphorus exchange between normal as well as algal free polyps and their surrounding media in the light and in the dark. This procedure should give evidence to support the hypothesis (Simkiss, 1964a, b, c) that zooxanthellae are capable of removing the inhibitory substance from the calcifying system.

With proper control of these two avenues of investigations, it should be possible to give additional evidence for sustaining the hypothesis that zooxanthellae have an intimate and necessary function in maintaining normal physiological functions of corals as exemplified in calcification.

II. MATERIALS AND METHODS

1. Experimental animals

The solitary coral, Fungia scutaria Lamarck, was used as the main experimental animal and, as a supplement to this, a branching colonial coral, Porites compressa Dana was used in some experiments. F. scutaria was chosen for the following reasons: (1) the single polyp makes it possible to study physiology of an individual; (2) it is one of the most common species of the Scleractinia in the shallow reef flats around Oahu; (3) it is easily collected, handled, and maintained in the laboratory.

A young polyp of F. scutaria is a cylindrical form attached to a common stock or rocky substratum. As it grows it assumes a mushroom shape; the discoid form (anthocyathus) remaining attached to the substratum for some time by a stalk (anthocaulus). The anthocyathus eventually (within a year or more — Edmondson, 1929) detaches from the anthocaulus and lives as a solitary coral lying loose on the bottom. Only detached polyps collected within a depth of five feet were used in the experiments. For reasons of economy and convenience of handling, the smallest available specimens (from 1 to 10 g wet weight) were used. It was desirable but not practical to keep the size of the polyps uniform. However, the possible size-dependent effects were minimized by replicating different sizes in all treatment groups. In this way the total mass of the polyps in a given volume of the incubating medium was also kept approximately uniform.

All the animals (Fungia and Porites) used for the present study were
collected at the edge of a coral reef flat along the southern coast of Kaneohe Bay, Oahu. The animals were carefully transported to the laboratory and maintained in tanks of circulating sea water until used in the experiments (from 1 to 14 days). In the course of this work, it was found that the polyps of *F. scutaria* can be maintained in a healthy condition in the laboratory without artificial feeding for at least half a year.

2. **Media and procedures used for incubation**

Sea water was collected from the same location and at the same time as the animals. It was first filtered through a Whatman #1 filter and then through an HA Millipore filter (Millipore Filter Corp.) with a pore size of 0.45μ. In all experiments this water was used as the standard incubating medium. When the effect of some constituent in the water was to be investigated, the standard medium was modified. When it was desirable to study the effects of calcium concentration, artificial sea water was prepared.

All the experiments were carried out in a room equipped with fluorescent lamps whose light intensity was controlled by a voltage regulator. The light was maintained constant at a given intensity for a given experiment (variation from one experiment to another ranged from 2,300 to 6,000 lux).

Individual experiments were performed using plastic containers of the 0.5- or 2.0-liter capacity. The containers were fitted with covers, and some of them were painted black to exclude light. Parallel incubations were made using the transparent containers and the dark containers as controls for measuring the effect of light on calcium uptake.

From two to five polyps were placed in a container. The volume of the media and mass of the polyps in each container were kept approximately the same in any given experiment in order to minimize the variability which would be caused by the difference in the size of individuals. The polyps were aerated for the interval of the experiments by bubbling a slow stream of air through the incubating medium.

Temperature was kept constant (24° ± 0.5°C) by keeping the containers partly immersed in a water bath supplied with circulating tap water. In those experiments in which temperature was altered, a low temperature (15°C or 18°C) was maintained by using a commercial low temperature water bath. High temperature was accomplished by using a water bath whose temperature was allowed to equilibrate with the room temperature (from 30° to 32°C).

Before initiating an experiment the polyps were acclimatized for a given period of time (from 1 to 24 hours). After acclimatization, a given quantity of Ca45 as CaCl2 or P32 as phosphate, or both were added to the medium. The initial activity of these radioisotopes in the medium was determined by counting three 0.2-ml aliquots from each container which were collected after allowing 20 minutes for complete mixing of the isotopes. Upon removal of polyps from the medium three 0.2-ml aliquots were pipetted from
each container to determine the final Ca\textsuperscript{45} or P\textsuperscript{32} activity. In order to establish suitable controls for measuring inorganic, isotopic exchange with the skeleton, clean, dead coralla were used in most experiments.

Following incubation for a given period of time with the radioisotope, the polyps and coralla were removed from the medium. They were washed by immersion for 5 minutes in two baths of non-radioactive sea water. The material was placed on Kleenex tissue to remove excess water, and immediately weighed on an analytical balance. The material was then dried for 24 hours in an oven at 115°C for dry weights, and ashed for 4 days in a muffle furnace at approximately 525°C. This period of ashing was found to be optimal.

3. Preparation and measurement of radiocalcium and radiophosphorus

After determining the ash weight, the coral ash was pulmerized and from it three aliquots, weighing from 20 to 50mg each, were placed on pre-weighed aluminum planchettes. The ash was evenly spread with the aid of water over approximately 3.1cm\textsuperscript{2} of the central portion. Afterward the samples were dried under infrared lamps, and the weight of the dry ash on the planchettes was determined. The radioactivity for each sample was detected using an endwindow G-M tube or gasflow detector connected to a commercial scaling unit.

In the experiments using the double-labelling technique (Ca\textsuperscript{45} and P\textsuperscript{32} simultaneously) the sample activities were determined employing a method similar to that described by Comar et al. (1951). The activity of each sample was counted with and without an aluminum absorber with the surface density of 60mg/cm\textsuperscript{2} inserted between the sample and the window of the detector. A preliminary study showed that this absorber completely absorbs the radiation from Ca\textsuperscript{45}. A correction factor for the absorption of the P\textsuperscript{32} activity by the absorber was determined by measuring the count rate of a P\textsuperscript{32} reference source with and without the absorber. The correction factor for the absorption of the P\textsuperscript{32} activity with this absorber was about two. The Ca\textsuperscript{45} activity was determined by calculating the difference between the count rate obtained without the absorber and the corrected P\textsuperscript{32} activity. When necessary, aliquots of acid digests (page 67) were also placed on aluminum planchettes, neutralized with NH\textsubscript{4}OH, dried and counted.

Dissolved organic and inorganic phosphate labelled with P\textsuperscript{32} (DOP\textsuperscript{32} and DIP\textsuperscript{32}) in the medium were determined by a modification of the method described by Johannes (1963, 1964a). Duplicate 5.0-ml samples of the filtered incubating medium were placed in 15-ml conical centrifuge tubes. To each tube, 0.8ml of saturated Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} were added, followed by 1.2ml of saturated Ca(OH)\textsubscript{2} in 10% CaCl\textsubscript{2}. After centrifugation, an additional 0.2ml of Ca(OH)\textsubscript{2} in 10% CaCl\textsubscript{2} was added and the mixture was again centrifuged. Duplicate 0.2-ml aliquots of the supernatant fluid (organic fraction A) were dried on aluminum planchettes and counted. The remaining
supernatant fluid was discarded, and the precipitate was suspended in 5.0ml of 10% trichloroacetic acid (TCA). One sample was filtered through an HA Millipore filter (0.45 µm pore diameter). The centrifuge tube was washed with about 2 ml of TCA and the rinsings were filtered. The filter was washed with about 2 ml of TCA, mounted on an aluminum planchette with a drop of acteate glue diluted with acetone, dried and counted (organic fraction B). The second sample was neutralized with saturated NaOH using phenolphthalein as an indicator. The sample was centrifuged and duplicate 0.2-ml aliquots of the supernatant fluid were dried on aluminum planchettes and counted (organic fraction C). Corrections were applied for dilution of the samples with reagents and contamination of the organic fraction with the inorganic P³² (Pages 88 and 99).

All samples were counted for a minimum of five minutes and corrected for background. The observed activity of Ca⁴⁵ was corrected for self-absorption. No corrections for self-absorption were necessary for the P³² sources. The correction for decay was made by counting, at the same time as the samples, a reference source of a known activity.

4. Chemical analyses for calcium, phosphorus, and nitrogen

Calcium in sea water was determined by a method described by Cloud (1962). This method included formation of a calcium-murexide (ammonium purpurate) color complex at alkaline pH and titration with a standard versene (disodium ethylenediaminetetraacetic acid) solution calibrated against a standard calcium solution.

Dissolved total phosphate (DTP) and dissolved inorganic phosphate (DIP) in sea water were determined by a method of Robinson and Thompson (1948) as modified by Strickland and Parsons (1960) and with a further modification by Van Landingham (1955). The latter modification includes the addition of sodium fluoride and hydrazine sulfate to the solution to stabilize the phosphomolybdate blue complex. The color was developed upon warming the sample solutions for one hour at about 50°C. The resulting blue color was stable for at least 24 hours. The sample solution was transferred to a 5-cm corex cell and the reduced phosphomolybdate was measured at 730 mµ using a Beckman Model B spectrophotometer.

Water samples for DTP and DIP analyses were filtered through HA Millipore filters and duplicate 50-ml portions were analyzed as soon as possible. When it was not practical to carry out DIP analysis immediately after taking samples, the sample was stored frozen in a refrigerator at about -5°C.

The Millipore filter through which water sample for DTP and DIP analyses was filtered was digested with perchloric acid (PCA) following the procedure similar to that described by Sherman (1942). The filter was placed in a 125-ml Erlenmeyer flask, 15ml of 70% PCA added and the mixture heated to boiling until 20 minutes after the solution became clear. After digestion was
complete, an excess of NH$_4$OH was added to neutralize the remaining acid. The excess base was then driven off as NH$_3$ by heating. The sample was diluted to a standard volume with redistilled water and analyzed for DIP. Reagent blanks and standards received the same digestion procedure and were run with every batch of samples.

Total phosphorus and nitrogen content of *F. scutaria* was analyzed using a digestion procedure similar to that described by Goreau (1963). A portion weighing about 200 to 300 mg in wet weight was cut off from each polyp after the total wet weight was determined. This was placed in a 125-ml Erlenmeyer flask, 5 ml of concentrated (12 N) HCl was added and heated to boiling after the initial burst of CO$_2$ subsided. When the volume was reduced by about half, the solution was cooled and 15 ml of 70% PCA was added. The solution was then gently heated until a dark greenish brown color was developed. As soon as the charring and foaming subsided, the temperature was raised to the boiling point and heating continued until the solution became colorless and clear. The entire process took less than one hour. After digestion was complete, the solution was made to 100 ml with redistilled water. Duplicate 1-ml aliquots were further diluted to 50 ml with redistilled water and analyzed for DIP. Blanks and standards which received the same digestion procedure were run with every batch of samples.

Total nitrogen content of the PCA digests was analyzed colorimetrically by a method similar to that described by Johnson (1941) and modified by Strickland and Parsons (1960). Duplicate 1-ml aliquots were placed in test tubes and 4 ml of 1.125 N NaOH were added to each sample. Minutes after the addition of NaOH, 4 ml of the Nessler reagent was added. The optical density of the solution was measured by a Beckman B spectrophotometer within 15 minutes in a 1-cm corex cell at a wave length of 430 μm.

5. **Radioautography**

Radioautographs for the P$^{32}$-labelled tissue sections of *F. scutaria* were prepared by a stripping film technique similar to that described by Pearse (1961). The tissue sections prepared as described on page 96 were mounted on a slide glass which has been previously dipped in an aqueous solution of 0.5% gelatine and 0.05% chrome alum, and then allowed to dry. After deparaffinizing, the slide was brought down to water through a graded series of alcohol solutions and an emulsion layer stripped from a Kadok AR.10 photographic plate was applied to it. The tissue-emulsion preparations were exposed for 2 or 4 weeks at about 20°C, and developed using Kodak D-19 developer in combination with Kodak acid fixer. The replicate slides were developed for 5, 10, and 15 minutes at about 20°C. After developing, some of the replicate slides were stained with 0.1% aqueous basic fuchsin while others were not stained at all. All the slides were mounted with balsam. The emulsion did not take up basic fuchsin stain.
III. RESULTS

1. The effect of some environmental factors on calcium uptake

Because of the postulated role of zooxanthellae in coral metabolism and calcification (Goreau 1959, 1961a, b, 1963), the effect of light on the rate of calcium uptake by F. scutaria was investigated under various conditions of the environment; temperature, salinity, and calcium and phosphate concentration of sea water. The rate of calcium uptake by the normal polyps and the polyps without zooxanthellae were compared in the light and in the dark. The effect of dinitrophenol, an uncoupler of phosphorylation, was also analyzed in the light and in the dark.

The procedures and results for each of these studies are presented in the following sections. The interactions and significance of each to the role of calcification in F. scutaria will be discussed subsequently.

a. Temperature

The effect of temperature on the rate of calcium uptake by F. scutaria was studied by subjecting the polyps to different temperatures both in the light and in the dark and subsequently determining the amount of calcium incorporated into the polyps within a particular period of time. The quantity of calcium taken up by the polyps was estimated by a tracer technique using Ca\(^{45}\).

Because the energy of Ca\(^{45}\) and P\(^{32}\) are greatly different, (0.245 Mev and 1.71 Mev, respectively) it was possible to design experiments for measuring the rates of both calcium and phosphorus concurrently. This was achieved by exposing the polyps to sea water containing both Ca\(^{45}\) and P\(^{32}\), and subsequently determining the radioactivity of both isotopes incorporated by the polyps. The method for concurrent determination of the Ca\(^{45}\) and P\(^{32}\) activity has been described in a preceding section (page 65). The results of the P\(^{32}\) measurements will be presented later in the consideration on phosphorus uptake (page 102).

Two series of experiments using Ca\(^{45}\) and P\(^{32}\) were performed employing two concentrations of phosphate in the medium (2.70 and 11.45 μg-at. P/l). In each series of experiments, the polyps maintained in the laboratory from two to four days were acclimatized to the experimental conditions from 16 to 22 hours. Four polyps were placed in each container having 1 liter of filtered sea water with a specified concentration of phosphate. Following acclimatization, the sea water of each container was changed once. One hour after changing sea water, approximately 40 μc of Ca\(^{45}\) and 12 μc of P\(^{32}\) were introduced to each container and the polyps were maintained under these conditions for 26 hours. Upon removal from the containers, the polyps were ashed and the quantities of calcium taken up were determined.

In a series of experiments employing the higher phosphate concentration (11.45 μg-at. P/l), the experimental temperature was set at 15°, 24°, and 31°C. The results obtained from this experiment are shown in Figure 1.
It was noted that the polyps exposed to low temperature (15°C) showed the symptoms of death (heavy secretion of mucus and partial disintegration of tissues) within 26 hours. This condition of the polyps is reflected in the low rate of calcium uptake as shown in Figure 1A. The experimental temperature of the second experiment was set at 18°, 24, and 30°C. At 18°C the polyps remained healthy during the entire period of the experiment. The results obtained from this experiment were combined with those obtained from the third experiment which was conducted under the same conditions as the second, except that P³² was not employed. These results are shown in Figure 1B.

Figure 1. The rate of calcium uptake as a function of temperature by the living polyp of *F. scutaria* in the light (3,000 lux) and in the dark, during 26-hour incubation in sea water containing 11.45 μg-at. P/1 (A) and 2.70 μg-at. P/1 (B) of dissolved inorganic phosphate. The vertical lines drawn through the points represent the standard deviations of the means. The rates of exchange of calcium by dead coralla are also included in the figures.
The results shown in Figures 1 A and 1 B reveal that in the dark the rate of calcium uptake by *F. scutaria* increased rectilinearly with increase in temperature, while in the light the maximum rate was attained at the intermediate temperature (24°C). From the data given in these figures, the Q₁₀ can be calculated by the formula,

\[
Q_{10} = \frac{K_2}{K_1}
\]

where \( K_1 \) is the rate of calcium uptake at 20°C and \( K_2 \) at 30°C. The \( K_1 \) and \( K_2 \) were determined by interpolation or extrapolation from the available data. The rectilinear relationship between the rate of calcium uptake and temperature in the dark made it possible to calculate a single value for the Q₁₀ over the entire range of temperature in the dark. On the other hand, in the light two values for the Q₁₀ were needed to characterize the rate-temperature relationship; one for the change in the rate between the lowest (15° or 18°C) and the intermediate (24°C) temperatures and the other for the change in the rate between the lowest and the highest (30° or 31°C) temperatures.

The following Q₁₀ values were obtained:

<table>
<thead>
<tr>
<th></th>
<th>low phosphate</th>
<th>high phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>dark</td>
<td>1.52</td>
<td>2.70</td>
</tr>
<tr>
<td>light (1)</td>
<td>1.55</td>
<td>2.45</td>
</tr>
<tr>
<td>light (2)</td>
<td>2.74</td>
<td>2.85</td>
</tr>
</tbody>
</table>

where low phosphate and high phosphate indicate the phosphate concentrations of the incubating media (2.70 and 11.45 µg-at. P / l, respectively ), and (1) indicates the entire range of temperature and (2) the range between the lowest (15°C or 18°C) and the intermediate (24°C) temperatures.

The Q₁₀ for the polyps kept in the medium with the higher phosphate concentration is consistently higher than that for the polyps kept in the medium with the lower phosphate concentration. However, as was noted before, the polyps kept at 15°C in the high phosphate medium were not in a healthy state. This may be the main cause for the higher Q₁₀ for the group of polyps kept in the high phosphate media.

b. Salinity

In its natural environment on the reef, *F. scutaria* is subjected to considerable fluctuation in salinity. During heavy precipitation dilution occurs and at periods of low tide on dry days evaporation may occur in pools. Because these changes in salinity may have some effect on the rate of calcification, this parameter was investigated.

The effect of salinity on the rate of calcium uptake by *F. scutaria* was studied both in the light and in the dark by placing the polyps in the medium with variable salinities. The media with five relative concentrations were employed; 125, 100, 75, 50 and 25% sea water. The sea water collected at the same place and at the same time as the polyps was diluted with redistilled water to obtain the media with low salinity (75, 50, and 25% sea water).
The 125% sea water was prepared by slow evaporation. This treatment did not cause any significant precipitation of salts, and the concentrated sea water was allowed to stand for 24 hours to bring dissolved gases into equilibrium with the air. The pH of the sea water was determined by a Beckman Model G pH meter prior to experimentation. Calcium concentration and total osmotic concentration were also determined. Osmotic concentration was determined by the Fiske Osmometer and expressed as milliosmoles. The analyses for normal, dilute and concentrated sea water are summarized in Table 1. Although the pH of the 125% sea water was slightly higher than the rest, no attempt was made to correct for it to avoid introduction of other variables.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Treatment (% sea water)</th>
<th>Osmotic Concentration (milliosmol)</th>
<th>Calcium Concentration (mg/1)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>269</td>
<td>102</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>520</td>
<td>207</td>
<td>8.07</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>776</td>
<td>304</td>
<td>8.04</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1032</td>
<td>403</td>
<td>8.02</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1288</td>
<td>519</td>
<td>8.22</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>212</td>
<td>8.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>418</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>506</td>
<td>204</td>
<td>8.11</td>
</tr>
<tr>
<td></td>
<td>50+Ca</td>
<td>522</td>
<td>398</td>
<td>8.18</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1012</td>
<td>410</td>
<td>8.00</td>
</tr>
</tbody>
</table>

Four living polyps and a clean corallum were placed in each container with one liter of sea water with a specified salinity. Parallel incubations in the transparent and opaque containers were performed for each salinity employed. The polyps kept in circulating sea water for six days were acclimatized for three hours in the light or dark containers to the experimental media and temperature, 23°C ± 0.2°C. The media were removed and replaced with the fresh media one hour prior to introducing 35.4 μc per liter of Ca⁴⁵, and the animals were incubated for 24 hours.

Following incubation with Ca⁴⁵, the coral polyps were removed from the containers and treated as described on page 65. The resulting calcium uptake rate under the light and dark conditions for each salinity is shown in Figure 2.
Figure 2. The rate of calcium uptake by *F. scutaria* and the rate of diffusion exchange by the dead coralla of the same animal as a function of salinity both in the light (6,000 lux) and in the dark. The vertical lines drawn through the points represent the standard deviation of the means.

Because it was noted that during incubation the water content of the polyps changed in relation to the osmotic concentration of the medium to which they were subjected, the rate of calcium uptake was expressed in terms of dry weight rather than wet weight. The relative effect on the water content of the polyps is shown in Figure 3. At low dilutions (below 75%) significant quantities of water were incorporated into the tissues of the polyps.

Figure 3. The ratio of wet weight to dry weight of the living polyp of *F. scutaria* as a function of salinity.
The data presented in Figure 2 shows that the rate of calcium uptake decreased with either decrease or increase in salinity. In the dark the rate of calcium uptake in 75, 50, and 25% sea water was 34, 20, and 13% of that of the controls (100% sea water), respectively. In the light the rate of calcium uptake decreased to the same extent as in the dark with decrease in salinity below 75% sea water, while in 75% sea water, the rate did not significantly differ from that of the controls. In 125% sea water the rate of calcium uptake was about 60% of that of the controls in the dark, while in the light the corresponding value was 82%. It appears that, except in 75% sea water, light does not alter the effect of salinity on the rate of calcium uptake.

That the lower rate of calcium uptake was not solely caused by the decrease in the ambient calcium concentration is shown in Figure 5A, in which the relative concentration ratio of calcium was plotted against calcium concentration or salinity. The concentration ratio as used here is defined by the equation:

\[
\text{Concentration ratio} = \frac{\text{Rate of calcium uptake (}\mu\text{g Ca/g/hr)}}{\text{Calcium concentration of medium (}\mu\text{g Ca/ml)}}
\]

The relative concentration ratio is taken to mean the concentration ratio at a given salinity (or calcium concentration) expressed as percent of that at normal salinity (or calcium concentration) in the light.

The effect of decreased salinity on the rate of calcium uptake was also studied independent of the effect of decreased calcium concentration. The rate of calcium uptake was measured in a group of the living and dead polyps and the dead coralla exposed to the normal and half-strength (50%) sea water and to 50% sea water with calcium concentration equivalent to normal sea water (ca 400 mg Ca/l). The calcium and osmotic concentrations, and the pH of these media are tabulated in Table 1 (Experiments 2 and 3). The acclimatization (for two hours) and incubation (for 25 hours) were carried out placing two individual polyps or coralla in 200 ml of the medium with a specified salinity and with 20 μc of Ca\(^{45}\) (added at the onset of incubation) under the constant temperature of 29.5\(^{\circ}\)\pm\ 0.5\(^{\circ}\)C and illumination of 3,300 lux. The results from these treatments are presented in Table 2.

The rate of calcium uptake of the living polyps kept in the 50% sea water was 31.1% and 34.0%, respectively, of that of the control groups in Experiments 2 and 3. Although, on the other hand, the rate of the group kept in the 50% sea water with normal calcium concentration in Experiment 3 was much higher than these, it was considerably lower than that of the control group (56.8%).

These data, then, offer an additional evidence to support a conclusion drawn from the results of the experiment previously described, namely altered salinity has an adverse effect on the rate of calcium uptake by *F. scutaria*. This effect is independent of the effect of altered calcium concentration.
Table 2. The rate of calcium uptake by the living polyps of F. scutaria and the rate of diffusion exchange of calcium by the dead polyps and coralla of the same animal in the normal (100%) and 50% sea water with added calcium under continuous illumination (3,300 lux) for 25 hours. The data were obtained in two independent experiments.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Treatment (% sea water)</th>
<th>Rate of Calcium uptake&lt;sup&gt;2)&lt;/sup&gt; (µg/g dry/hr) mean ± S. D.</th>
<th>Rate of Diffusion Exchange&lt;sup&gt;3)&lt;/sup&gt; (µg/g dry/hr) mean ± S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100</td>
<td>9.25±4.54 (4)</td>
<td>1.92±0.04 (2)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.88±2.12 (8)</td>
<td>1.07±0.32 (4)</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>7.01±3.43 (4)</td>
<td>5.34±2.12 (2)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.38±0.82 (4)</td>
<td>2.82±0.54 (2)</td>
</tr>
<tr>
<td></td>
<td>50+Ca&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>3.98±1.42 (4)</td>
<td>5.00±3.25 (2)</td>
</tr>
</tbody>
</table>

1) Sufficient CaCl<sub>2</sub> was added to the sea water to provide approximately 400 mg Ca/1 (equivalent to the concentration in normal sea water).
2) The rate of calcium uptake by living polyps; figures in parentheses indicate numbers of individual polyps in each treatment group.
3) The rate of diffusion exchange of calcium by dead coralla (upper two rows) and by dead polyps which were killed and fixed in 10% formalin for 24 hours and washed thoroughly in running tap water (bottom three rows); figures in parentheses indicate numbers of individual coralla or dead polyps.

c. Calcium concentration

The preceding work has shown that salinity has a marked effect on the rate of calcium uptake and that the effect is independent of calcium concentration. The next logical step is to study whether or not the ambient calcium concentration has some effect on the rate of calcium uptake by corals. For this purpose the polyps of F. scutaria were exposed to different ambient calcium concentrations and the rate of calcium uptake was measured. Artificial sea water was prepared based on the formula proposed by Lyman and Flemming (1940) as given by Sverdrup et al. (1942), but the calcium concentration was varied from 0 to 596 mg/l. The varied calcium concentration was established by replacing a specified quantity of CaCl<sub>2</sub> with a corresponding quantity of NaCl or vice versa. Changes in the quantity of NaCl would bring about the least changes in the relative ionic composition of the artificial sea water, for Na<sup>+</sup> and Cl<sup>-</sup> are the most abundant elements in sea water.

Determination of osmotic concentration of the sea water thus prepared showed that it was slightly less than that of the water from the natural habitat of F. scutaria (9.5% to 12.6%, cf. Tables 1 and 3). Because
salinity difference, ±25% of the normal sea water effected little change on the rate of calcification, it was assumed that this difference in osmotic concentration would not interfere measurably with the purpose of the present experiment. The calcium concentration and pH of the artificial sea water are presented, along with the osmotic concentration, in Table 3. A trace amount of calcium (6 μg/1) was introduced as Ca<sup>45</sup> to the calcium-free sea water. A little difference in pH (0.17 units in range) was not corrected in order to avoid introducing other possible variables.

Table 3. Some properties of the artificial sea water used in an experiment in which the effect of calcium concentration on the rate of calcium uptake by F. scutaria was studied.

<table>
<thead>
<tr>
<th>Calcium Concentration (μg/1)</th>
<th>Osmotic Concentration (milliosmol)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.006</td>
<td>934</td>
<td>8.08</td>
</tr>
<tr>
<td>39.706</td>
<td>932</td>
<td>8.12</td>
</tr>
<tr>
<td>198.006</td>
<td>928</td>
<td>8.08</td>
</tr>
<tr>
<td>397.006</td>
<td>924</td>
<td>7.95</td>
</tr>
<tr>
<td>596.006</td>
<td>912</td>
<td>8.03</td>
</tr>
</tbody>
</table>

The coral polyps previously maintained in circulating sea water for two weeks were acclimatized to the experimental artificial sea water media (24.4° ±0.5°C, 6,000 lux) for two hours, four polyps being placed in a liter of the media. The acclimatizing sea water was replaced by the fresh but chemically similar water one hour prior to the addition of 70μc/1 of Ca<sup>45</sup>, and then incubated for 24 hours. In a separate group of containers containing 7μc of Ca<sup>45</sup> per 200ml of the artificial sea water, two pieces of clean coralla were incubated under the same conditions as the polyps.

The rate of calcium uptake as a function of the ambient calcium concentration is shown in Figure 4. The rate of calcium uptake in the calcium-free sea water (0.006mg Ca/1) was too small to be entered in the figure. The rate obtained was 0.00017 ± 0.00006 μg Ca/g dry/hr for the polyps in the light and 0.00016 ± 0.00005 μg Ca/g dry/hr for those in the dark, and the rate of diffusion exchange by the coralla was 0.00005 μg Ca/g dry/hr.

The rate of calcium uptake increased linearly with the increase in the ambient calcium concentration from 0 to 400mg Ca/1 in the light and from 0 to 200mg Ca/1 in the dark. The slopes for the corresponding regression lines were 0.0258 ± 0.0018 and 0.0272 ± 0.0042, respectively. These two slopes were significantly different from zero, but one was not significantly different from the other.

In the upper range of calcium concentrations, two separate regression lines
were fitted for animals kept in the dark; one connecting three levels of calcium concentration (200, 400, and 600 mg/l), and the other connecting two levels of calcium concentration (400 and 600 mg/l). These regression lines are defined by the coefficients, 0.0077 ± 0.0070 and 0.0154 ± 0.0120. Neither of these differs significantly from zero (P = 0.3). This might be taken as an indication that the rate of calcium uptake in the dark reaches a maximum value at a calcium concentration of about 200 mg/l. The maximum velocity will then lie somewhere between 6 and 9.5μg Ca/g dry/hr.

Over the range of calcium concentration beyond 200mg/l, some factor other than the substrate concentration is acting as the rate limiting factor in the dark. Because light affects the photosynthetic activities of zooxanthellae, the most logical rate limiting factor would involve the zooxanthellae. Cramer and Dueck (1962) and Cramer (1963) observed a similar relationship between the rate of calcium absorption and the calcium concentration in the intestine of dogs. He interpreted his results as showing that the intestinal calcium absorption is not due solely to passive diffusion, but utilizes a carrier system with the character of facilitated transport. Because the rate of calcium uptake in *F. scutaria* as determined in the present work is the sum of the rate of transport and the rate of deposition of calcium carbonate, the same interpretation as that of Cramer's may not necessarily be applicable to the data obtained in the present work. Instead the present data should be regar-
ded as satisfying the hypothesis of Goreau's (1959) that the removal of carbon dioxide from the calcifying system shifts the equilibrium reaction toward deposition of calcium carbonate. It follows then that without photosynthesis in operation, the rate of calcium uptake reaches a steady state at a calcium concentration equivalent to half the normal concentration.

Figure 5. The relative concentration ratio of calcium in F. scutaria; to that in sea water ($\frac{\mu g \text{Ca/g dry}}{\mu g \text{Ca/ml}}$) as a function of the ambient calcium concentration after 24-hour incubation in the light and in the dark in the sea water with the altered salinity (A) and in the sea water with a constant salinity (B).

Figure 5 B shows the relative concentration ratio as a function of calcium
concentration. The concentration ratio was expressed as percent normal (the concentration ratio for the polyps kept in the light at a calcium concentration of 400 mg/l). The figure shows that in the dark over the range of calcium concentrations 400 to 600 mg/l, the system is operating half as effectively as in the lower range, while in the light the effectiveness increases drastically as the calcium concentration increases from 400 to 600 mg/l.

d. Phosphate concentration

Because phosphate has been known to act as a crystal poison for calcium carbonate (Simkiss, 1964 a, b, c) on one hand, and to enhance (at lower concentrations) or inhibit (at higher concentrations) photosynthetic fixation of carbon dioxide (Losada and Arnon, 1964) on the other hand, it was considered that the rate of calcification in corals may be increased or decreased with changes in the phosphate concentration of the surrounding medium.

Hence experiments were performed to show the effect of phosphate concentration on the rate of calcium uptake by *F. scutaria*. In addition to *F. scutaria* *Porites compressa* Dana, a colonial coral was also employed in this study.

The incubating medium was prepared by adding varying concentrations of sodium dihydrogen phosphate (NaH$_2$PO$_4$) to the natural sea water. The phosphorus concentration of the sea water used as the control medium for these experiments ranged from $1.56 \times 10^{-7}$ to $2.44 \times 10^{-7}$ M, determined as inorganic phosphate. The addition of NaH$_2$PO$_4$ to the sea water provided phosphate concentrations in the experimental medium, varying by the factors of 10, to a maximum of $10^{-2}$ M. The phosphate concentrations of $10^{-4}$ M added to sea water did not significantly change its pH from the normal values of 8.06 to 8.18. However, at higher concentrations the pH was decreased considerably ($6.69$ at $10^{-3}$ M and $5.40$ at $10^{-2}$ M). It was not feasible to adjust pH at these concentrations without causing precipitation of salts from the medium. Consequently, the experiments using media with high concentrations of phosphate were performed at that pH without adjusting it to normal.

The polyps of *F. scutaria* for the experiment were maintained in circulating sea water for three days in the laboratory prior to use. Groups of five polyps were first acclimatized for two hours in 200 ml sea water with the phosphate concentrations ranging from $10^{-7}$ to $10^{-2}$ M, followed by addition of 8.6 $\mu$C Ca$^{45}$ to each, and incubated in the light (3,300 lux) or in darkness for a period of 12 hours. Temperature was maintained at 22.8° ± 0.3°C. The dead corallum controls were incubated in the separate vessels. All polyps, including those exposed to the low pH ($10^{-2}$ M phosphate) remained healthy during this short incubation period.

The rate of calcification in the light and in the dark by *F. scutaria* at varying phosphate concentrations is shown in Figure 6. The rate of diffusion exchange by the corallum remained constant over the entire range of phosphate
Figure 6. The rate of calcium uptake by *F. scutaria* as a function of phosphate concentration of sea water during 12-hour incubation in the light (3,300 lux) and in the dark. The rate of diffusion exchange of calcium by the dead coralla of the same animal under the same condition is also included in the figure. Each point represents the rate for an individual polyp.

The rate of calcium uptake was the lowest in both light and darkness at the highest phosphate concentration (10^-2 M). As implied before, the decreased rates at this phosphate concentration should be interpreted as related to the low pH (5.40). However, because during the incubation period all polyps remained healthy at this phosphate concentration, and because the tissue phosphate content was proportional to the external phosphate concentration (see following and Figure 7), it is concluded that pH is not solely responsible for the decreased rates.

The rates of calcium uptake obtained in the intermediate phosphate concentrations (10^-5 - 10^-4 M) are aggregated into groups with narrow deviation, as if two groups of animals reacting to a common stimulus with opposing responses. Nevertheless, the data show that there is a general trend for the rate of calcium uptake to be inversely proportional to the environmental phosphate concentration. The effect of phosphate concentration on the rate of calcium uptake as seen in Figure 6 is not much different in the light and in darkness, except in the sea water having the low phosphate concentration (10^-7 M).
The phosphorus content of the polyps of *F. scutaria* expressed in terms of unit weight of nitrogen is shown in Figure 7 as a function of phosphate concentration of sea water. This figure shows that the phosphorus content of the polyps incubated in sea water containing high phosphate concentrations was significantly higher than that found in the polyps incubated in the natural water or in moderately enriched sea water ($10^{-5}$ M). It is proposed from the information presented in Figure 7 that increase in phosphorus content of the polyps is proportional to the logarithmic increase in the external phosphate concentration and no significant difference was produced by illumination.

The relationship between the phosphorus content and the rate of calcium uptake is shown in Figure 8. Although no good correlation between the two variables could be shown, it can be postulated that the rates of calcium uptake higher than $10 \mu g / g$ dry/hr are limited to a narrow range of the phosphorus content (between 4.5 and 5.5 $\mu g$-at. P/mg N). This suggests that the polyps with higher phosphorus content have lower rate of calcium uptake.

Because it is assumed that the colonial corals exhibit less individual variation in the calcification rates than the solitary corals (i.e. *Fungia scutaria*), it is of interest to compare the two. Experiments were performed with *Porites compressa* Dana for comparison. This species was chosen because it lives under the same environmental conditions as *F. scutaria*.

In the field the branches weighing from 14 to 20 grams (dry weight) were carefully cut out of a colony. These pieces were kept in the circulating sea water for one day, after which they were acclimatized and incubated according to the method used for the experiments with *F. scutaria*. A branch with 4 to
Figure 8. The rate of calcium uptake by *F. scutaria* as a function of phosphorus content (expressed as protein ratio) during 12-hour incubation in the light (3,300 lux) and in the dark in sea water containing different concentrations of phosphate.

5 sub-branches was placed in each container with 200 ml of the medium having a phosphate concentration of $1.56 \times 10^{-7}$, $10^{-5}$, $10^{-4}$ or $10^{-3}$ M. Paired opaque and transparent containers were used for each concentration. Approximately 7 $\mu$C/200 ml of Ca$^{45}$ was added to each experimental container. Temperature was maintained constant at $23.3^\circ \pm 0.3^\circ$C and light intensity at 3,000 lux. Duration of incubation was 24 hours.

After drying at 115°C for 24 hours and weighing, the tips of the subbranches were severed at 5mm from the distal end. These pieces were weighed and dry ashed in a muffle furnace at 525°C. The dry weights of these pieces ranged from 100 to 300 mg and contained from 50 to 100 polyps. Because it has been shown that there is a striking gradient in the rate of calcification from the tip of a branch to the base in a branching coral (Goreau, 1959), it was necessary to remove samples from homologous parts of the colonies. This was best accomplished by using samples removed from the tips of the branches.

The rate of calcium uptake in the light and in the dark as a function of phosphate concentration of the medium is shown in Figure 9. It can be seen in this figure that the rate of calcification for a group treated in a specific manner is less variable than was obtained for *F. scutaria* treated in a like manner. Because the medium with the phosphate concentration of $10^{-3}$ M or higher produced the low pH (6.69), it must be emphasized that
Figure 9. The rate of calcium uptake by *P. compressa* as a function of phosphate concentration of sea water during 24-hour incubation in the light (3,300 lux) and in the dark. The vertical lines drawn through the points represent the standard deviation of the means.

However, Figure 9 shows clearly that the rate of calcium uptake decreases with the increasing concentration of phosphate. The rate of the decrease in calcification is proportional to the logarithm of the concentration. Excluding measurements for the highest phosphate concentration, the following coefficients of regression were obtained; $-9.31$ (µg Ca/g dry/hr/10 M P in log scale) in the light and $-11.17$ (µg Ca/g dry/hr/10 M P in log scale) in darkness. The statistical analysis shows that there is no significant difference between the two slopes with a common slope of $-10.15$ ($P > 0.25$). The difference in elevation of the two regressions is highly significant ($P < 0.001$).

Although statistically not significant, the difference in the rate of calcium uptake between the light and dark treatments is the largest at the intermediate concentration of phosphate ($10^{-5}$ M). This may be taken to mean that the effect of light in increasing the rate of calcium uptake is the largest at this phosphate concentration.

2. The effect of the removal of zooxanthellae on calcium uptake

The effect of zooxanthellae on calcium uptake by *F. scutaria* was studied by comparing the rate of calcium uptake of the polyps from which zooxanthellae were removed with that of the polyps with zooxanthellae. Comparisons were made both in the light and in the dark. The possible interaction between the normal polyps and the polyps from which zooxanthellae were removed (blea-
ched polyps) was studied by placing a group of both the normal and bleached polyps in a single container. The rate of calcium uptake of these polyps (mixed group) was compared with that of their counterparts which were kept in the separate containers (separate group).

In order to remove zooxanthellae from the tissues of *F. scutaria*, a group of polyps were placed in plastic aquaria supplied with circulating sea water under complete darkness for 50 days. This period of time was found sufficient for nearly complete removal of zooxanthellae from the tissues of *F. scutaria*. Examination of the histological sections prepared from the bleached polyps revealed that only very few numbers of healthy cells of zooxanthellae survived this period of darkness (Pl. Fig. 4). The metabolic state of zooxanthellae is suggested by the presence of pyrenoid which is positive to basic fuchsin stain. The control group of the normal polyps were kept for 15 days under constant illumination of 6,000 lux in a fiber glass tank receiving circulating sea water.

The water from the same source as that circulated through the aquaria in which both the normal and bleached polyps had been kept was used as the incubating medium after filtering through an HA Millipore filter. Five normal polyps were placed in each of a pair of the light and dark containers containing one liter of the filtered water, and five bleached polyps were placed in a similar pair of containers. Into each of a third pair of containers, three normal and three bleached polyps were placed. A piece of clean, tissue-free skeleton of *F. scutaria* was placed in each container.

All the containers were placed in a water bath with water temperature maintained at 24° ± 0.2°C, and the coral polyps were acclimatized to this condition for two hours. Light intensity was kept constant at 6,000 lux during this period and the following incubation period. At the end of the two-hour acclimatization period, 54.5 μC of Ca⁴⁵ and 113.4 μC of P³² were added to each container and the polyps were incubated for an additional 24 hours. At the end of the incubation period, all the polyps and the pieces of corallum were removed from each container and treated in the manner previously described (page 65). The activity of P³² and Ca⁴⁵ taken up by the polyps and skeleton pieces were determined on the samples.

When the polyps and corallum pieces were removed, the sea water from each container (1 liter) was filtered through an HA Millipore filter. The filter and its retained particulate matter was digested with perchloric acid as previously described (page 66), and the acid digest was diluted to 100ml with redistilled water. Three 0.2-ml aliquots of the diluted digest was counted to determine Ca⁴⁵ retained on the filter together with the particulate P³². Three 0.2-ml aliquots from the filtrate were counted to determine Ca⁴⁵ in solution and the final activity of Ca⁴⁵ in the medium was estimated from the total activities determined from the filter and filtrate. The filtering process
was performed not particularly to determine the final Ca\textsuperscript{45} activity in the medium but rather to determine the P\textsuperscript{32} activity in the different fractions (particulate, dissolved organic, dissolved inorganic fractions). The filtrate was further treated to determine the P\textsuperscript{32} - labelled dissolved organic phosphorus compounds. The method of determining Ca\textsuperscript{45} and P\textsuperscript{32} activities from the common samples is described in a section on methods (page 65).

The mean rates of calcium uptake for three or five polyps and the standard deviations for each treatment are summarized in Table 4. The rate of phosphorus uptake and associated P\textsuperscript{32} activities in the media are presented in Table 5. The mean rates of calcium uptake for five or three polyps in a group and the standard deviations from the means are entered in the table (A single rate for skeleton is available for each container). The ratio of the mean rate of the normal polyps to that of the bleached polyps within the separate or mixed groups and the ratio of the mean rate in the light to that in the dark are also shown in the table.

**Table 4. The effect of the removal of zooxanthellae from tissues on the rate of calcium uptake by *F. scutaria* in light and darkness.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate of Calcium Uptake ((\mu gCa/g dry/hr))</th>
<th>Ratio N / B</th>
<th>Ratio L / D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal (N)</strong></td>
<td>49.56 ± 14.59</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Separate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached (B)</td>
<td>4.11 ± 1.39</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light (L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>45.70 ± 11.40</td>
<td>2.47</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>2.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>3.69 ± 0.20</td>
<td>12.38</td>
<td>1.07</td>
</tr>
<tr>
<td>Corallum</td>
<td>2.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Darkness (D)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23.99 ± 8.93</td>
<td>8.48</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>4.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>2.83 ± 1.24</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>3.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>3.44 ± 1.14</td>
<td>5.37</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>3.10</td>
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</tr>
</tbody>
</table>

*Mean ± standard deviation.*

The data show that the removal of zooxanthellae from the tissues of *F. scutaria* caused a significant drop in the rate of calcium uptake. The difference in the rates is more pronounced in the light (approximately 12 times) than in the dark (approximately 5 – 8 times) reflecting insensitivity of the bleached polyps to light.

There was no statistically significant difference \((P > .5)\) in the mean rates between the normal polyps kept separately and the normal polyps mixed with
the bleached polyps both in the light and in the dark. There was no significant difference (P > .5) between the bleached polyps kept separately and those kept mixed with the normal polyps, both in the light and in the dark. However, in the dark, when the rate of calcium uptake was corrected for physical diffusion exchange of calcium (by the dead coralla), the rate of calcium uptake of the normal polyps kept together with the bleached polyps was considerably lower than that of the normal polyps kept separately, while the rate of the bleached polyps increased when kept with the normal polyps. In the light both the "mixed" normal polyps and "mixed" bleached polyps had lower rate of calcium uptake than their counterparts kept separately. In the dark, then, there is some indication of interaction between the normal and bleached polyps in the rate of calcium uptake.

Examination of the histological sections of the bleached polyps (Pl. Fig. 4) shows that apart from loss of zooxanthellae, the only visible difference detectable between these and normal polyps is the reduction in the height of the columnar epidermal cells. Whether this change has any effect on the rate of calcium uptake or not is not known from the results of the present experiment alone.

3. The effect of dinitrophenol on calcium uptake

The effect of dinitrophenol (DNP) on calcium uptake by *F. scutaria* was studied employing three concentrations of 2,4-DNP (10^-5, 10^-4 and 10^-3 M). This range of concentrations of DNP has been reported to have inhibitory effects on calcium deposition of an oyster, *Crassostrea virginica* (Maroney et al., 1957).

In the present experiment, 32 polyps of *F. scutaria* were divided into eight groups of four individuals. Each group was placed in one of the plastic containers (4 transparent and 4 opaque) containing 200 ml (or a volume which was made up to 200 ml by later addition of DNP solution) of filtered sea water. Following two-hour acclimatization to the experimental conditions (23.6° + 0.3°C and 2,300 lux), a predetermined volume of 0.01-M 2,4-DNP solution of sea water was added to the experimental sea water. The final concentrations of DNP of the three experimental pairs of containers thus obtained were 10^-5, 10^-4 and 10^-3 M, respectively. One pair did not receive DNP and served as control.

Immediately following the addition of DNP, 3.2µc Ca^{45} was added to each container. Addition of the Ca^{45} solution was taken as the start of incubation. After allowing 16.5 hours for incubation, the polyps were removed from the containers and processed in the standard way.

A piece of clean tissue-free corallum of *F. scutaria* was incubated in a container containing 50 ml of filtered sea water or sea water with 10^-5 or 10^-3 M DNP. All three containers received 1.3 µc Ca^{45}, and the corallum
pieces were treated in the same way as the coral polyps.

The pH of the sea water did not change significantly after adding DNPa at the concentrations tested, as indicated by the following pH values obtained; 8.09 (normal sea water) 8.03 (10^{-5} M DNP) and 8.05 (10^{-3} M DNP).

The rate of calcium uptake for each treatment with or without DNP obtained for this experiment is summarized in Figure 10. The results show a significant difference in the rate of calcium uptake between the control and experimental groups, except for the group incubated with 10^{-5} M DNP in the light. Although 10^{-5} M DNP did not appear to have any significant effect on the rate of calcium uptake in the light, it caused a complete inhibition in the dark. There was no difference between the rate of calcification in this group of animals and that which was attributed to physical diffusion in the corallum.

Figure 10. The effect of dinitrophenol on the rate of calcium uptake by *F. scutaria* in the light (3,300 lux) and in the dark. The polyps were treated with dinitrophenol for 16 hours and the rate of calcium uptake was determined during this period of time. The rate of diffusion exchange by the dead corallum of *F. scutaria* under the same condition is also included in the figure.
The figure suggests that there is a trend of increase in the rate with the increase in DNP concentration from $10^{-5}$ M (except light treated animals) to $10^{-3}$ M. This increase follows a regression line with a slope of 1.39 ($\mu g Ca /g/hr$ per each logarithmic increase in DNP concentration). This is significantly different from 0 ($P < .001$), although one can attribute this to physical diffusion, because the corallum also shows this phenomenon.

4. The effect of light on uptake and release of phosphorus

a. Phosphorus uptake

The effect of light on phosphorus uptake by F. scutaria was studied in several experiments employing $P^{32}$. The labeled phosphorus in the medium and animals were examined to establish changes in phosphorus concentration with time as a consequence to phosphorus movement. The early work of Yonge and Nicholls (1931 a, b) has implied that the zooxanthellae derive phosphorus for an energy source from that excreted by the host animal.

Before investigating the mechanism of this movement, it was desirable to measure $P^{32}$ uptake in the light and in the dark over a period of 12 hours. The polyps were kept in circulating sea water in the laboratory for three days, and acclimatized to the experimental conditions (24.4° ± 0.3 °C and 3,300 lux) for an additional eight-hour period. In the acclimatization and the following incubation with $P^{32}$, polyps were divided into ten groups of three polyps (immersed in 200 ml sea water in the paired light and dark containers). At the end of the acclimatization period, polyps were transferred to a new set of containers with 200 ml of sea water containing 2.7 $\mu g$-at. P/1 of phosphate. After an additional hour in this solution the polyps were exposed to approximately 178 $\mu c$ $P^{32}$ per liter.

Analysis of the $P^{32}$ activity in the corals were made on three polyps removed from both light and dark containers at the intervals of 1, 2, 4, 6, and 12 hours. Wet weights were determined and then two small pieces were cut off with scissors or cutting pliers from one specimen from each group. The cuts were made parallel to the septa so that homologous pieces of triangular shape were obtained. After wet weight was determined for each piece, one of the pieces was placed in a solution of 10% neutral formalin in sea water and processed for histological preparation. The remaining pieces were digested with perchloric acid (PCA). A piece of approximately equal mass was also removed from each of the other two polyps of each group and digested with PCA. Wet weights of these ranged from 300 to 600 mg. The acid digest was used for both chemical analysis of phosphorus and the determination of the $P^{32}$ activity. The remaining main portion of each polyp was dried and ashed in a muffle furnace at about 525°C. The activity of $P^{32}$ taken up by the polyp
was determined on the ash samples.

The entire incubating medium from each container was filtered through an HA Millipore filter (pore size of 0.45 μ). In transferring the medium, the wall of each container was carefully scraped with a rubber policeman to free any adhering material. The filter was placed in a 125-ml Erlenmeyer flask and digested with PCA in the same way as the coral samples. The acid digest was diluted with distilled water to 100 ml, and three 0.2-ml aliquots were placed on aluminum planchettes, dried and counted to determine the particulate P32 (PP32).

Three 0.2-ml aliquots of the filtered medium were counted to determine the total P32 in solution (DTP32). Duplicate 5.0-ml samples of the filtrate were placed in 15-ml conical centrifuge tubes and the dissolved organic P32 (DOP32) was determined on these samples employing the method described on page 65. The total P32 in the medium (TP32) was obtained by adding DTP32 and PP32. The dissolved inorganic P32 (DIP32) was obtained by subtracting DOP32 from DTP32. Duplicate 50-ml samples of the filtrate were analyzed chemically for dissolved inorganic phosphate (DIP) and a 50-ml sample was digested with perchloric acid and analyzed for total phosphate (TP) according to the procedure given on page 66.

An adequate sample of the incubating medium not exposed to the corals was set aside at the beginning of the incubation and kept frozen until the above analyses were made on the experimental media. The values for the P32 determinations and the chemical analyses of phosphorus obtained from this sample served as the control for all the experimental groups. The P32 activity retained on an HA Millipore filter through which the control medium was filtered was used to correct PP32 of the experimental media for contamination with the dissolved P32. The correction factor thus obtained was 0.5% of TP32. Contamination of DOP32 with DIP32 in the experimental media was also corrected by the factor obtained from this control medium. The extent of contamination of the control solution was 0.56% for DTP32 (A) 0.33% for DOP32 (B) and 0.51% for DOP32 (C).

The P32 activity of the polyps was determined on both the dry ash samples and the acid digests. Because two sets of determinations of the P32 activity of polyps varied within the range of individual variation and because it was believed that statistical significance can be increased by pooling all the available informations, the two sets of determinations were pooled together to obtain the mean P32 activity of the polyps. The P32 activity of polyps was checked against the activity removed from the medium as measured by the difference between the initial and final TP32. As is shown in Figure 12 the P32 activity of polyps is consistently slightly lower than the activity calculated as being removed from the medium.

The specific activity of the polyps and the initial specific activity of the medium were calculated from the following equations:
Specific activity of polyps = \( \frac{\text{cpm} \, P^{32} \, / \, \mu \text{g-at. P/g wet wt.}}{\mu \text{g-at. P/g wet wt.}} \)  

Specific activity of medium = \( \frac{\text{cpm} \, P^{32} \, / \, \mu \text{g-at. P/ml}}{\mu \text{g-at. P/ml}} \)  

The specific activity of the polyps divided by the initial specific activity of the medium gives the concentration ratio (Equation 4). The concentration ratios are plotted against time in Figure 11. Each point in the figure represents a mean for three polyps. It can be seen in Figure 11 that the rate of phosphorus uptake is greater in the light than in the dark (approximately 2 to 1 at the 12th hour of incubation). Although the concentration ratio for both light and dark animals steadily increased with time during the first 12 hours, it is tempting to suggest that if the experiment were extended in time, the uptake of \( P^{32} \) in the dark may level off earlier than in the light.

As will be pointed out later the concentration ratio as defined here is equal to the ratio obtained by comparing the amount of phosphorus taken up by animals in a given period of time to the total phosphorus content of animals. Because of this relationship, the slope of the lines in Figure 11 would give the relative.

![Figure 11](image-url)
rate of phosphorus uptake. This was calculated to be 0.40 (μg-at. P taken up /μg-at. P in animal /hr) for the light group and 0.17 for the dark group over the period between the sixth and twelfth hour of incubation.

It was mentioned earlier that the incubating medium was enriched with phosphate. Chemical determinations made on the control medium have shown the inorganic phosphate (DIP) concentration to be 2.7 μg-at. P/1. This concentration was chosen because it was found that with higher phosphate concentrations an adverse effect on coral's metabolism occurred (pages 79 and 82). Similar effects on P³² uptake of an amphipod were reported by Johannes (1963). The absolute quantity of dissolved inorganic phosphate of the incubating medium (0.54 μg-at. P/Container) was small compared with the total phosphorus content of the polyps; this ranged from 103.2 to 159.3 μg-at. P for three polyps from each container. Thus, the quantity of dissolved inorganic phosphate of the incubating medium was 0.34 to 0.52% of the total phosphorus contained in the polyps. During the twelve-hour incubation, the polyps accumulated approximately 90% dissolved inorganic phosphate in the light. It can be concluded that under conditions of the experiment, the period of twelve hours is nearly maximum for incubation without causing depletion of the available phosphate in the medium.

Absolute quantities of phosphorus taken up by F. scutaria can be estimated in two ways. One method is to convert the P³² measurements to absolute quantities by the use of the isotope dilution equation:

\[
\text{Phosphorus taken up by animal (μg-at. /g wet wt.)} = \frac{\text{Total P in medium (μg-at. /ml)}}{\text{P³² in medium (cpm/ml)}} \times \frac{\text{P³² in animal (cpm/g wet wt.)}}{\text{P³² in medium (cpm/g wet wt.)}}
\]

(3)

It should be pointed out here that the concentration ratio previously presented is essentially the ratio of the increment of phosphorus calculated in this way to the total phosphorus content of animals. Because,

\[
\text{Concentration ratio} = \frac{\text{P³² in animal /Total P in animal}}{\text{P³² in medium /Total P in medium}},
\]

(4)

it follows that

\[
\text{Relative P uptake} = \frac{\text{P taken up by animal /Total P in animal}}{\text{P³² in animal /Total P in animal}} \times \frac{\text{P³² in medium /Total P in medium}}{\text{P³² in medium /Total P in animal}}
\]

(5)
The present method takes into consideration the net exchange of the labelled phosphorus compounds between animal and the surrounding medium, but it does not take into consideration the exchange of stable phosphorus compounds unless the system to be studied has reached an equilibrium state. As can be seen in Figure 11, an equilibrium state was not reached within 12 hours. Therefore absolute quantities of phosphorus taken up by *F. scutaria* as calculated by this method should be regarded as approximate estimates of the gross uptake of phosphorus. In Figure 12, the quantities of phosphorus taken up by the polyps, as determined by this method are plotted against time. In this figure, values for each group of polyps, calculated from a mean P$^{32}$ activity determined on the ash samples and on the acid digests, were connected by a solid line. The other set of values, calculated from the P$^{32}$ activity removed from the medium as measured by the difference between the initial and final total P$^{32}$ activity, were connected by a broken line.

![Graph showing phosphorus uptake by *F. scutaria*](image)

Figure 12. Uptake of phosphorus by *F. scutaria* from sea water containing 2.70 µg-at. P / l of dissolved inorganic phosphate in the light (3,300 lux) and in the dark, as estimated from the P$^{32}$ recovered in the polyps (solid line) and from the P$^{32}$ removed from the medium (broken line).

The second method of estimating absolute quantities of phosphorus taken up by the polyps is to determine the phosphorus concentration of the medium at the beginning and end of incubation, and to estimate from the difference the quantity of phosphorus taken up by the polyps. Depending upon the form of phosphorus compounds measured, quantities of each form can be estimated. Quantities calculated by this method reflect net exchange (or uptake as in the present experiment) rather than gross exchange at a given time of incubation irrespective of the distribution of P$^{32}$, whether it became homogene-
ous inside and outside the animals.

The data presented in Figure 13 are based on the determinations of dissolved inorganic phosphate, and hence the information is limited to this particular form of phosphorus compound. An attempt to determine total phosphate of the medium was not successful due to loss of the fractions of some sample solutions during digestion with perchloric acid.

![Figure 13](image)

Figure 13. Uptake of dissolved inorganic phosphate (+) and release of phosphorus (−) by *F. scutaria* in the light (3,300 lux) and in the dark. The quantity of phosphorus released was estimated from the difference between the quantity absorbed as estimated from P\(^{32}\) removed from medium and the quantity of dissolved inorganic phosphate absorbed by *F. scutaria*.

As mentioned before the quantities of phosphorus taken up by corals as determined from the P\(^{32}\) activities of corals by using the isotope dilution equation are gross quantities and those calculated from the difference between initial and final phosphate concentrations of the medium are net quantities. It follows then that the difference between the two measures of the phosphorus uptake are the estimates of the amount of phosphorus excreted by the corals. The quantities of phosphorus excreted by corals as determined in this manner are given in Figure 13. The calculations are based on the gross quantities estimated from the P\(^{32}\) activities removed from the medium rather than from the P\(^{32}\) activities recovered in the coral samples.

The graphs of Figure 13 indicate that in the dark more phosphorus was excreted than taken up during the first two hours (0.0015 μg-at. P/g wet uptake, 0.005 μg-at. P/g wet release). During the following ten hours the quantities of phosphorus excreted slowly increased, but the quantity of phosphorus taken up was greater than that released. Phosphorus excreted in the light was much less than that in the dark, and the quantity decreased...
with time. These results agree with those obtained from the experiments designed for studying the effect of light on the release of phosphorus by the same animal (pages 98 to 102).

Figure 14. Relative changes with time in DIP$^{32}$, DOP$^{32}$, and PP$^{32}$ of sea water during incubation with *F. scutaria* in the light (3,300 lux) and in the dark. All P$^{32}$ fractions are expressed as percent of the initial activity in counts per minute per ml.

The changes in the relative quantities of DIP$^{32}$, DOP$^{32}$, and PP$^{32}$ in the medium are presented in Figure 14. While DIP$^{32}$ decreased with time, DOP$^{32}$ and PP$^{32}$ increased. At the end of 12 hours, approximately the equal quantities of TOP$^{32}$ (DOP$^{32}$ + PP$^{32}$) were present in the media containing the polyps incubated in the light (5.57% of the initial activity) and in the dark (7.01%). However, DIP$^{32}$ in the media containing the polyps incubated in the light was much less than that in the dark (L/D = 1/24.6). Consequently, the ratio of TOP$^{32}$ to DIP$^{32}$ in the media containing the polyps kept in the light was much higher (4/1) than that in the dark (0.22/1). These relationships can also be seen in the change of the specific activity of the medium with time (Figure 15). The specific activities of the medium at each period were calculated for DIP$^{32}$ and TP$^{32}$ according to the following equations:

$$\text{Specific activity of DIP}^{32} \ (\text{SA:DIP}^{32}) = \frac{\text{DIP}^{32}}{\text{DIP}}$$

$$\text{Specific activity of TP}^{32} \ (\text{SA:TP}^{32}) = \frac{\text{TP}^{32}}{\text{DIP}}$$

The percent differences from the initial specific activity were plotted against time in Figure 15. The changes in SA:TP$^{32}$ show the effect of dilution of
P\textsuperscript{32} by DIP and the change in SA : DIP\textsuperscript{32} the effect of dilution of DIP\textsuperscript{32} by TOP\textsuperscript{32} superimposed upon the dilution effect of DIP.

![Graph showing relative changes with time in the specific activity of TP\textsuperscript{32} (SA : TP\textsuperscript{32}) and DIP\textsuperscript{32} (SA : DIP\textsuperscript{32}) of sea water during incubation with F. scutaria in the light (3,300 lux) and in the dark. All values are expressed as percent of the initial specific activity (cpmTP\textsuperscript{32}/\mu g-at. P-DIP and cpm DIP\textsuperscript{32}/\mu g-at. P-DIP).

The results of radioautographs prepared from a polyp of each group failed to show any significant accumulation of P\textsuperscript{32} in the tissues. The quantity of P\textsuperscript{32} accumulated by these polyps may not have been large enough to leave sufficient activity for radioautography. It was found that only about 10 to 20\% of the initial activity survived the processes for histological preparation.

Information is available on phosphorus uptake over a long time interval (102 hours) for F. scutaria kept under constant illumination. This was obtained from the experiment in which the rate of phosphorus release was studied. Ten liters of sea water containing 8.9 \mu g-at. P/1 of dissolved inorganic phosphate was placed into two plastic aquaria of approximately 30-liter capacity. Twenty-seven polyps were placed into each aquarium to which 226 \mu c of P\textsuperscript{32} was added after one-hour acclimatization period. After the sea water was thoroughly mixed, three 0.2-ml samples were taken from each aquarium for determination of the initial P\textsuperscript{32} activity. At the end of 6, 12, 24, 30, 70 and 102 hours, one to two polyps were treated in the standard way and ashed in a muffle furnace at 525°C. The P\textsuperscript{32} activity was determined on the ash samples. During the period of acclimatization and incubation, the water temperature was kept constant (28° ±0.5°C) and light intensity at about 6,000 lux.

Because the experimental conditions of the two aquaria were approximately
identical and the changes in the $^{32}\text{P}$ activity of both aquaria followed a similar pattern, the $^{32}\text{P}$ activities of the polyps incubated for the same period of time in two aquaria were averaged. The concentration ratio (Equation 4) was calculated from these values and are presented in Figure 16. The graph shows that the concentration ratio increases at a constant rate from the 30th to 102nd hour. This indicates that the rate of phosphorus uptake by *F. scutaria* did not reach a steady state within 102 hours under the conditions of the experiment.

![Graph showing concentration ratio vs time](image)

**Figure 16.** The $^{32}\text{P}$ uptake by *F. scutaria*, expressed as concentration ratio, as a function of time; *F. scutaria* was kept in sea water containing 8.9 µg-at. P/1 of the initial phosphate concentration under constant illumination (6,000 lux).

**b. Distribution of radiophosphorus in tissues**

The distribution of $^{32}\text{P}$ in the tissues of *F. scutaria* was studied by radioautography on the polyps placed in the sea water containing $^{32}\text{P}$ for 13 and 24 hours in the light and in the dark, using the transparent and opaque containers, respectively.

Eight polyps of *F. scutaria* which had been kept for eight hours in circulating sea water in the laboratory were divided into four groups of two each. Each group was placed in a container with 200 ml of filtered seawater and acclimatized for one hour to the experimental conditions (23.5°C and 2,100 lux). The polyps were transferred to a new set of containers with 100ml filtered sea water containing 18.5 µc $^{32}\text{P}$ and 0.27 µg-at. P/1 dissolved inorga-
nic phosphate, and incubated under the same conditions as above.

At the end of 13 and 24 hours, two polyps from an opaque and two polyps from a transparent container were removed. From each polyp two homologous portions weighing about one gram were cut off with a pair of scissors. One portion from each coral was immediately placed in a 20-ml solution of 10% formalin in sea water. The other portion was digested with perchloric acid for the determination of total phosphorus content of the polyp. The remaining portion of the polyp was dried, ashed and counted to determine P\textsuperscript{32} taken up. The incubating medium was analyzed for dissolved inorganic phosphate. The P\textsuperscript{32} activity was determined on three 0.2-ml aliquots of the medium. No attempt was made to determine the different fractions of the labelled phosphate.

The portions of the coral polyps which were fixed for 24 hours in formalin were transferred to 100 ml solutions of 20% acetic acid in 10% neutral formalin (diluted with distilled water) and decalcified at room temperature. At the end of 37 hours, all specimens were completely decalcified. Each decalcified piece of tissue was washed in running tap water for six hours, and dehydrated in a graded series of alcohol. The tissue was then cleared in xylene and embedded in paraffin at 60°C. The tissue blocks were sectioned at eight microns and processed for radioautography as previously described.

The loss of P\textsuperscript{32} from the tissues during the processes of histological preparation was estimated by counting two 0.2-ml aliquots of the fixative, decalcifying solution and each alcohol solution. The activity determined for each solution was expressed as percentage of the original activity determined on the ash samples of each polyp. The following results were obtained: fixative, 45%; decalcifying solution, 8.4%; alcohol solutions, 7.0%.

Because the activity of P\textsuperscript{32} recovered in 100% alcohol was very low (1–8 counts per minute /0.2-ml aliquot above the background), further determinations on the other solutions were not made. The activity lost to tap water could not be determined. The total loss measured was 60% of the original activity, and because another fraction is assumed to have been lost to tap water and the other solutions, only 10% to 20% of the original activity can be regarded as having survived all the processes.

The concentration ratio as determined according to Equation 4 was 0.00020 for a group incubated in the dark for 13 hours and 0.00018 for the remaining three groups. This indicates that the exchange of P\textsuperscript{32} between the polyps and the media reached a steady state during the first 13 hours. The changes in the P\textsuperscript{32} activity of the medium (Figure 17) leads to the same conclusion. As much as 95% of the initial activity was removed during the first 13 hours, both in the light and in the dark, no further removal of P\textsuperscript{32} occurred beyond this point.
Despite the rapid uptake of P$^{32}$, there was no net uptake of DIP (Figure 17). From the determinations on the activity of the ash samples, absolute quantities of phosphorus taken up by the polyps were calculated according to Equation 3 (Figure 17).

The results obtained by radioautography are illustrated by the photomicrographs (Pl. figs. 1 and 2). The most extensive accumulation of P$^{32}$ was observed in the tissues of the polyps exposed to the radioactive medium for 24 hours in the light (Pl. fig. 1). In these polyps, zooxanthellae contained in the endoderm of the oral body wall, mesenteries and mesenterial filaments,
and in the endoderm lining the skeletal cavities (the coelenteron bounded by the sclerosepta, synapticula and theca) were generally the most positive. However, zooxanthellae contained in the endoderm of the aboral body wall were not significantly positive.

The heavy accumulation of P\textsuperscript{32} was also found in the mucous glands of the endoderm such as the mesentery and the inner zone of the mesenterial filaments, and in the gland cells of the glandular marign of the mesenterial filaments and the acontia. The mucous glands of the outer body wall (ectoderm) did not show any significant accumulation of P\textsuperscript{32}.

The polyps subjected to the P\textsuperscript{32}-containing medium for 24 hours in the dark also exhibited a detectable concentration of P\textsuperscript{32}, and with similar pattern of distribution as described above. No P\textsuperscript{32} accumulation was detectable in the polyps incubated with the radioactive medium for 13 hours in the light or in the dark (Pl. fig. 2). Because a large fraction of P\textsuperscript{32} accumulated by the polyps was lost during the processes of fixation, washing, dehydration and possibly clearing and embedding, the negative results obtained for these polyps do not necessarily prove that these polyps did not accumulate P\textsuperscript{32} in their tissues. The same thing also can be said with respect to the tissues (of the 24-hour treatment groups) in which no autograph of the P\textsuperscript{32} activity was obtained.

It has been shown that both absorption and excretion in corals are carried out by the cells of the inner part of the mesenterial filaments (Yonge, 1930) and that digestive enzymes are secreted by the gland cells of the glandular margin of the mesenterial filaments (Yonge, 1931). It was not possible to detect the sites of absorption and excretion of P\textsuperscript{32} in the present experiment. However, the fact that the mucous glands and the gland cells were positive to P\textsuperscript{32} activity suggests that some fraction of phosphorus taken up by the polyps of F. scutaria was incorporated into the secretory products by these cells and eventually secreted into the coelenteron.

The accumulation of P\textsuperscript{32} in the cells of zooxanthellae contained in the polyps incubated with the radioactive medium in the dark needs to be explained, for utilization of inorganic phosphorus by plant cells has been known to be light dependent (Arnon, 1956). This will be explained by diffusion exchange of the isotopes P\textsuperscript{32} and P\textsuperscript{31}. Because the specific activity of the incubating medium was high, it is expected that there was a rapid exchange between P\textsuperscript{32} present in the medium and P\textsuperscript{31} present in zooxanthellae. Because there was no net uptake of DIP by the polyps, the possibility of the active absorption of P\textsuperscript{32} by zooxanthellae is weak.

c. Phosphorus release

It was suggested previously that F. scutaria takes up less and excretes more phosphorus in the dark than in the light. This relationship can be more
clearly demonstrated by transferring the coral polyps labelled with $P^{32}$ into nonradioactive medium and subsequently measuring the amount of $P^{32}$ lost from the corals in the light and in the dark.

To accomplish this, 40 polyps of $F. scutaria$ were kept for 72 hours in two plastic aquaria with 10 liters of sea water and 266 $\mu$C of $P^{32}$ as previously described (page 94). After this incubation period, the polyps were transferred, in groups of four, to ten small plastic containers (five transparent and five opaque) with 200 ml sea water filtered through an HA Millipore filter. The chemical analysis established that this water contained 0.14 $\mu$g-at. P/1 of dissolved inorganic phosphate. These containers were kept under constant illumination (approximately 6,000 lux) and at a constant temperature ($23.4^\circ \pm 0.3^\circ$C). After incubating for periods of 1, 3, 7, 17 and 30 hours, four polyps from a transparent and four polyps from an opaque container were removed, treated by the standard procedure and their $P^{32}$ activity determined on the ashed samples.

Upon removal of polyps, the entire volume of the medium from each container was filtered through an HA Millipore filter with pore size of 0.45 $\mu$. The filter was mounted on an aluminum planchette with a drop of acetate glue diluted with acetone. Each sample was then counted to determine $P^{32}$. Determinations of DIP$^{32}$ and DOP$^{32}$ and subsequent calculations of DTP$^{32}$ and TP$^{32}$ were made on the filtrate as previously described.

Two 200-ml control samples of sea water were taken from the same stock as the experimental medium. One sample (A) received approximately 0.5 $\mu$C and the other (B) 1.0 $\mu$C of $P^{32}$. They were kept frozen and later analyzed, together with the experimental media, for the different $P^{32}$ fractions and for dissolved inorganic phosphate. The $P^{32}$ activity retained on the filter after filtering the control solutions averaged 3.68% of the total activity. This control was used to correct for contamination of PP$^{32}$ with DIP$^{32}$ of the experimental media. The correction factors for contamination of three fractions of DOP$^{32}$ were obtained by running DOP$^{32}$ analyses on these two control solutions. The mean values, shown below, were applied to all the experimental media:

<table>
<thead>
<tr>
<th></th>
<th>DOP$^{32}$ (A)</th>
<th>DOP$^{32}$ (B)</th>
<th>DOP$^{32}$ (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>2.5%</td>
<td>0.12%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Control B</td>
<td>2.7%</td>
<td>0.10%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Mean</td>
<td>2.6%</td>
<td>0.11%</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

The total $P^{32}$ activity recovered in the medium (TP$^{32}$/200ml) was added to the total $P^{32}$ activity determined for four polyps in a container to obtain the initial activity of animals. All the fractions of $P^{32}$ were expressed as the percentage of the initial activity. Figure 18 summarizes the relative quantities of DIP$^{32}$, DOP$^{32}$ and PP$^{32}$ released by the corals. The histograms in the figure show that $F. scutaria$ releases the labelled phosphorus into its surrounding medium at a much higher rate in the dark than in the light. This was
already suggested from the results of the preceding experiment. There is no significant difference in the quantities of DOP\textsuperscript{32} and PP\textsuperscript{32} released between the light and dark treated animals. The sharp difference was found in the quantities of DIP\textsuperscript{32} released; the main portion of P\textsuperscript{32} released in the dark is attributable to DIP\textsuperscript{32}. The ratio of DIP\textsuperscript{32} to DOP\textsuperscript{32} released at the 30th hour was about 9 : 1 in the dark and 1 : 1 in the light.

![Diagram showing relative activities of DIP, DOP, and PP at different times in light and dark conditions.](image)

**Figure 18.** The changes with time in the relative quantities of DIP\textsuperscript{32}, DOP\textsuperscript{32}, and PP\textsuperscript{32} in sea water during incubation in the light (6,000 lux) and in the dark with *F. scutaria* labelled with P\textsuperscript{32}. The P\textsuperscript{32} fractions are expressed as percent of the initial activity of the polyps.

The change in the quantities of dissolved inorganic phosphate as determined spectrophotometrically roughly paralleled that of DIP\textsuperscript{32} in the dark, whereas in the light the change in the quantities of dissolved inorganic phosphate was somewhat irregular, though there was a general trend of decrease with time. These relationships are shown in Figure 19 as the changes in the specific activities. Two values of the specific activities were calculated as before (page 93), SA : TP\textsuperscript{32} (TP\textsuperscript{32} / DIP) and SA : DIP\textsuperscript{32} (DIP\textsuperscript{32} / DIP). The unusually high value of the specific activities for the light treated animals at the seventh hour of incubation is related to the very small quantity of DIP (0.07 µg-at./l) present in the medium. According to Strickland and Parsons (1960), this concentration of phosphate is lower than the lower limit of the sensitivity of the analytical technique (0.08 µg-at.P/1). Hence, a small error which might have been introduced during the analytical process could have been exaggerated. Except for these values there is a general trend of increase with time in two values of the specific activity in the light. This can be interpreted
as showing that there is a constant release of $P^{32}$ from the polyps kept in the light with the simultaneous absorption of dissolved inorganic phosphate.

![Figure 19](image)

Figure 19. The changes with time in the specific activities of TP$^{32}$ (SA: TP$^{32}$) and DIP$^{32}$ (SA: DIP$^{32}$) of sea water during incubation in the light (6,000 lux) and in the dark with F. scutari labelled with $P^{32}$.

This relationship is more clearly shown in Figure 20 as the changes in the "concentration ratio":

$$\frac{\text{DIP}^{32} \text{ released}}{\text{DIP} \text{ released}} \div \frac{\text{Initial TP}^{32} \text{ in animal}}{\text{Initial TP in animal}}$$

Because there was no significant increase in DIP in the light (Figure 23), the constant increase in the "concentration ratio" indicates that the system did not reach a steady state within 30 hours, that is, the distribution of $P^{32}$ did not become homogeneous inside and outside the polyps by the exchange processes.

The absolute quantities of phosphorus released by corals are shown in Figure 21 as measured from the differences in the initial and final dissolved inorganic phosphate of the medium. The relatively higher values for the first three hours both in the light and in the dark can be accounted for by the quantities of dissolved inorganic phosphate which had been adsorbed to the polyps. Except for these values, there is a constant increase of the quantities of dissolved inorganic phosphate released in the dark. The net release rate as calculated from these data is maintained at a constant value of 0.0029 $\mu$g-at. P/g wet/hour between the 7th and 30th hour of incubation in the dark. Although there is a definite exchange of phosphorus between the polyps kept in the light and their medium, as shown in Figures 19 and 20, no significant net release of dissolved inorganic phosphate occurred in the light.
Figure 20. The release of DIP$^{32}$ expressed as concentration ratio \( \frac{\mu \text{c DIP}^{32} \text{ released}}{\mu \text{g-at. P DIP released}} \) by F. scutaria in the light (6,000 lux) and in the dark in sea water containing 0.14 \( \mu \text{g-at. P} \) of DIP but no P$^{32}$.

Figure 21. The release of DIP by F. scutaria in the light (6,000 lux) and in the dark as a function of time.

5. The effect of temperature on phosphorus uptake

The effect of temperature on phosphorus uptake by F. scutaria was studied in two series of experiments in which the rates of incorporation of Ca$^{45}$ and P$^{32}$ were simultaneously measured at different temperatures.
The initial activity of $P^{32}$ of the incubating medium was determined by counting three 0.2-ml aliquots placed and dried on aluminum planchettes. Upon removal of polyps, the incubating medium was filtered through an HA Millipore filter (pore size of 0.45μ), and the final activity of $P^{32}$ was determined by combining the activity retained on the filter and that recovered in the filtrate. Duplicate 50-ml samples of the filtrate were analyzed for dissolved inorganic phosphate. The initial phosphate concentration was determined on two 50-ml samples taken from the stock solution of the incubating medium and kept frozen until analyzed together with the experimental media. The initial phosphate concentration of the medium used in the first series of experiments were 11.45 μg-at. P/1, and that in the second series 2.70 μg-at. P/1.

![Figure 22](image-url). The rate of phosphorus uptake by *F. scutaria* as a function of temperature in the light (3,300 lux) and in the dark in seawater containing 11.45 μg-at. P/1 (A) and 2.70 μg-at. P/1 (B) of dissolved inorganic phosphate. The rate of phosphorus exchange by the dead corallum of *F. scutaria* under the same condition is also included in the figure.
The absolute quantities of phosphorus taken up by the polyps were obtained from the data on the P\textsuperscript{32} determinations for the ash samples by using Equation 3. Figure 22A shows the results of the experiment employing 11.45 \( \mu g\)-at. P/1, and Figure 22B those employing 2.70 \( \mu g\)-at. P/1. The low values for the rate of phosphorus uptake by the polyps kept at 15\(^\circ\)C (Figure 22A) reflect the moribund condition of these polyps. Except for these polyps, the rate increased linearly in the dark with the increase in temperature. In the light, on the other hand, the rate of phosphorus uptake was highest at 24\(^\circ\)C and lowest at 18\(^\circ\)C. There was no difference in the effect of temperature and light between high and low phosphate media, except that the rate was generally higher in the medium with higher phosphate concentration. The similar effect of temperature, light, and phosphate concentration were observed on the rate of calcium uptake (Figures 1A and 1B).

Figure 23. The rate of uptake or release of dissolved inorganic phosphate by \textit{F. scutaria} as a function of temperature during 26-hours incubation in sea water containing 11.45 \( \mu g\)-at. P/1 (High PO\textsubscript{4}) and 2.70 \( \mu g\)-at. P/1 (Low PO\textsubscript{4}) of dissolved inorganic phosphate in the light (3,300 lux) and in the dark.
The results on the determination of dissolved inorganic phosphate (DIP) is shown in Figure 23. The changes in DIP are expressed as the quantities taken up or released by unit wet weight of corals per hour. There was net uptake of DIP in the light at all temperatures, again with the highest rate at 24°C. No net uptake of DIP was observed in darkness except for a group of polyps kept at 24°C in the low phosphate medium.

6. The effect of the removal of zooxanthellae on phosphorus uptake

The effect of the removal of zooxanthellae from the tissues of *F. scutaria* on phosphorus uptake was studied in the experiment in which both the normal polyps and the polyps from which zooxanthellae had been removed were exposed to P$^{32}$ and Ca$^{45}$ (pages 82 to 85).

The results on the rate of phosphorus uptake determined by measuring P$^{32}$ absorbed by these animals are summarized in Table 5. As was the case with the rate of calcium uptake, the "bleached" polyps absorbed phosphorus at a considerably lower rate than the normal polyps. There was again no significant difference in the light between the normal polyps kept separately and those kept mixed with the bleached polyps, and likewise, between the bleached polyps kept separately and those kept mixed with the normal polyps. However, in the dark the normal polyps kept separately absorbed phosphorus at significantly higher rate than those mixed with the bleached polyps ($P=0.01$), and, on the contrary, the bleached polyps kept mixed with the normal polyps absorbed phosphorus at significantly higher rate than those kept separately ($P=0.025$). In the dark, some interaction can be suspected between the normal and bleached polyps when kept together in the same container. The suspected interaction caused the rates of phosphorus uptake to approach an intermediate value.

Since both in the light and in the dark, the rate of diffusion exchange of phosphorus in the container having both the normal and bleached polyps was considerably lower than that of the container having either normal or bleached polyps, corrections for this difference is necessary to make more meaningful comparisons between the different treatment group. When this correction was made by subtracting the rate of diffusion exchange from the rate of phosphorus uptake, the rate of the normal polyps kept in the same container as the bleached polyps in the light was found to be lower than that of the normal polyps kept in a separate container. On the other hand, the rate of phosphorus uptake by the bleached polyps incubated together with the normal polyps in the light was higher than that of the bleached polyps kept in a separate container. The same relationships were also found in the dark. It appears then safe to conclude that, when both the normal polyps and the polyps from
Table 5. The effect of the removal of zooxanthellae from tissues on the rate of phosphorus uptake by *F. scutaria* in light and darkness.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate of Phosphorus Uptake* ( (\mu g\text{-at.} \text{P/g wet/hr}) )</th>
<th>Ratio</th>
<th>N/B</th>
<th>L/D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Separate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (N)</td>
<td>0.00937 ± 0.00246</td>
<td>1.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>0.00105</td>
<td>8.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached (B)</td>
<td>0.00114 ± 0.00017</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>0.00111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light (L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.00879 ± 0.00028</td>
<td>2.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>0.00101 ± 0.00041</td>
<td>8.70</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>0.00076</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Darkness (D)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.00530 ± 0.00036</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>0.00152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>0.00088 ± 0.00017</td>
<td>6.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>0.00174</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td>0.00116 ± 0.00007</td>
<td>3.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corallum</td>
<td>0.00098</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*mean ± standard deviation

which zooxanthellae were removed were kept in the same container, the former exerts stimulatory effect on the phosphorus uptake of the latter while the latter exerts inhibitory effect on the former, both in the light and in the dark. The difference in the rate of phosphorus uptake between the “separate” groups and the “mixed” groups were greater in the dark than in the light. Regarding the nature of these effects nothing can be said on the basis of the data obtained from the present experiment.

The significant finding in this experiment is the fact that the normal polyps absorbed phosphorus in the dark at a considerably higher rate than the bleached polyps. More significant is the fact that there was a net absorption of DIP by these polyps whereas net release was observed for the bleached polyps under the same condition; the absorption rate for the normal polyps was 0.0013 \( \mu g\text{-at.} \text{P/g wet/hr} \), and the release rate for the bleached polyps was 0.0010 \( \mu g\text{-at.} \text{P/g wet/hr} \). The comparable uptake rates of the normal polyps and the bleached polyps in the light were 0.0065 and 0.0052 \( \mu g\text{-at.} \text{P/g wet/hr} \), respectively.

The release rate of DOP\(^{32}\) was also estimated by the method given on
The following results were obtained: normal, light: 1.45%, bleached, light: 0.66%, normal, dark: 1.19%, bleached, dark: 1.09% of the initial $^{32}$P activity of the incubating medium. These results show that more organic phosphorus was released by the normal than the bleached polyps, both in the light and in the dark.

The tissue radioautographs prepared from some of the normal and bleached polyps are shown in Pl. figs. 3 and 4. The normal polyps kept in the light show accumulation of silver grains over the mucous glands of the oral epidermis, zooxanthellae in the oral gastrodermis. The bleached polyp is shown to have no positive tissue. It is also shown in these photomicrographs that the oral epidermis of the bleached polyp was reduced in thickness, that is in the height of columnar cells. Whether this is an artifact or not is not known from the histological preparations made in this experiment. That zooxanthellae were lost almost completely from the bleached polyps is also observed on these photomicrographs.

IV. DISCUSSION

1. Factors controlling calcification
   a. Temperature

A considerable amount of information has been accumulated on the effects of temperature on respiration and on temperature tolerance of corals. Mayer (1916) working with the West Indian corals, reported that at about 16°C all corals tested lost the power to catch food and died within one hour at temperatures 3° to 5°C lower than this. The higher death temperature (just sufficient to kill the corals within one hour) ranged from 36.4° to 38.2°C (Mayor, 1918). For the Hawaiian corals, the upper range of temperature at which the feeding activity ceased was found to be 31.5° to 35.5°C and the lower range 11.5° to 7.9°C (Edmondson, 1928). The temperatures 32°C and 15°C tested in the present work are considered to be within the normal physiological range, or deviating little from it.

The effect of temperature on the rate of calcium uptake by corals has not previously been investigated; hence information for comparison of the results from the present work with other species is wanting. The data obtained in the present work reveal a distinct difference in the effect of temperature between the light and dark conditions. In the dark, the rate of calcium uptake increased linearly with increase in temperature within the range of temperatures investigated (15° to 31°C), while in the light an optimum rate was attained at an intermediate temperature (24°C). Consequently, the greatest difference in the rate of calcium uptake between the light and dark conditions was obtained at 24°C (Figure 24A).
Figure 24. The net effect of light on the rate of calcium uptake by *F. scutaria* (A-E) and *P. compressa* (F) under various conditions. The net effect of light is expressed as the difference in the rate of calcium uptake in light from that in darkness and corrected for physical diffusion where data are available. High PO₄ and low PO₄ in A stand for phosphate concentrations of the incubating media, 11.45 and 2.70 μg P/l respectively.

Because photosynthesis may be regarded as the only activity sensitive to light in *F. scutaria*, the increased rate in the light should be interpreted in relation to zooxanthellae. Measurements of phosphorus uptake as shown in Figure 22 may be regarded as an index of photosynthesis, because phosphorylation is an integral part of photosynthesis. Because nearly parallel effects
of light and temperature were obtained on the rates of calcium and phosphorus uptake, both calcification in the animal body and photosynthesis by the algae may be regarded as operating optimally at 24°C or a temperature close to this.

Working with the developing embryos of the Hawaiian sea urchin, *Colobocentrotus atratus*, Hsiao (1961) found the optimum temperature for radiocalcium uptake to be 30°C. At 35°C radiocalcium uptake of his animal was lower than at the room temperature (ca. 25°C), and at 40°C the gastrula failed to develop further. This information suggests that the rate of calcium uptake by *F. scutaria* may eventually reach an optimum value in the dark with a little increase in temperature beyond 31°C.

b. Salinity

The only available information on the effect of altered salinity on the growth of corals was obtained by Edmondson (1929). *Lepistrea purpurea* weighing 120.48 g lost 1.54 g within four months, when exposed to 80% sea water (changed daily). Similarly *Fungia scutaria* weighing 225.15 g lost 47.16 g during the first month of exposure to 75% sea water and a total of 58.6 g within ten months. Because the animals used in the above experiments were not fed during the experimental period and no control groups for comparing the effect of fasting were provided, no valid conclusion can be made from these results on the effects of the lowered salinity on growth. However, *F. scutaria* could endure 66.3% and 75% sea water for a period of four months (Edmondson, 1928). From his experiment, Edmondson (1928) concluded that *F. scutaria* among other species can live in 75% sea water for an indefinite time interval if sufficient food is available.

The results of the present work agree with this conclusion. In the light the rate of calcium uptake in 75% sea water did not significantly change from that in 100% sea water. Furthermore, it is assumed that hydration of tissues was relatively insignificant in 75% sea water and that the physiological state of *F. scutaria* was not appreciably changed by exposure to 75% sea water under the conditions of the experiment. In the dark, however, the rate of calcium uptake in 75% sea water was significantly lower than that in 100% sea water. This suggests that the zooxanthellae have an important role in maintaining the normal rate of calcification.

c. Calcium concentration

By analogy to an enzyme-catalysed reaction, a study on the relationships between the rate of calcium uptake and calcium concentration could reveal the characteristics of the overall reaction of calcification. Such a study has never been performed on corals nor on any other invertebrate calcifying system, although some descriptive observations have been made on the effect
of calcium concentration on the calcifying tissues.

Bevelander and Benzer (1948) reported that the regenerating shell of a mollusc, *Pedalion*, failed to deposit calcium carbonate in sea water containing one-eighth of the normal calcium concentration. Okazaki (1956) studied the effect of altered calcium concentration on spicule formation by the larval sea urchin. Her observation has shown that calcifying systems can operate within a wide range of calcium concentration. She found that within the range of calcium concentrations 40 to 600% of that of normal sea water, spicules with roughly normal appearance were formed; and that even in sea water with a calcium concentration as low as 5% of the normal concentration, the spicules, once formed, continued to grow.

One of the most interesting findings of the present work is the fact that the rate of calcification responded differently to the changes in calcium concentration under different light conditions. In the dark the rate of calcium uptake changed in much the same way as an enzyme-catalyzed reaction. On the other hand, in the light the rate continued to increase beyond the concentration (200 mg Ca/l) where a steady state was reached in the dark. This difference can again be best attributed to the presence of zooxanthellae.

The sharp increase in the rate of calcium uptake with the increase in calcium concentration from 400 to 600 mg/l is difficult to explain. The change in calcium concentration resulted in the altered ionic ratio, Mg/Ca: 3.2 in the medium containing 400 mg Ca/l and 2.1 in the medium containing 600 mg Ca/l. The antagonizing effect, if any, of Mg++ on the transport of Ca++ through tissues is assumed to be decreased by this change; and consequently, acceleration in calcium uptake resulted. Whether the results are due to changes in Mg/Ca or not needs to be critically studied. In this respect, the observation by Okazaki (1961) that increase in the concentration of magnesium caused the same effect on the spicule formation of the sea urchin larva as the decreased calcium concentration, is worth mentioning.

d. Phosphate concentration

The effect of phosphate concentration on calcium uptake by corals requires discussion from two viewpoints, namely, the effect of the ambient phosphate concentration on photosynthesis and the proposed inhibitory effect of phosphorus compounds on calcification (Simkiss, 1954a, b, c).

Gibbs and Calo (1960) found that carbon dioxide fixation in the reconstituted spinach chloroplast was increased by a factor of 14 when the phosphate concentration of the medium was increased from 5 x 10^{-6} to 10^{-2} M. However, further increase in phosphate concentration (10^{-2} to 5 x 10^{-2} M) resulted in inhibition of carbon dioxide fixation. They also found that in the intact chloroplast, the threshold for inhibition was at the lower phosphate
concentration \( (2 \times 10^{-3} \text{ M}) \).

At present, nothing is known of the photosynthesis of zooxanthellae in this respect. If phosphate is assumed to have a similar effect on zooxanthellae as the spinach chloroplast, this would be reflected in calcification. This would result in an increase in the rate of calcification in the light with increase in phosphate concentration up to about \( 10^{-3} \text{ M} \), and then a decrease with the further increasing concentration of phosphate. Because photosynthesis is not in operation in the dark, calcification in the dark would be independent of the phosphate concentration.

Taking the opposing viewpoint, if phosphate has an inhibitory effect on calcification as suggested by Simkiss (1964 a, b, c), and if local accumulation of phosphate is prevented by photosynthetic activity of zooxanthellae, then the inhibitory effect of phosphate would be manifested in calcification in the dark, but not in the light.

If we accept the possibility of dual controlling mechanism, the rate of calcium uptake would increase in the light up to the phosphate concentration of about \( 10^{-3} \text{ M} \) and then decrease beyond this concentration, whereas in the dark the rate would decrease without break with the increase in phosphate concentration.

The data obtained for \( P. \) compressa (Figure 9) show that the graph for the polyps kept in the light does not satisfy any of the above assumptions. However the graph for the polyps maintained in the dark satisfies the second and third assumptions. When the mean differences in the rates of calcification (light–dark) were plotted against phosphate concentration (Figure 24F), the greatest differences were found in the intermediate phosphate concentrations \( (10^{-6} - 10^{-5} \text{ M}) \). This might be taken as indicating that the optimum phosphate concentration for photosynthesis of zooxanthellae lies within this range. Because the rate of the light group adjusted against that of the dark group as is shown in Figure 24F is more meaningful with respect to the role of zooxanthellae, it may be concluded that the data for \( P. \) compressa support the assumption that phosphate controls calcification through its stimulatory effects on photosynthesis and inhibitory effects on calcium carbonate deposition.

The data obtained for \( F. \) scutaria (Figure 6) sustain the above conclusion. The graph for polyps kept in the dark satisfies the second and third assumptions except for a few individual points at the intermediate phosphate concentrations \( (10^{-5} - 10^{-4} \text{ M}) \). The fact that in the light the rates of calcification of some polyps remained at the level of the control group (the group exposed to the phosphate concentration of \( 10^{-7} \text{ M} \)), while those of the other polyps decreased significantly, may be taken as suggesting that the dual control mechanism is causing some disturbance in the metabolic activities of these polyps.
Although not easily distinguishable from the graph (Figure 8), examination of individual values for P/N ratio reveals that the individuals with high calcification rates in the intermediate phosphate concentrations had low values for P/N ratio (about 5 μg-at. P/mg N). If it is assumed that the high P/N ratio results from some physiological disturbance due to high phosphate concentration, then these polyps can be regarded as being able to overcome the disturbing effects, while those with the reduced rates of calcification were unable to do so. The definite increase in P/N ratio (Figure 7) with the increase in phosphate concentration supports the above assumption.

As a summary, a diagram illustrating the overall effect of phosphate on calcification was constructed (Table 6). The stimulatory effect of phosphate on photosynthesis and the inhibitory effect on calcium carbonate deposition were assigned arbitrary numbers at each phosphate concentration in the light and in the dark. The effect of phosphate was obtained by subtracting "inhibition" from "photosynthesis".

Table 6. An illustration of the overall effect of phosphate on calcification as a function of phosphate concentration both in the light and in the dark. (The effect on calcification is obtained by subtracting the assigned number expressing the effect of inhibition on calcium uptake from the corresponding number for the stimulatory effect on photosynthesis.)

<table>
<thead>
<tr>
<th>Phosphate Concentration</th>
<th>Light</th>
<th>Photosynthesis</th>
<th>Inhibition</th>
<th>Calcification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(0 - 10^{-7} M)</td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate (10^{-7} - 10^{-4} M)</td>
<td>Yes</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>No</td>
<td>0</td>
<td>1</td>
<td>-1</td>
</tr>
</tbody>
</table>

The effect of light on the rate of calcium uptake was also investigated in relation to some other factors, and these are summarized in Figure 24. The net effect is expressed as the difference of the rate of calcium uptake in the dark from that in the light. When the information on the rate of physical diffusion by dead coralla was available, the rate of calcification was corrected for this.

The results obtained from the experiment in which the effect of the removal of zooxanthellae was studied agree with the results reported by Goreau (1959); that is, the rate of calcium uptake of the polyps without zooxanthellae was considerably lower than that of the normal polyps.
present work includes two additional findings. In the first place, although not statistically significant, there was some indication of interaction between the normal polyps and those without zooxanthellae in the dark. This interaction was manifested in decreasing the rate of calcification of the normal polyps when these were kept with the polyps without zooxanthellae, and in increasing the rate of the polyps without zooxanthellae when these were kept with the normal polyps. The similar but more definite interaction was detected for the rate of phosphorus uptake both in the dark and in the light. It appears as if inhibitory substance present in the polyps without zooxanthellae is affecting the calcium and phosphorus uptake by the normal polyps and the stimulatory agent of the normal polyps, in turn, influencing the zooxanthella-free polyps when both are present side by side. These interactions seem to be more pronounced in the dark than in the light. At present nothing can be proposed on the nature of these interactions. A further study using suitable ahermatypic corals would provide some information on the nature of such interactions.

The second important finding is that there were nearly parallel changes in calcification and phosphorus uptake caused by the removal of zooxanthellae. However, there was a significant difference in the ratio of normal to bleached polyps between calcium and phosphorus uptake. This ratio was 1.47 (in the light) and 1.41 (in the dark) times greater for the rate of calcium uptake than for the rate of phosphorus uptake. This is interpreted to mean that calcium uptake was more strongly affected by the removal of zooxanthellae than phosphorus uptake; or conversely calcium uptake was stimulated more strongly than phosphorus uptake by the presence of zooxanthellae. This is, then, an additional evidence to show that the stimulatory effect of zooxanthellae is not solely due to photosynthetic activity but that some other factor is also involved.

The fact that the bleached polyps took up DIP from the medium in the light but released it in the dark may be taken as indicating residual activity of zooxanthellae in the bleached polyps. This may account for the slightly higher rate of calcium uptake (1.45/1) and phosphorus uptake (1.30/1) by the bleached polyps in the light than in the dark.

The effect of dinitrophenol on the rate of calcium uptake in *F. scutaria* is similar to that on the calcium deposition in the oyster (Maroney *et al.*, 1957), except that in *F. scutaria* the concentration of $10^{-5}$ M inhibited calcium uptake in the dark while in the oyster this concentration was not effective. The finding that dinitrophenol has an inhibitory effect on calcium uptake shows that coral calcification includes some reaction which is dependent upon phosphorylation. The present study suggests that photophosphorylation is less sensitive to dinitrophenol than oxidative phosphorylation.
2. Phosphorus metabolism in relation to calcification

One of the major questions raised in this work concerning phosphorus metabolism in corals was whether or not zooxanthellae can absorb, in the dark, excess phosphorus excreted by the host coral.

Yonge and Nicholls (1931a, b) have shown that corals such as *Porites* and *Favia* absorb phosphate from sea water containing 0.11 and 65.7 μg-at. P/1 phosphate, whereas the same species of corals, when zooxanthellae were removed, excreted phosphate into sea water to the same extent as a coral naturally lacking zooxanthellae (*Dendrophyllia*). From this finding, they concluded that zooxanthellae can utilize the metabolic wastes of the host corals. However, their experiments were conducted under natural conditions of illumination and therefore, nothing can be said about the state of phosphorus metabolism in the dark.

In order to explain the finding by Goreau (1959) that, in the dark, the normal corals calcified faster than the corals without zooxanthellae, Simkiss (1964a, b, c) proposed that zooxanthellae aided calcification by removing excess quantities of phosphorus compound which he regarded as an inhibitor of the deposition of calcium carbonate.

It has been known that in the dark algae do not absorb phosphate in considerable amount (Arnon, 1956). Gest and Kamen (1948) reported that *Chlorella* and *Scenedesmus* absorbed phosphate at a considerably lower rate in the dark than in the light. Rice (1953) regarded any amount of phosphate absorbed by the photosynthetic algae in the dark as due to physical exchange between algae and the medium. Whether or not zooxanthellae behave like the free living algae is a question to be solved in the present work.

The results of the present work have shown that under conditions of the experiment the polyps of *F. scutaria* with zooxanthellae can absorb phosphate in the dark. This was shown not only in terms of gross uptake but also in terms of net uptake, estimated by DIP determinations. Because the normal polyps absorbed P$^{32}$ in the dark six times faster than the polyps without zooxanthellae suggests that this absorption is due to the presence of zooxanthellae in the normal polyps. Although the net uptake of DIP by the normal polyps in the dark was only 14% of the corresponding value in the light, the finding that in the dark the polyps without zooxanthellae rather excreted phosphate into the medium supports this suggestion.

It is therefore tentatively concluded that zooxanthellae absorb phosphate in the dark though at a lower rate than in the light. This conclusion needs to be supported by further studies in which phosphorus metabolism of the isolated zooxanthellae is investigated. Such studies also have shortcomings, because the physiological conditions of the isolated zooxanthellae cannot arbitrarily be considered to be the same as they are in the host corals.
V. SUMMARY

1. The rate of calcium uptake of *F. scutaria* increased, in the dark, linearly with increase in temperature within the range 15°-31°C, while in the light the rate attained a maximum value at the intermediate temperature (24°C). The change in the rate of phosphorus uptake with changing temperature paralleled that of the rate of calcium uptake both in light and in darkness.

2. The rate of calcium uptake decreased considerably in the dark with decreasing salinity. In the light the rate decreased to the same extent as in the dark with decrease in salinity below 75% sea water, but in 75% sea water the rate remained the same as in 100% sea water. With increase in salinity to 125% sea water, the rate of calcium uptake decreased both in the dark and in the light with the decrease in the dark being more pronounced than in the light.

3. In the dark, with increase in calcium concentration from 0 to 200 mg/1, the rate of calcium uptake increased linearly and reached a steady state. In the light, the linear increase exhibited the same slope as in the dark but continued to 400 mg Ca/1, and above this concentration of calcium the rate started to increase sharply with increasing calcium concentration.

4. The rate of calcium uptake in the dark decreased linearly with the logarithmic increase in the ambient phosphate concentration (10^-7 - 10^-2 M), while in the light the decrease in the rate of calcium uptake was more rapid. As a net result, the difference in the rate of calcium uptake in the light was greater at the lower range of phosphate concentration. Similar results were obtained for a colonial coral, *P. compressa*.

5. Dinitrophenol completely inhibited calcium uptake at concentrations of 10^-4 M and higher both in the light and in the dark. At a concentration of 10^-5 M complete inhibition of calcification occurred in the dark, while no effect occurred in the light.

6. The removal of zooxanthellae from the animal resulted in a decrease in the rate of calcium uptake in the light to 1/12 the control value and in the dark to 1/8. There was no significant difference in the rate of calcium uptake in either light or darkness in the polyps devoid of zooxanthellae. The parallel change in phosphorus uptake occurred upon removal of zooxanthellae from the animal. The ratio, zooxanthella-free to normal polyps for phosphorus uptake were 1/8 and 1/6 in the light and in the dark, respectively.

7. A weak indication of interaction was observed between the polyps with or without zooxanthellae placed in the same container in darkness; the rate of calcium uptake of the normal polyps was lower when incubated together with the polyps without zooxanthellae than when incubated in separate containers, while the rate of polyps without zooxanthellae was higher when incubated together with the normal polyps than when incubated in separate
containers. This interaction was stronger for phosphorus uptake than for calcium uptake.

8. Phosphorus was absorbed by *F. scutaria* in the dark from sea water supplemented with phosphate (2.7 μg-at. P/1). However, the rate of phosphorus uptake in the dark was approximately one half that in the light. At this phosphate concentration, net uptake (as DIP) was observed both in the light and in the dark, while in natural sea water (0.27 μg-at. P/1) net release (as DIP) rather than uptake was observed.

9. The polyps devoid of zooxanthellae as well as the normal polyps incubated in the light also absorbed DIP (net uptake) from sea water with phosphate concentration of 7.9 μg-at.P/1. The normal polyps incubated in the dark under the same condition absorbed DIP at a reduced rate than in the light, while those without zooxanthellae released DIP.

10. Nine more times inorganic phosphorus was released by the normal polyps in the dark than in the light. Approximately the equal quantities of organic phosphorus were released in the light and in the dark. The normal polyps released more organic P$^{32}$ than those without zooxanthellae, the ratio was 1/2.6 (zooxanthella-free to normal) in the light and 1/1.6 in the dark.

11. From the results summarized above, it was concluded that: (a) zooxanthellae accelerate calcification in corals under various environmental conditions, and the adverse effects of environment on calcium uptake is reduced by the presence of zooxanthellae in the coral tissue. (b) This effect of the presence of zooxanthellae is achieved through two processes; the removal of carbon dioxide from the carbon dioxide-carbonate system, and the removal of phosphorus compounds, a possible inhibitor of calcification, from the site of calcium carbonate deposition.

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VII LITERATURE CITED


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EXPLANATION OF PLATE FIGURES

Abbreviations

C: Coelenteron
E: Epidermis of the oral body wall
G: Gastrodermis of the oral body wall
GC: Gastrodermis lining calicoblastic layer
GM: Glandular margin of the mesenterial filament
M: Mesentery
MG: Mucous gland
IM: Inner region of the mesenterial filament
S: Decalcified skeleton
Z: Zooxanthellae

Figure

1. Historadioautograph showing accumulation of P$^{32}$ in the gland cells of the glandular margin and the mucous glands of the inner region of the mesenterial filament, and in zooxanthellae contained in the oral gastrodermis of a polyp of F. scutaria kept for 24 hours in the light in sea water containing P$^{32}$. 120X.

2. Historadioautograph showing a failure of accumulation of P$^{32}$ in tissues of a polyp of F. scutaria kept for 13 hours in the light in sea water containing P$^{32}$. 120X.

3. Historadioautograph showing accumulation of P$^{32}$ in the mucous gland of the oral epimis and in zooxanthellae of the oral gastrodermis of a polyp of F. scutaria kept for 24 hours in the dark in sea water containing Ca$^{45}$ and P$^{32}$. 120X.

4. Historadioautograph showing a failure to accumulate P$^{32}$ in tissues of a polyp of F. scutaria from which zooxanthellae were removed, and kept for 24 hours in the dark in sea water containing Ca$^{45}$ and P$^{32}$. 120X.
PLATE