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CALCIFICATION IN A SOLITARY CORAL, FUNGIA SCUTARIA

LAMARCK IN RELATION TO ENVIRONMENTAL FACTORS

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CALCIFICATION IN A SOLITARY CORAL, <u>FUNGIA</u> <u>SCUTARIA</u>

LAMARCK IN RELATION TO ENVIRONMENTAL FACTORS

By Kiyoshi Yamazato

A dissertation submitted to the Graduate Division of the

University of Hawaii in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy.

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 <u>Fungia</u> <u>scutaria</u>

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 Ca/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 Ca/l and with a steeper slope from 400 to 600 mg Ca/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 ug-at. P/1) 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 ug-at. P/1). 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 P-32 was released by the normal polyp than by the zooxanthella-free polyps under both light and dark conditions.

CALCIFICATION IN CORALS

Coral reefs are 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 were Agassiz (1890), Vaughan (1919), 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 $CaCO_3$ (Vinogradov, 1953), deposition of $CaCO_3$ 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 (Goreau

and Bowen, 1955) and radiocarbon (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 all 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 the site of deposition.

(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 (Stephens, 1962) which are absorbed by corals from sea water. It has been suggested that the general body surface serves as the site of entrance of these ions on the basis of electronmicroscopic observations of epithelial cells (Goreau and Philpott, 1956). As yet 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 calcium in the tissues of corals is only 0.88 that in sea water (Goreau and Bowen, 1955). It has therefore been postulated that the coral tissue serves to provide a simple medium for calcium transport, and is intimately associated with both the regulation of 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-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-14
carbonate taken up by corals was recovered in the organic
fraction of tissues. By the concurrent exposure of corals
to Ca-45 and $C-14CO_3$, he observed that the quantity of
exchangeable carbonate in the tissue was 2 to 15 times
greater than that of exchangeable calcium, further substan-
tiating the irreversibility of calcium transport.

Histological evidence indicates that calcium carbonate
is deposited on the organic matrix secreted by the calico-
blastic epidermis (Matthai, 1918; Hayashi, 1937; Goreau,
1956a, b). In the studies by Goreau he identified an
acid-mucopolysaccharide-like substance in the organic matrix
and subsequently (Goreau, 1959) proposed that Ca^{++} was
adsorbed by ion exchange on an 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 evidence has 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 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 was 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 the difference in the rate of calcification in the dark between normal corals and corals devoid of zooxanthellae on the basis of his proposal, 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.

OUTLINE OF THE PRESENT WORK

As was 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 was 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 work 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 zooxanthelae.

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 obtain 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.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

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

A young polyp of <u>F</u>. <u>scutaria</u> 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. How-
ever, 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 may be maintained in a
healthy condition in the laboratory without artificial
feeding for at least half a year.

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 0.45 μ. 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 as described on page 35.

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 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 media and mass of the polyps in each container were approximatly equal in any given experiment in order to minimize the variability due to 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 ($24° \pm 0.5°$ 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 ($15°$ 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 Ca-45 as $CaCl_2$ or P-32 as PO_4, 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, 0.2 ml aliquots (triplicate) were pipetted from each container to determine the final Ca-45 or P-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.

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 50 mg each, were placed on pre-weighed aluminum planchettes. The ash was evenly spread with the aid of water over approximately 3.1 cm^2 of the central portion. Afterwards the samples were dried under infra-red lamps, and the weight of the dry ash on the planchettes was determined. The radioactivity for each sample was detected using an end-window G-M tube or gas-flow detector connected to commercial scaling unit.

In the experiments using the double-labelling technique (Ca-45 and P-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 (60 mg/cm^2) inserted

between the sample and the window of the detector. A pre-

liminary study showed that this absorber density completely

absorbs radiation from the Ca-45. A correction factor for

absorption of P-32 by the absorber was determined by measuring

the count rate of a P-32 reference source with and without

the absorber. The correction factor for the absorption of

the P-32 activity with this absorber was about 2. The Ca-45

activity was determined by calculating the difference between

the count rate obtained without the absorber and the corrected

P-32 activity.

When necessary, aliquots of acid digests (page 19) were

also placed on aluminum planchettes, neutralized with NH_4OH,

dried and counted.

Dissolved organic and inorganic phosphorus labelled with

P-32 (DOP-32 and DIP-32) in the medium were determined by a

modification of the method described by Johannes (1963, 1964a).

Duplicate 5.0 ml samples of filtered incubating medium were

placed in 15 ml conical centrifuge tubes. To each tube, 0.8

ml of saturated $Ca_3(PO_4)_2$ were added, followed by 1.2 ml of

saturated Ca(OH)$_2$ in 10% CaCl$_2$. After centrifugation, an

additional 0.2 ml of Ca(OH)$_2$ in 10% CaCl$_2$ 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 5.0

ml of 10% trichloroacetic acid (TCA). One sample was filtered

through an HA Millipore filter (0.45 u 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 acetate 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 inorganic P-32 (pages 64 and 87).

All samples were counted for a minimum of five minutes

and corrected for background. The observed activity of Ca-45

was corrected for self-absorption. No corrections were

necessary for self-absorption of the P-32. The correction

for decay was made by counting a reference source of a known

activity.

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 included 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 phosphomo-

lybdate was measured at 730 mu 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 $-5^{\circ}C$.

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, 15 ml 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_4OH 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 Erlen-
meyer 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 received the same digestion procedure were run with
every batch of samples.

Total nitrogen content of the solutions prepared above
were 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.
Ten minutes after the addition of NaOH, 4 ml of Nesler reagent

was added. The extinction 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 mu.

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 7 9 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 Kodak AR.

ten 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

fuschsin while others were not stained at all. All the slides

were mounted with balsam. The emulsion did not take up basic

fuchsin stain.

EFFECT OF SOME ENVIRONMENTAL FACTORS ON CALCIUM UPTAKE

Because of the postulated role of the 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 thelight 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 the 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.

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 (0.254 Mev and

1.71 Mev, respectively) are greatly different, 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 15). The results of

the P-32 measurements will be presented later in the consi-

deration on phosphorus uptake (page 93).

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 ug-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/1), 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 the P-32 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 $\underline{F}$. scutaria in the light (3,300 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 rate of exchange of calcium by the dead corallum are also included in the figures.
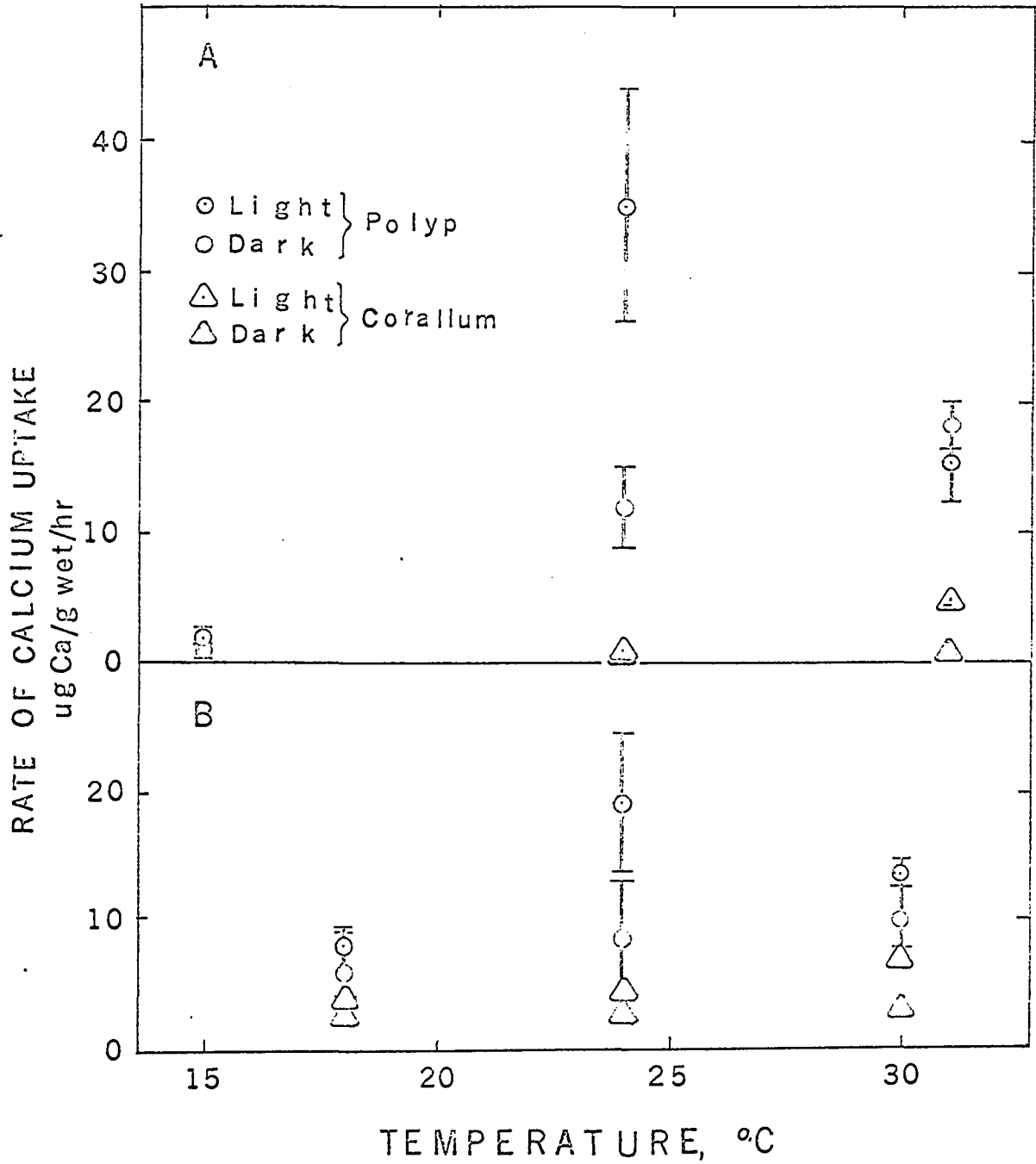
The results shown in Figures 1A and 1B reveal that in the dark the rate of calcium uptake by $\underline{F}$. $\underline{scutaria}$ increased rectilinearly with increase in temperature, while in the light the maximum rate was attained at the intermediate temperature (24° C). From the data given in these figures, the Q_{10} can be calculated by the formula,

$$Q_{10} = \frac{k_2}{k_1} \text{ ,}$$

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 Q_{10} over the entire range of temperature in the dark. On the other hand, in the light two values for the Q_{10} 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 Q_{10} values were obtained;

	low phosphate	high phosphate
dark	1.52	2.70
light*	1.55	2.45
light**	2.74	2.85,

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 * indicates the entire range of temperature and ** the range between the lowest (15° or 18° C) and the intermediate (24° C) temperatures.

The Q_{10} for the polyps kept in the medium containing higher phosphate concentration is consistently higher than that for the polyps kept in the medium with lower phosphate concentration. However, as was noted before, the polyps kept at 15° C in the medium with higher phosphate concentration were not in a healthy state. This may be the main cause for the higher Q_{10} for the group of polyps kept in the medium with higher phosphate concentrations.

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

for a known period of time and subsequently determining by

a tracer technique the quantity of calcium taken up by the

polyps.

Salinities of five relative concentrations were employed;

125, 100, 75, 50 and 25% sea water. Sea water collected at

the same place and at the same time as the polyps was

diluted with redistilled water to obtain sea water 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 concen-

trated 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 concen-

tration was determined by the Fiske Osmometer and expressed

as millimoles. 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.

Four living polyps and a clean corralum were placed in each container with one liter of sea water of a specified salinity. Parallel incubations in transparent and opaque containers were performed for each salinity employed.

Coral polyps kept in circulating sea water for six days were acclimatized for three hours in light and dark containers with previously prepared solution of sea water at $23^{\circ} \pm 0.2^{\circ}C$. The solution was removed and replaced with a fresh solution one hour prior to introducing 35.4 μc per liter of Ca-45, and the animals were incubated for 24 hours.

Following incubation with Ca-45, the coral polyps were removed from the containers and treated as described on page 14. The activity of Ca-45 taken up by polyps were determined as previously described (page 14). The resulting calcium uptake rate under light and dark conditions for each salinity is shown in Figure 2.

Because it was noted that during incubation the water

Table 1. Osmotic concentration, calcium concentration and
pH of the incubating media in three experiments (1 - 3) in
which the effect of salinity on the rate of calcium uptake
by _F_. _scutaria_ was studied.

Experiment No.	Treatment (% sea water)	Osmotic Concentration (milliosmol)	Calcium Concentration (mg/1)	pH
1	25	269	102	7.95
	50	520	207	8.07
	75	776	304	8.04
	100	1032	403	8.02
	125	1288	519	8.22
2	50		212	8.26
	100		418	8.20
3	50	506	204	8.11
	50 + Ca	522	398	8.18
	100	1012	410	8.00

Figure 2. The rate of calcium uptake by F. scutaria and the rate of diffusion exchange by the dead corallum 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.

Figure 3. The ratio of wet weight to dry weight of the living polyp of F. scutaria as a function of salinity.
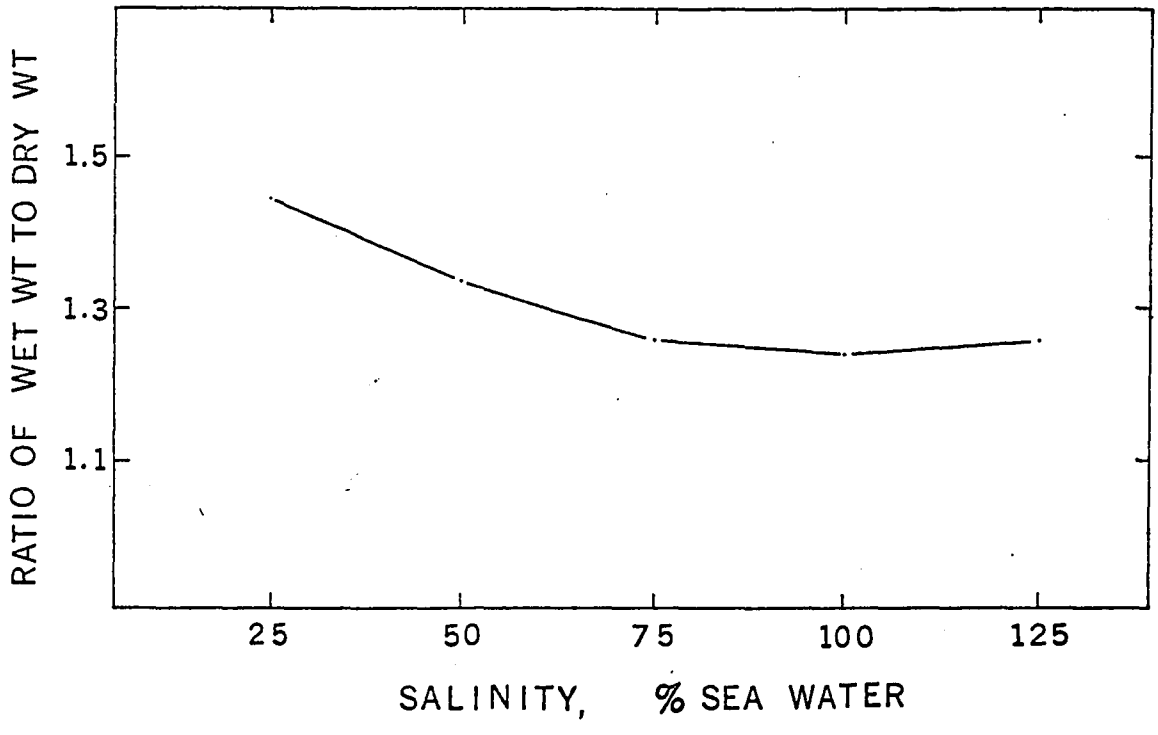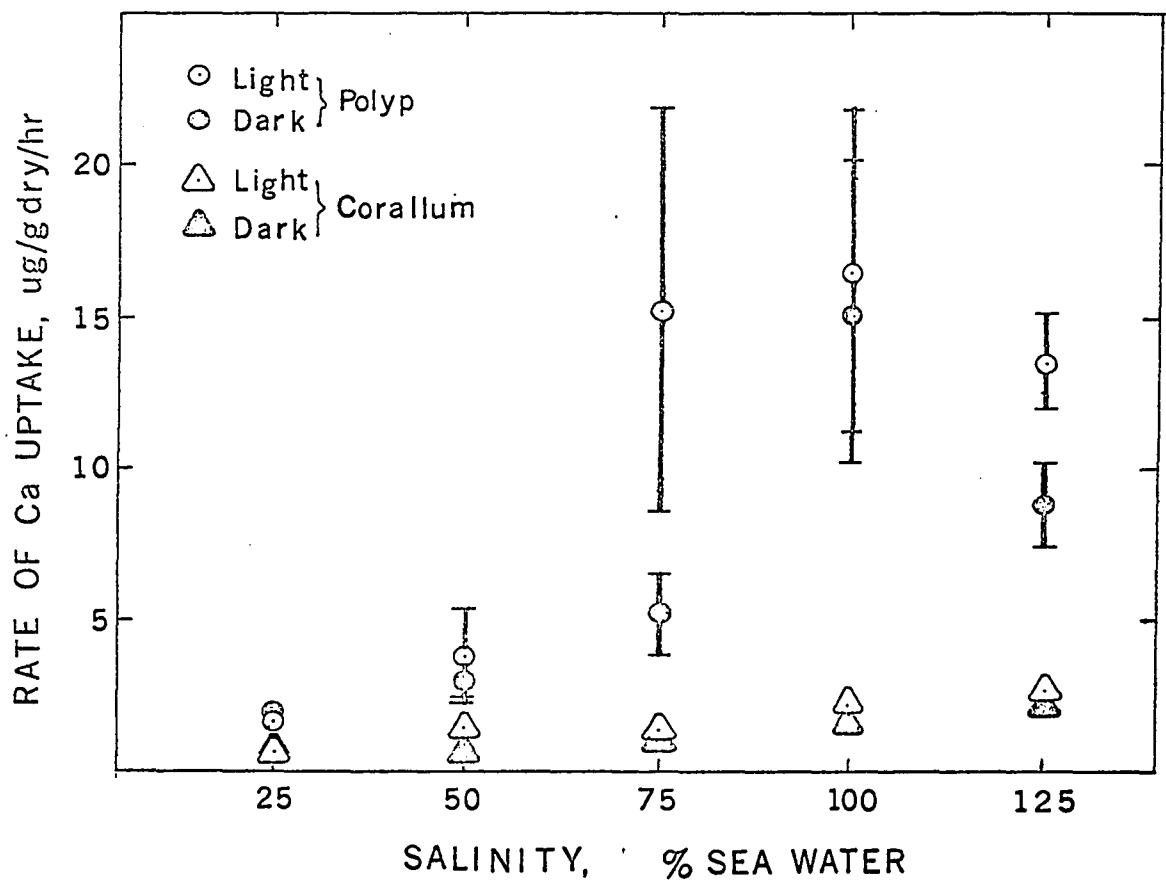
content of polyps changed in relation to the osmotic concentration of the medium to which they were subjected, the rate of calcium uptake was expressed in terms of dry weight rather than wet weight. The relative effect on the water content of the polyps is shown in Figure 3. At low dilutions (below 75%) significant quantities of water were incorporated into the tissues of the polyps.

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 in 100% sea water. 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:

$$\text{Concentration ratio} = \frac{\text{the rate of calcium uptake (ug Ca/g/hr)}}{\text{calcium concentration of medium (ug Ca/ml)}}$$

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 living and dead polyps and dead

coralla exposed to normal (100%) and half-strength (50%)

sea water and in 50% sea water with calcium concentration

equivalent to normal sea water (ca 400 mg Ca/l). The

calcium concentration as well as osmotic concentration and

pH of these media are tabulated in Table 1. The acclimati-

zation (for two hours) and incubation (for 25 hours) were

carried out placing two individual polyps or coralla in 200

ml of the medium with specified salinity and with 20 uc of

Table 2. The rate of calcium uptake by living polyps of F. scutaria and the rate of diffusion exchange of calcium by dead polyps and coralla of the same animal in normal (100%) and 50% sea water and 50% sea water with added calcium under continuous illumination (3,300 lux) for 25 hours. The data obtained in two independent experiments.

Experiment No.	Treatment (% sea water)	Rate of Calcium uptake** (ug/g dry/hr) mean ± S.D.	Rate of Diffusion Exchange*** (ug/g dry/hr) mean ± S.D.
2	100	9.25 ± 4.54 (4)	1.92 ± 0.04 (2)
	50	2.88 ± 2.12 (8)	1.07 ± 0.32 (4)
3	100	7.01 ± 3.43 (4)	5.34 ± 2.12 (2)
	50	2.38 ± 0.82 (4)	2.82 ± 0.54 (2)
	50 + Ca*	3.98 ± 1.42 (4)	5.00 ± 3.25 (2)

* Sufficient $CaCl_2$ was added to the dilute sea water to provide approximately 400 mg Ca/l (equivalent to the concentration in normal sea water).

** The rate of calcium uptake by living polyps; numbers in parentheses indicate number of individual polyps in each treatment group.

*** 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); numbers in parentheses indicate number of individual coralla or dead polyps.

Ca-45 (added at the onset of incubation) under 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 by living polyps in 50% sea water was 31.1% that of the control (100% sea water) group. The rate of the group kept in the 50% sea water added with calcium was higher: 56.8% of the control group. But the value of uptake rate was considerably less than that of the control.

These data, then, offer 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.

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 by using Ca-45 as a tracer.

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 $CaCl_2$ 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 artificial sea water, for

Na and Cl are the most abundant elements in sea water.

Determination of osmotic concentration of artificial

sea water thus prepared showed that it was slightly less

than that of sea water from the natural habitat of F. scutaria

(9.5% to 12.6%, cf. Tables 1 and 3). Because salinity

difference ± 25% of 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 with osmotic concentration in Table 3. A trace

amount of calcium (6 μg/l) was introduced as Ca-45 to the

Table 3. Some properties of the artificial sea water used

in an experiment in which the effect of calcium concentra-

tion on the rate of calcium uptake by F. scutaria was

studied.

Calcium Concentration (mg/l)	Osmotic Concentration (milliosmol)	pH
0.006	934	8.08
39.706	932	8.12
198.006	928	8.08
397.006	924	7.95
596.006	912	8.03

calcium-free sea water. A little difference in pH (0.17
units in range) was not corrected in order to avoid intro-
ducing other possible variables.

The coral polyps previously maintained in circulating
sea water for two weeks were acclimatized to the experimental
artificial sea water media ($23.4^\circ \pm 0.5^\circ$ C, 6,000 lux) for
two hours; four polyps being placed in a liter of the media.
The acclimatizing sea water was replaced by fresh but chemi-
cally similar water one hour prior to the addition of 70
μc/l of Ca-45, and then incubated for 24 hours. In a
separate group of containers containing 7 μc Ca-45 per 200
ml of the artificial sea water, two pieces of clean corallum
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 for calcium-free sea water (0.006 mg Ca/l)
was too small to be entered in the figure. The rate obtained
was 0.00017 ± 0.00006 μg Ca/g dry/hr for polyps in the light
and 0.00016 ± 0.00005 μg Ca/g dry/hr for polyps in the dark,
and the rate of diffusion exchange by the corallum was
0.00005 μg Ca/g dry/hr.

The rate of calcium uptake increased linearly with the

Figure 4. The rate of calcium uptake by the living

polyp of _F_. _scutaria_ in the light (6,000 lux) and in

the dark as a function of the ambient calcium concen-

tration during 24-hour incubation in artificial sea

water. The rate of calcium exchange by the dead

corallum of the same animal is also shown as a function

of calcium concentration.
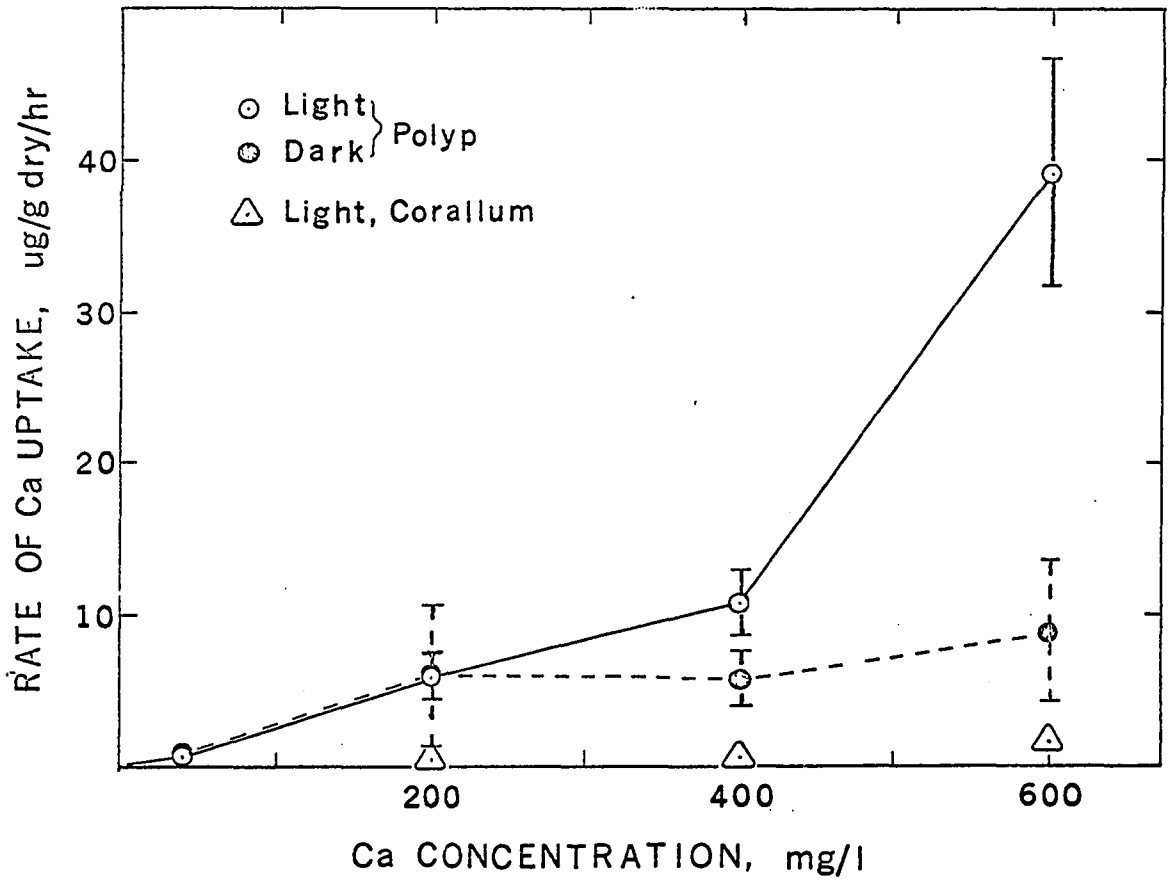
increase in the ambient calcium concentration from 0 to 400

mg Ca/l in the light and from 0 to 200 mg Ca/l 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 concentra-

tion (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, $0.0077 \pm$

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 calcium concentration of about

200 mg/l. The maximum velocity will then lie somewhere

between 6 and 9.5 µg Ca/g dry/hr.

Over the range of calcium concentration beyond 200 mg/l,

some factor other than 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 interpre-
ted his results as showing that the intestinal calcium
absorption is not due solely to passive diffusion, but uti-
lizes 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 regarded 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 5B shows the relative concentration ratio as
a function of calcium concentration. The concentration ratio

Figure 5. The relative concentration ratio of calcium

in F. scutaria to that in sea water ($\frac{\text{ug Ca/g dry}}{\text{ug Ca/ml}}$) as a

function of the ambient calcium concentration after 24-

hour incubation in the light and in the dark in sea

water with altered salinity (A) and in sea water with

a constant salinity (B).

A Salinity Altered
⊙ Light
○ Dark

B Salinity Constant
⊙ Light
○ Dark

RELATIVE RATE OF Ca UPTAKE, %CONTROL

Ca CONCENTRATION, mg/l

SALINITY, % SEA WATER
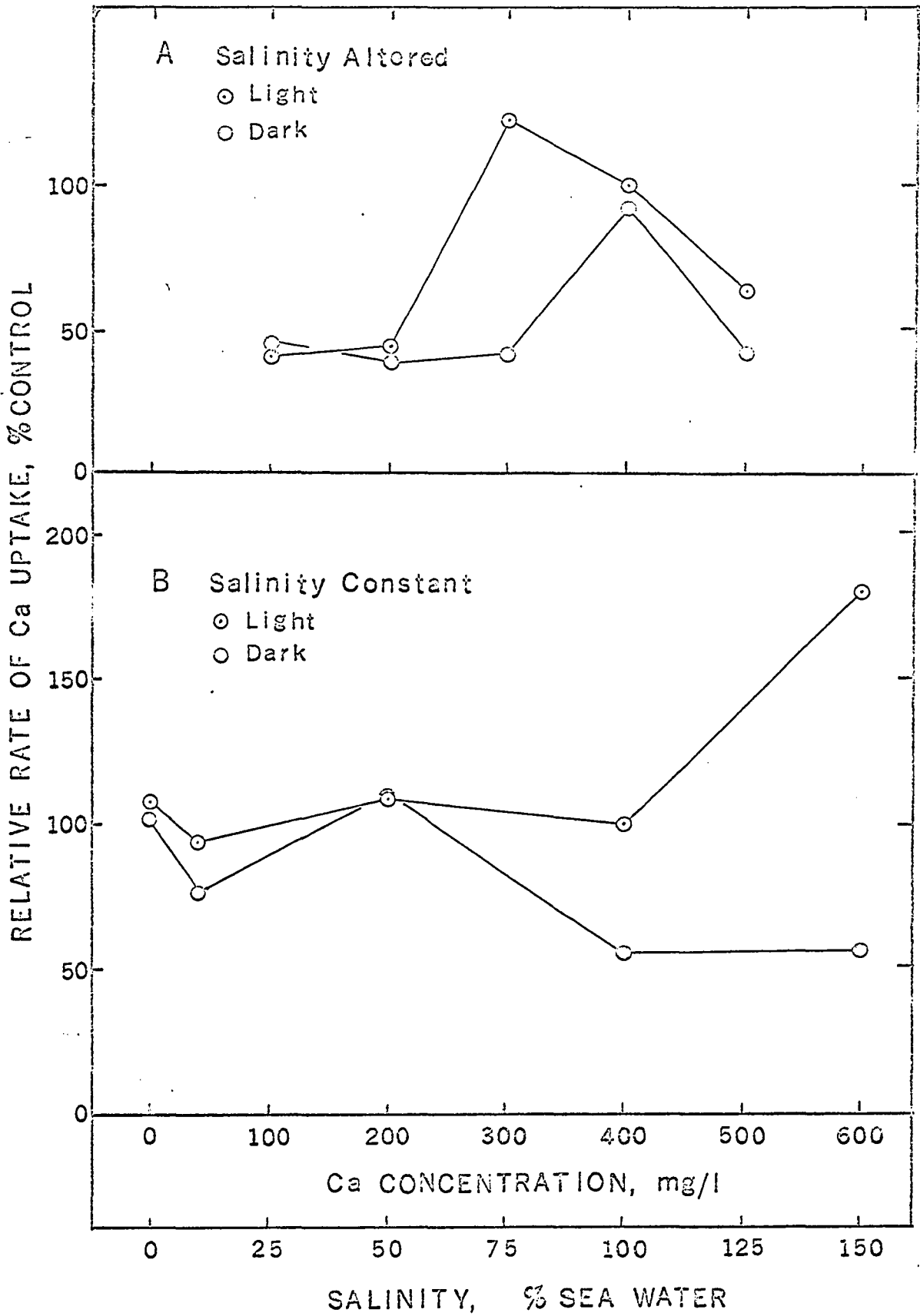
was expressed as percent normal (the concentration ratio
for the polyps kept in thelight at a calcium concentration
of 400 mg/l). The figure shows that in the dark over the
range of calcium concentrans 400 to 600 mg/l, the system
is operating half as effectively as in the lower range, while
in the light the effectiveness increases drastically as the
calcium concentration increases from 400 to 600 mg/l.

Phosphate Concentration.

Because phosphate has been known to act as a crystal
poison for calcium carbonate (Simkiss, 1964a, 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 may be
increased or decreased with change in phosphate concentration
in the surrounding medium of corals.

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_2PO_4) to natural sea water. The phosphorus concentration of natural sea water used as a control medium for these experiments ranged from 1.56×10^{-7} to 2.44×10^{-7}, determined as inorganic phosphate. The addition of NaH_2PO_4 to the sea water provided phosphorus concentrations in the experimental medium, varying by factors of 10, to a maximum of 10^{-2} M. Phosphorus 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} 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 this 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 phosphate phosphorus ranging from 10^{-7} to 10^{-2} M, followed by addition of 8.6 μc Ca-45 to each, and incubated in illumination

(3,300 lux) or complete darkness for a period of 12 hours.
Temperature was maintained at $22.8° \pm 0.3°$ C. The dead
corallum controls were incubated in separate vessels. All
polyps, including those incubated in subnormal pH (10^{-2} M
phosphate) remained healthy during this short incubation
period.

The rate of calcification in the light and in the dark
by $\underline{F}$. $\underline{scutaria}$ at varying phosphate concentrations are shown
in Figure 6. The rate of diffusion exchange by the corallum
remained constant over the entire range of phosphate con-
centration. The rate of calcium uptake was 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 phosphorus 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

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 corallum of the same animal under the same

condition is also included in the figure. Each point

represents the rate for an individual polyp.

Figure 7. The phosphorus content of F. scutaria

(expressed as protein ratio) as a function of phosphate

concentration of sea water at the end of 12-hour incu-

bation in the light (3,300 lux) and in the dark. The

vertical lines drawn through the points represent the

standard deviation of the means.
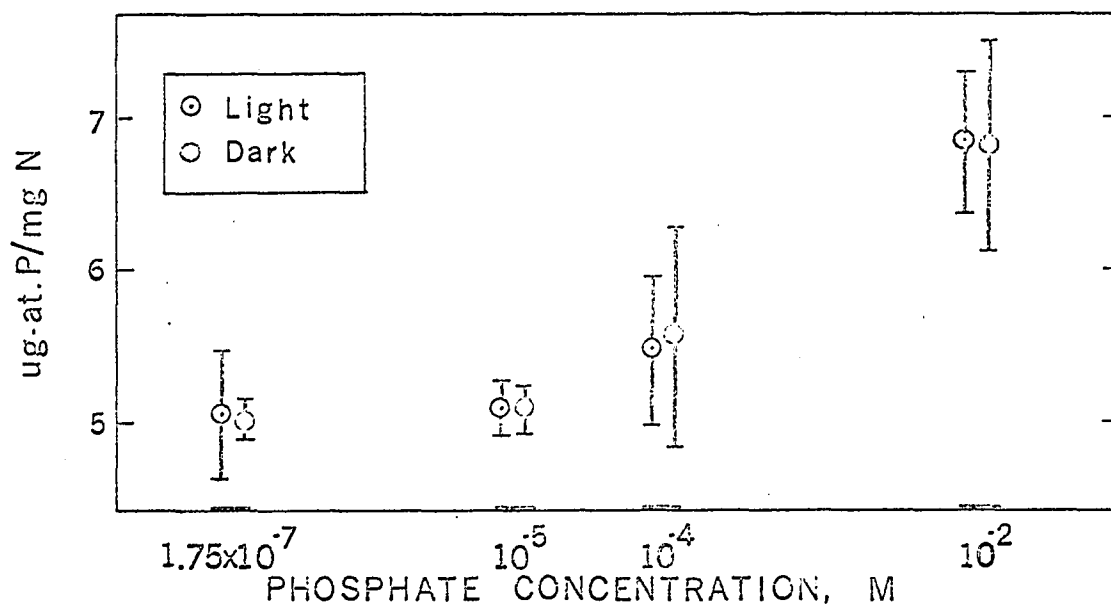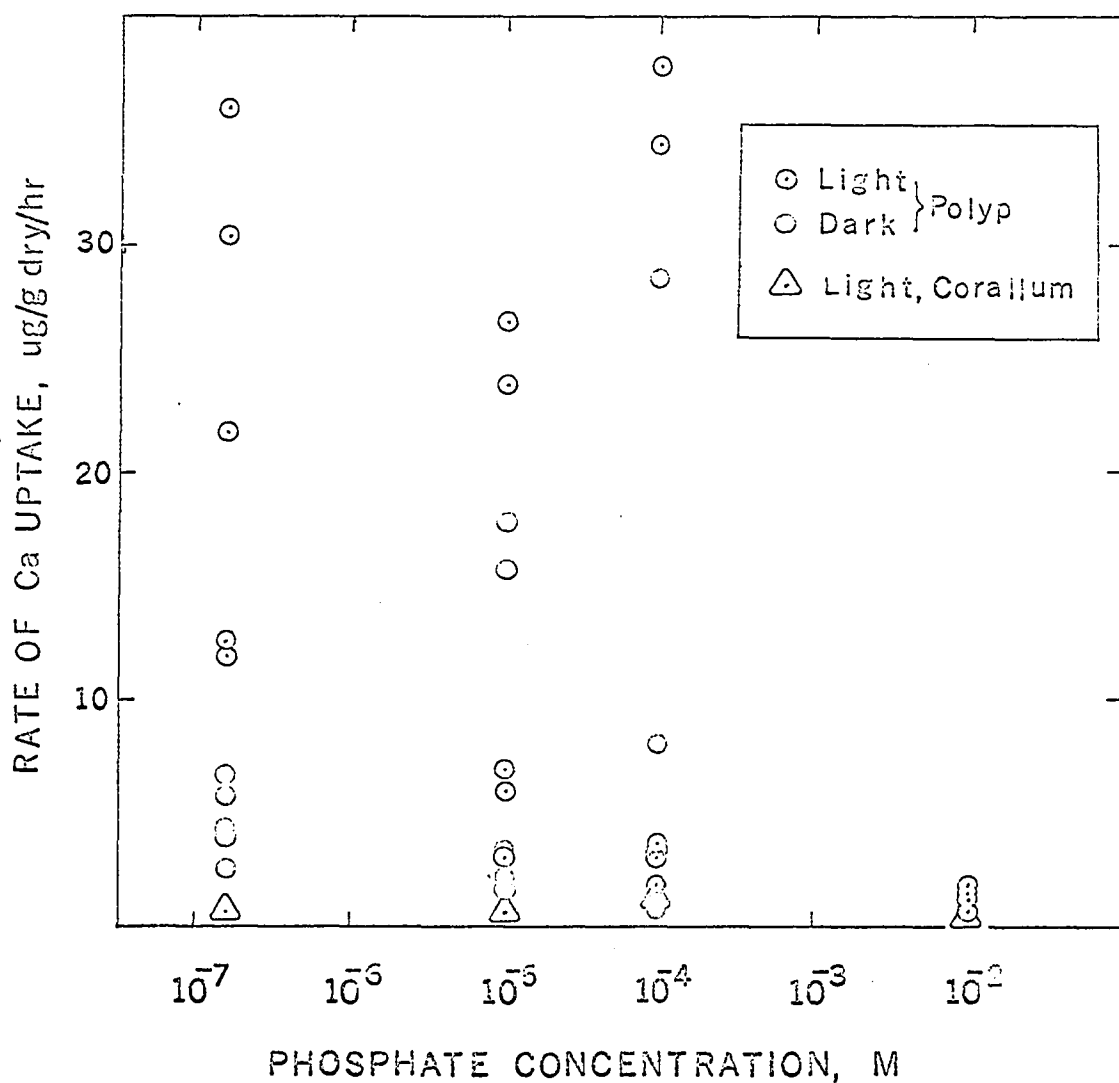
groups with narrow deviation, as if two groups of animals reacting to a common stimulus with opposing responses. Nevertheless, the data show that there is a general trend for the rate of calcium uptake to be inversely proportional to environmental phosphate concentration. The effect of phosphate concentration on the rate of calcium uptake as seen in Figure 6 is not different in light and in darkness, except in sea water having phosphate at low concentration $(10^{-7}$ M).

The phosphorus content of the polyps of $\underline{F}$. 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 natural sea 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 in $\underline{F}$. scutaria is proportional to the logarithmic increase in external phosphate concentration, and furthermore no significant difference was produced by illumination.

The relationship between 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 rates of calcium uptake higher than 10 µg/g dry/hr are limited to a narrow range of phosphorus content (between 4.5 and 5.5 µg-at. P/mg N). This suggests that polyps with higher tissue 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 branches weighing between 14 and 20 grams (dry weight) were carefully cut out of a colony. These pieces were retained in 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 5 sub-branches was placed in each container with 200 ml incubating medium having a phosphate concentration of 1.56×10^{-7}, 10^{-5}, 10^{-4} or 10^{-3} M. Paired opaque

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.
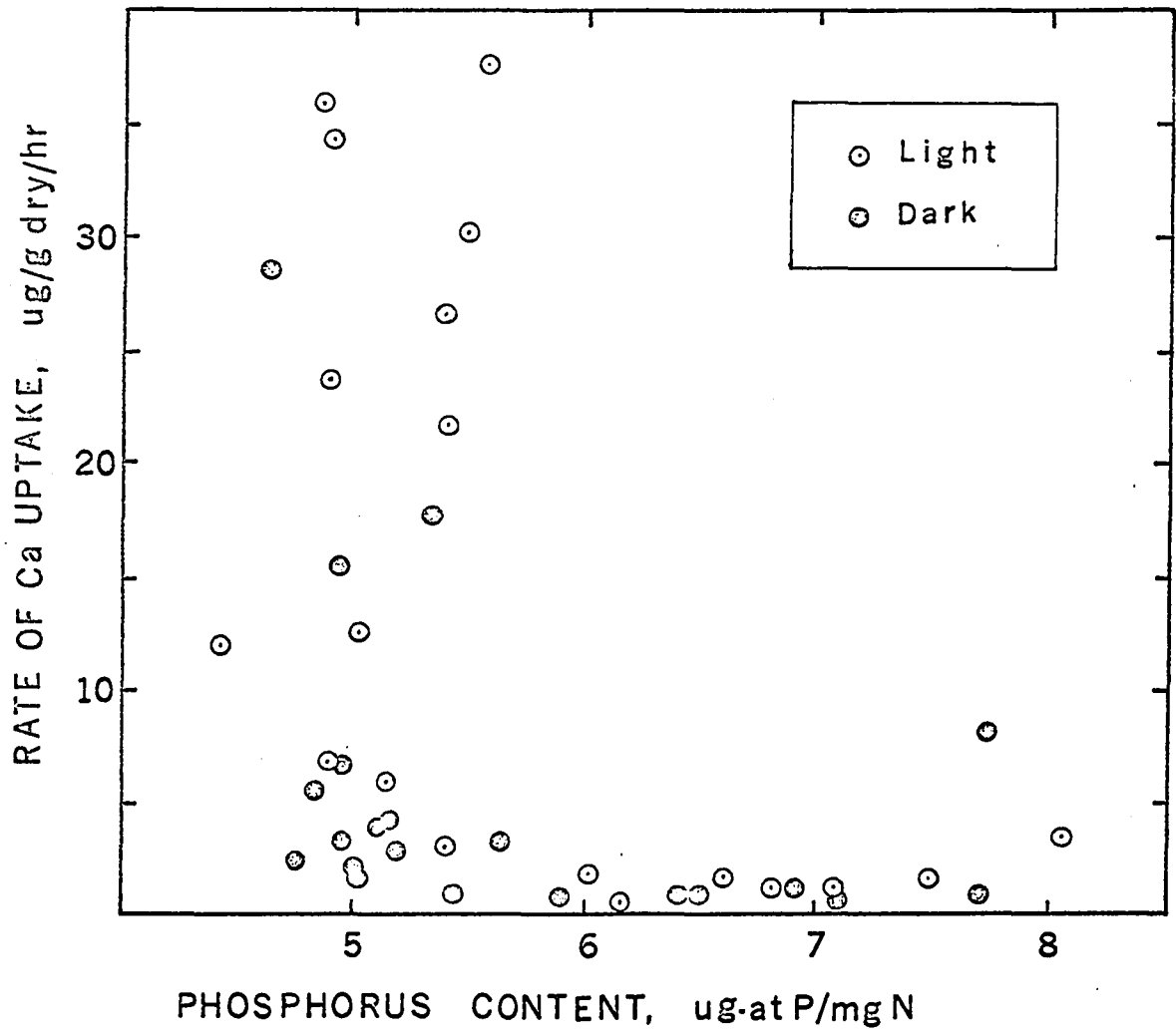
and transparent containers were used for each concentration. Approximately 7 μc/200 ml of Ca-45 was added to each experimental container. Temperature was maintained constant at 23.3° ± 0.3° C and light intensity at 3,000 lux. Duration of incubation was 24 hours.

After drying at 115° C for 24 hours and weighing, the tips of the sub-branches were severed 5 mm 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 phosphorus concentration in 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 incubating medium

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 repre-

sent the standard deviation of the means.

RATE OF Ca UPTAKE, ug/gdry/hr

PHOSPHATE CONCENTRATION, M

10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3}

⊙ Light
○ Dark
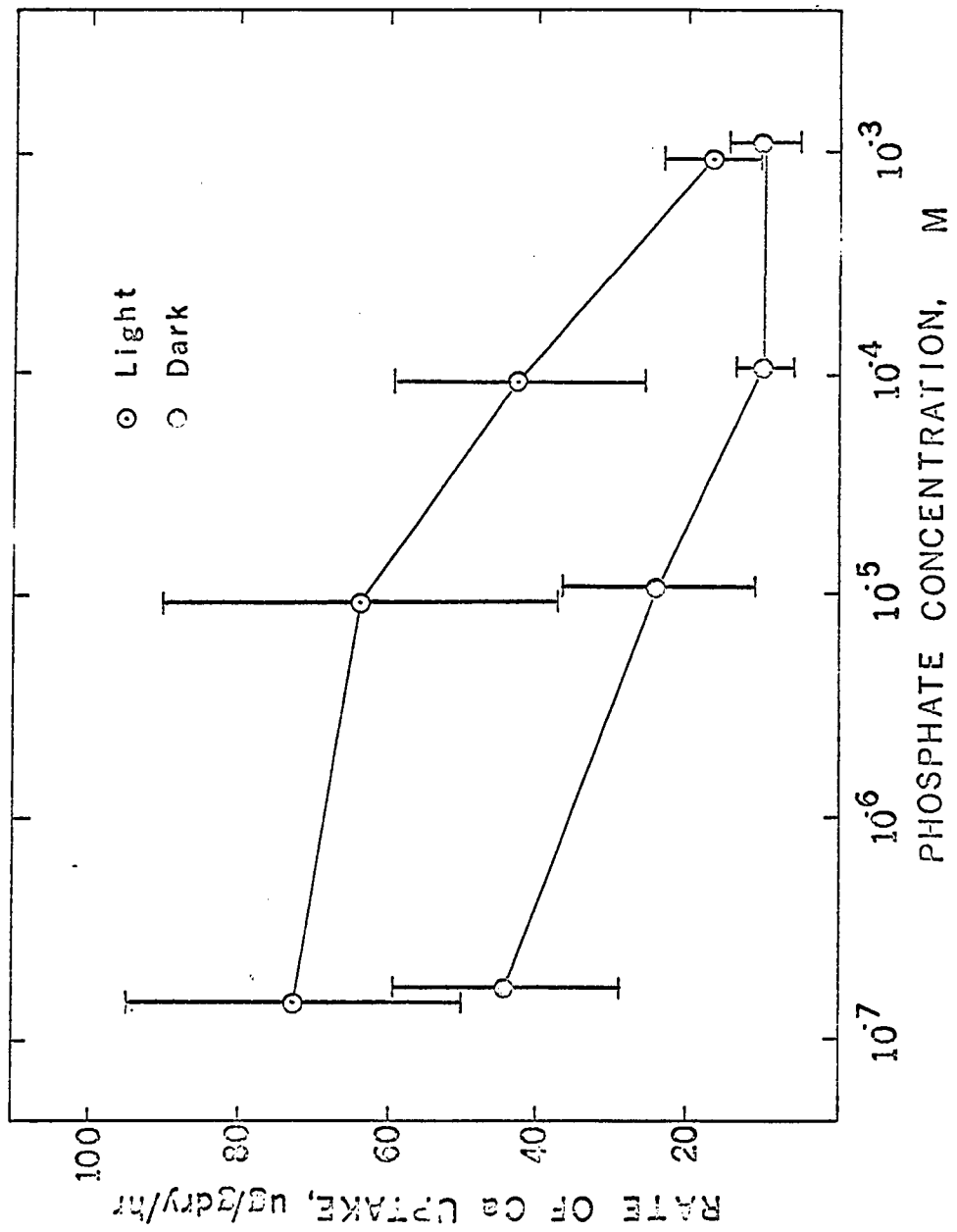
with phosphorus concentrations 10^{-3} M or higher produced subnormal pH (6.69), it must be emphasized that interpretation of these results must be viewed with caution.

However, Figure 9 shows clearly that the rate of calcium uptake decreases with increasing concentration of phosphate. Furthermore, the rate of decrease in calcification is proportional to the logarithm of the concentration. Excluding measurements for the highest phosphorus concentration, the following coefficients of regression were obtained; -9.31 (μg Ca/g dry/hr/10 M P in log scale) in 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 two slopes with common slope of -10.15 ($P \gg 0.25$). The difference in elevation of the two regressions is highly significant ($P \ll 0.001$). Although statistically not significant, the difference in the rate of calcium uptake between light and dark treatments is largest at 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 largest at this phosphate concentration.

EFFECT OF THE REMOVAL OF ZOOXANTHELLAE ON CALCIUM UPTAKE

The effect of zooxanthellae on calcium uptake by Fungia was studied by comparing the rate of calcium uptake of polyps from which zooxanthellae were removed with the rate obtained for plyps with zooxanthellae. Comparisons were made both in the light and in the dark. The possible interaction between normal polyps and polyps from which zooxanthellae were removed (bleached polyps) was studied by placing a groups of both normal and bleached polyps in a single container. The rate of calcium uptake of these polyps (mixed group) was compared with the rate for their counterparts which were kept in the separate containers (separate group).

In order to remove zooxanthellae from 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 tissues of F. scutaria. Examination of histological sections prepared from the bleached polyps revealed that only very few numbers of healthy cells of zooxanthellae survived this period of darkness (Figure 27). The metabolic state of zooxanthellae

is suggested by the presence of pyrenoid which is positive to basic fuchsin stain. The control group of normal polyps were kept for 15 days under constant illumination of 6,000 lux in a fiber glass tank receiving circulating sea water.

Sea water from the same source as that circulated through the aquaria in which both normal and bleached polyps had been kept was used as the incubating medium after first filtering through an HA Millipore filter. Five normal polyps were placed in each of a pair of the light and dark containers containing 1 liter of filtered sea 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 $\underline{F}$. scutaria was placed in each container.

All the containers were placed in a water bath with water temperature maintained at 24° C $\pm$ 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 Ca-45 and 113.4 μc of P-32 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 14). The activity of P-32 and Ca-45 taken up by 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 18), and the acid digest was diluted to 100 ml with redistilled water. Three 0.2 ml aliquots of the diluted digest was counted to determine Ca-45 retained on the filter together with the particulate P-32. Three 0.2 ml aliquots from the filtrate were counted to determine Ca-45 in solution and the final activity of Ca-45 in the medium was estimated from the total activities determined from the filter and filtrate. The filtering process was not performed. particularly to determine the final Ca-45 activity in the medium but rather to determine the P-32 activities in the different fractions (particulate, dissolved organic, dissolved inorganic fractions). The filtrate was further

treated to determine P-32 dissolved organic phosphorus
compounds as described on page 17 . The method of
determining Ca-45 and P-32 activities from the common samples
is described in a section on methods (page 15).

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-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 ratios of the mean
rates of normal to bleached polyps within the separate or
mixed groups and the ratios for light to dark rates are also
shown in the table.

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

There was no statistically significant difference

(P > .5) in the mean rates between normal polyps kept se-
parately and normal polyps mixed with bleached polyps in the
light or in the dark. There was no significant difference
(P > .5) between bleached polyps kept separately and those
kept mixed with normal polyps, both in the light and in the
dark. However, when the rate of calcium uptake was corrected
for physical diffusion exchange of calcium (by the dead coral-
la), in the dark, the rate of calcium uptake of the normal
polyps kept together with the bleached polyps was considera-
bly lower than that of normal polyps kept separately, while
the rate of the bleached polyps increased when kept with the
normal polyps. In the light both "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 normal and
bleached polyps in the rate of calcium uptake.

Examination of histological sections of bleached polyps
(Figure 27) 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 epider-
mal 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.

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

Treatments		Rate of Calcium Uptake* (µg Ca/g dry/hr)	Ratio N/B	Ratio L/D
Light (L)	Separate			
	Normal (N)	49.56 ± 14.59		2.06
	Corallum	1.55	12.06	
	Bleached (B)	4.11 ± 1.39		1.45
	Corallum	1.55		
	Mixed			
	Normal	45.70 ± 11.40		2.47
	Bleached	3.69 ± 0.20	12.38	1.07
	Corallum	2.35		
Darkness (D)	Separate			
	Normal	23.99 ± 8.93		
	Corallum	4.14	8.48	
	Bleached	2.83 ± 1.24		
	Mixed			
	Corallum	3.82		
	Normal	18.49 ± 6.50	5.37	
	Bleached	3.44 ± 1.14		
	Corallum	3.10		

* Mean ± standard deviation.

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}). This range of concentration 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 four 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 addition of DNP, 3.2 µc Ca-45 was added to each container. Addition of the Ca-45 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 (page 14).

A piece of clean tissue-free corallum of $\underline{F}$. $\underline{scutaria}$ 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 sea water with different concentrations of DNP was estimated by determining pH of the solutions prepared in the same way as the incubating media. The pH of sea water did not change significantly after adding DNP at the concentrations tested, as indicated by the following pH values obtained; 8.09 (normal sea water), 8.03 (10^{-5} M/l DNP) and 8.05 (10^{-3} M/l DNP).

The rates of calcium uptake for each treatment with or without DNP obtained for this experiment are 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/l DNP in light. Although 10^{-5} M DNP does not appear to have any significant effect on the rate of calcium uptake in the light, in the dark, inhibition of calcium uptake was complete. There was no difference between the rate of calcification in

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 dinitro-
phenol 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.
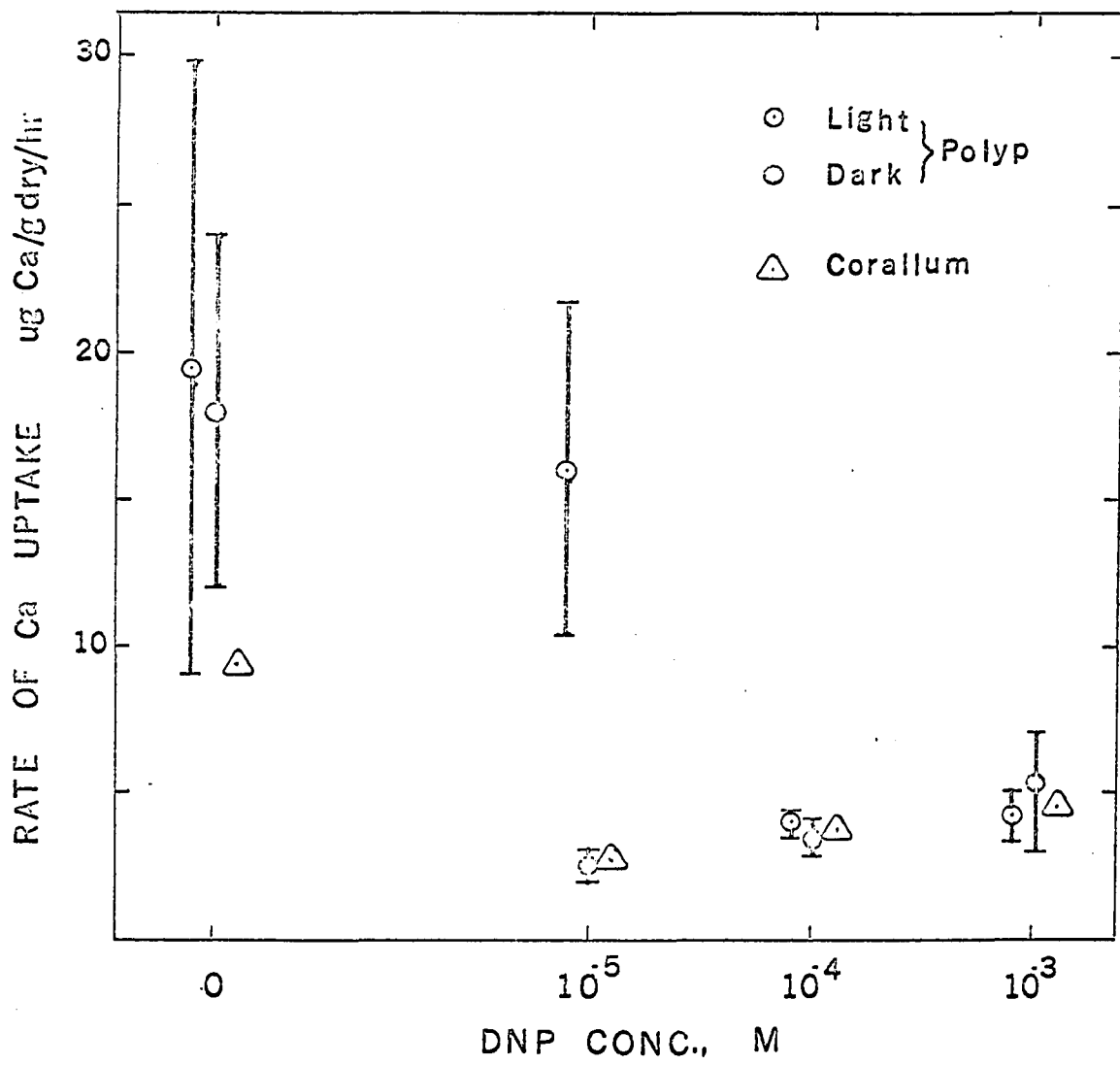
this group of animals and that attributed to physical diffussion in the corallum.

The figure suggests that there is a trend of increase in the rate with the increase in DNP concentration from 10^{-5} M/l (except light treated animals) to 10^{-3} M/l. This increase follows a regression line with a slope of 1.39 (ug Ca/g/hr per each logarithmic increase of 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.

EFFECT OF LIGHT ON UPTAKE AND RELEASE OF PHOSPHORUS

Phosphorus Uptake. - The effect of light on phosphorus uptake by F. scutaria was studied in several experiments employing P-32. The labelled phosphorus in the medium and the animals were examined to establish changes in phosphorus concentration with time as a consequence to phosphorus movement. The early work of Yonge and Nicholls (1931a, 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 ($24.4° \pm 0.3°$ 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 and 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 μg-at. P/1 of phosphate. After an additional hour in this solution the polyps were exposed to approximately 178 μg P-32 per liter.

Analysis of P-32 activity in the corals were made on three polyps removed from both light and dark containers at 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 as described on

page 78. The remaining pieces were digested with perchloric acid (PCA) (page 19). 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 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 polyps 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 P-32 (PP-32).

Three 0.2-ml aliquots of the filtered medium were counted to determine the total P-32 in solution (DTP-32).

Duplicate 5.0-ml samples of the filtrate were placed in 15

ml conical centrifuge tubes and the dissolved organic P-32

(DOP-32) was determined on these samples employing the

method described on page 16. The total P-32 in the

medium (TP-32) was obtained by adding DTP-32 and PP-32.

The dissolved inorganic P-32 (DIP-32) was obtained by

subtracting DOP-32 from DTP-32. Duplicate 50-ml samples of

the filtrate were analyzed chemically for dissolved inorganic

phosphorus (DIP) and a 50-ml sample was digested with

perchloric acid and analyzed for total phosphorus (TP)

according to the procedure given on page 17.

An adequate sample of the incubating medium not exposed

to the corals was set aside at the beginning of the incuba-

tion and kept frozen until the above analyses were made on

the experimental media. The values for the P-32 determina-

tions and the chemical analyses of phosphorus obtained for

this sample served as the control for all the experimental

groups. The P-32 activity retained on an HA Millipore filter

through which the control medium was filtered was used to

correct PP-32 of the experimental media for contamination

with the dissolved P-32. The correction factor thus obtained

was 0.5% of TP-32. Contamination of DOP-32 with DIP-32 in

the experimental media was also corrected by the factor

obtained from this control medium. The extent of contami-

nation of the control solution was 0.56% of TDP-32 for DOP-

32(A), 0.33% for DOP-32(B) and 0.51% for DOP-32(C).

The P-32 activity of the polyps was determined on both

the dry ash samples and the acid digests. Because two sets

of determinations of the P-32 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 mean P-32

activity of the polyps. The P-32 activity of polyps was

checked against the activity removed from the medium as

measured by the difference between the initial and the final

TP-32. As is shown in Figure 12 the P-32 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:

$$\text{Specific activity of polyps} = \frac{\text{cpm P-32/g wet}}{\text{μg-at. P/g wet}} \quad (1)$$

$$\text{Specific activity of medium} = \frac{\text{cpm P-32/ml}}{\text{μg-at. P/ml}} \quad (2)$$

The specific activity of the polyps divided by the initial specific activity of the medium gives the concentration ratio (Equation 4, page 69). The concentration ratios are plotted against time in Figure 11. Each point in the figure represent a mean from three polyps. It can be seen in Figure 11 that the rate of phosphorus uptake is greater in the light than in the dark (apprximately 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 (page 69), 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 peɨod 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 rate of phosphorus uptake. This was calculated to be 0.40 (ug-at. P taken up/ ʌg-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 (DTP)

concentration to be 2.7 µg-at. P/l. This concentration was

chosen because it was found that with higher phosphate

concentrations an adverse affect on coral's metabolism

occurred (pages 46 and 51). Similar effects on P-32

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 incuation without causing depletion of the available

phosphate in the medium.

Figure 11. The effect of light on P-32 uptake by

F. scutaria from sea water containing 2.70 µg-at. P/1

dissolved inorganic phosphate, shown by the changes

in concentration ratio ($\frac{\text{cpm/µg-at. P/g wet}}{\text{cpm/µg-at. P/ml}}$) with

time. Each value plotted represent a mean of three

determinations.
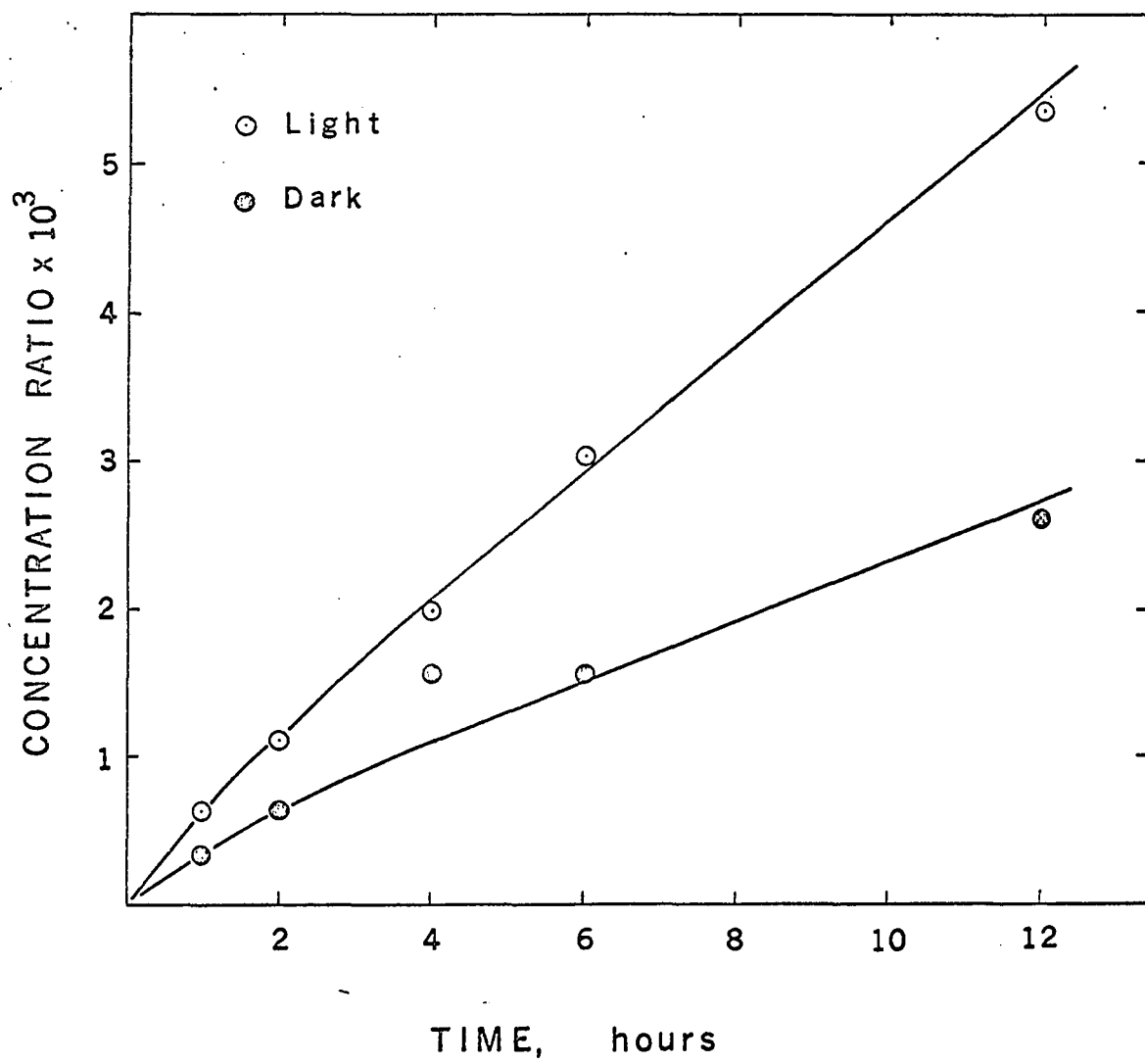
Absolute quantities of phosphorus taken up by _F._

scutaria can be estimated in two ways. One method is to

convert the P-32 measurements to absolute quantities by the

use of the isotope dilution equation:

Phosphorus taken up by animal (µg-at/g wet)

$$= \frac{\text{total P in medium (µg-at/ml)}}{\text{P-32 in medium (cpm/ml)}} \text{ X } \begin{array}{l} \text{P-32 in animal} \\ \text{(cpm/g wet)} \end{array} \quad (3)$$

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,

Concentration ratio

$$= \frac{\text{P-32 in animal/total P in animal}}{\text{P-32 in medium/ total P in medium}} \quad (4)$$

it follows that

Relative P uptake

= P taken up by animal/total P in animal

$$= \text{P-32 in animal X} \frac{\text{total P in medium}}{\text{P-32 in medium}} \text{ X } \frac{1}{\text{total P in animal}}$$

$$= \frac{\text{P-32 in animal/total P in animal}}{\text{P-32 in medium/total P in medium}}$$

= Concentration ratio.

The present method takes into consideration net exchange of

the labelled phosphorus compounds between animal and the

surrounding medium, but it does not take into consideration

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 phospho-

rus taken up by $\underline{F}$. $\underline{scutaria}$ as calculated by this method

should be regarded as approximate estimates of the gross

uptake of phosphorus. In Figure 12, 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.

The second method of estimating absolute quantities of

phosphorus taken up by the polyps is to determine phosphorus

concentration of the medium at the beginning and the end of

incubation, and to estimate from the difference the quantity

of phosphorus taken up by the polyps. Depending upon the f

form of phosphorus compounds measured, quantities of each

Figure 12. Uptake of phosphorus by F. scutaria from

sea water containing 2.70 µg-at. P/1 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).

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 inor-

ganic phosphate absorbed by F. scutaria.

12413
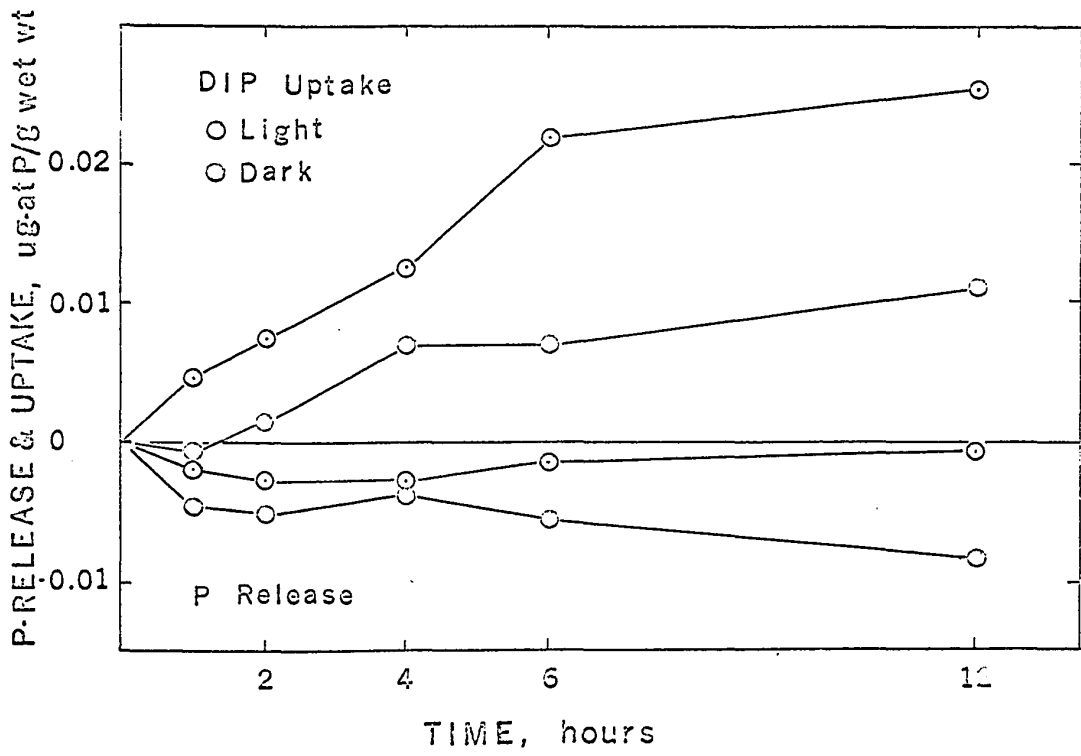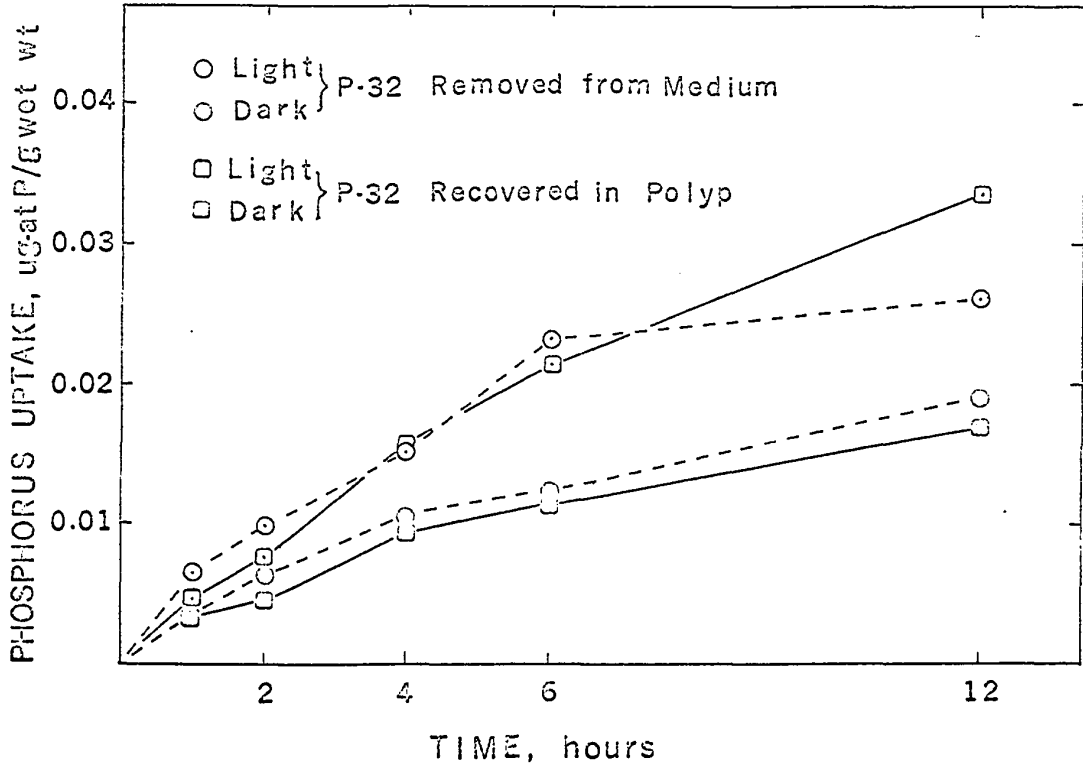
form can be estimated. Quantities calculated by this method reflect net exchange (or uptake as in the present experiment) rather than gross exchange at a given time of incubation irrespective of the distribution of P-32 , whether it became homogeneous inside and outside the animals.

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.

As mentioned before (page 70), 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 differences between the 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 (.0015 µg-at. P/g wet uptake, .005 µg-at. P/g wet release). From two hours until 12 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 excreted 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 86 and 93).

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 equal quantities of TOP-32 was present in media for polyps incubated in the light (5.57% of the initial activity) and in the dark (7.01%). However, DIP-32 in media for polyps incubated in the light was much less than in the dark (L/D = 1/24.6). Consequently,

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 initial activity in counts per minute per ml.

Figure 15. Relative changes with time in the specific activity of TP-32 (SA: TP-32) and DIP-32 (SA: DIP-32) of sea water during incubation with F. scutaria in the light (3,300 lux) and in the dark. All the values are expressed as percent initial specific activity (cpm TP-32/µg-at. P DIP and cpm DIP-32/µg-at. P DIP).
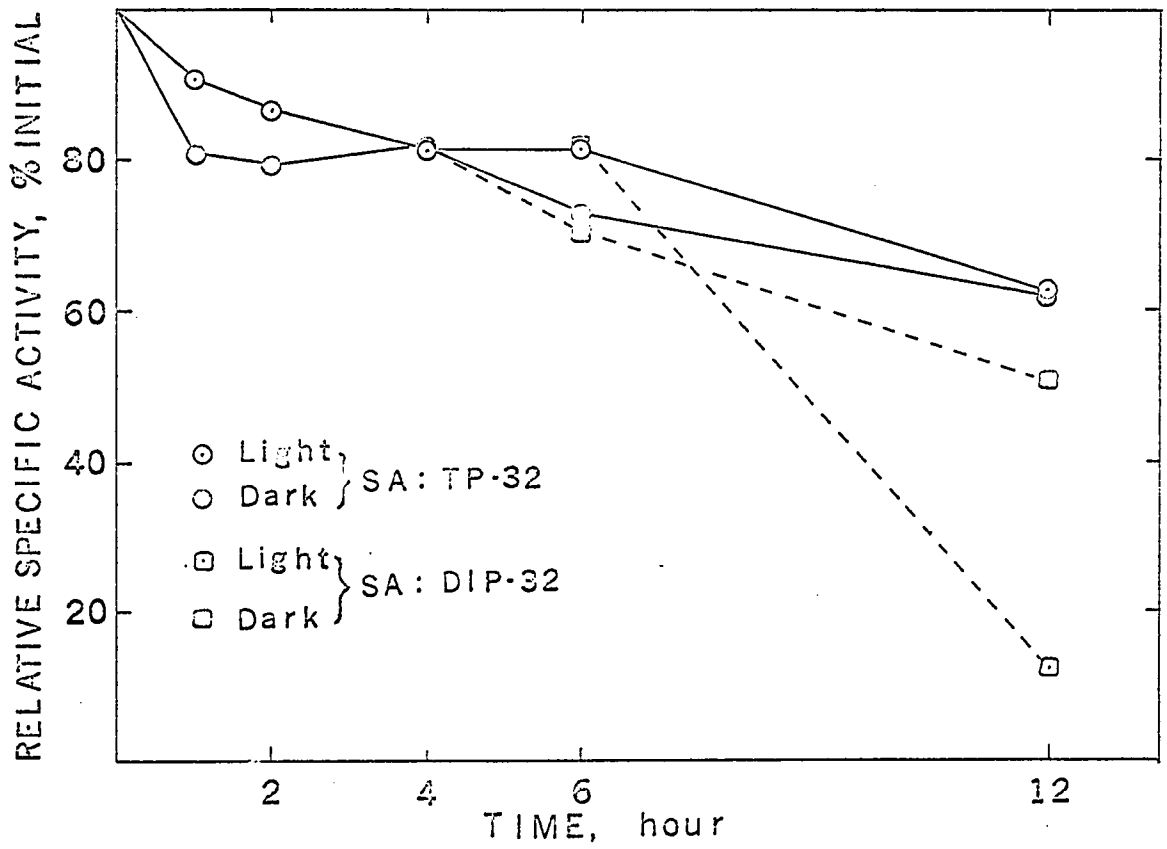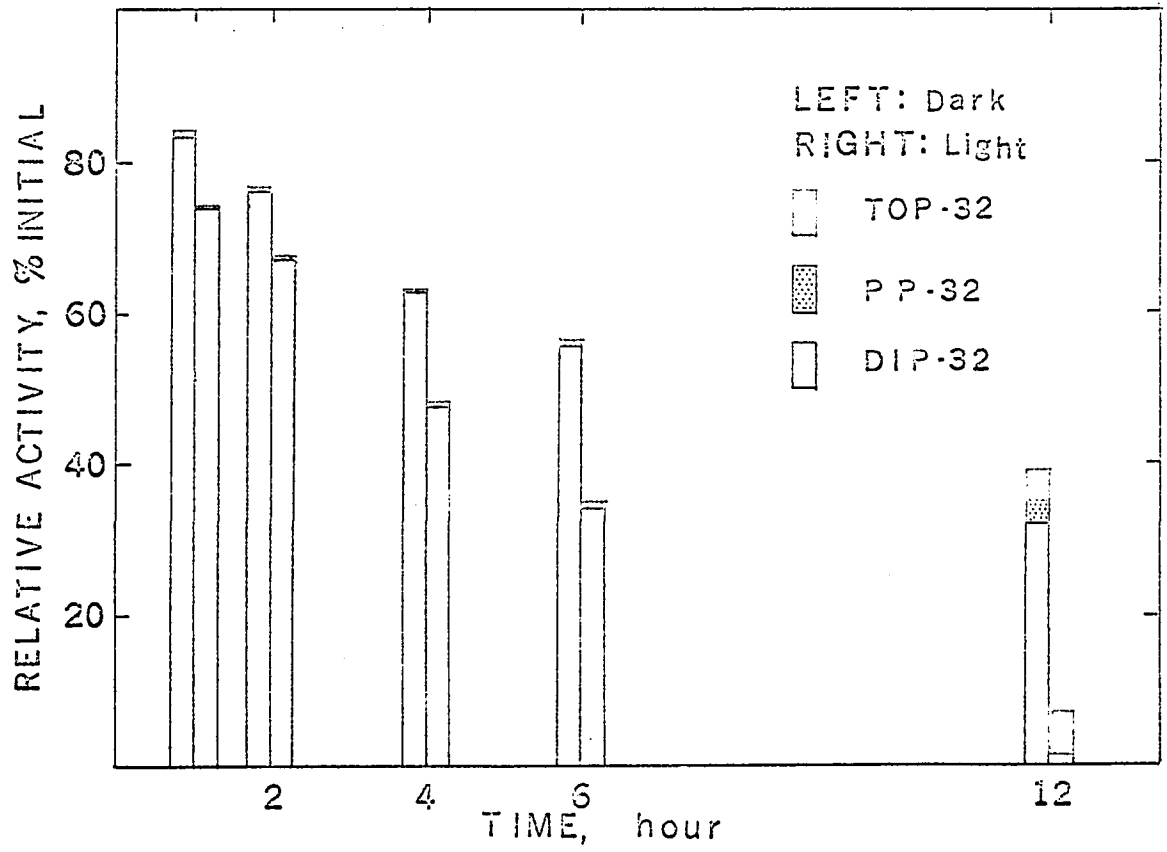
the ratio of TOP-32 to DIP-32 in media for polyps kept in the light was much higher (4/1) than that in the dark (0.22/1). These relationships can also be seen in the changes of the specific activity of the medium with time (Figure 15). The specific activities of the medium at each period were calculated for DIP-32 and TP-32 according to the following equations;

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

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

The per cent differences from the initial specific activity was plotted against time in Figure 15. The changes in SA:TP-32 show the effect of dilution of P-32 by DIP and the changes in SA:DIP-32 the effect of dilution of DIP-32 by TOP-32 superimposed upon the dilution effect of DIP.

The results of radioautographs prepared from a polyp of each group failed to show any significant accumulation of P-32 in the tissues. The quantity of P-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 (page 80).

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 µg-at. P/l 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 µc of P-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-32. At the end of 6, 12, 24, 30, 70 and 102 hours, one to two polyps were treated in the standard way (page 14) and ashed in a muffle furnace at 525° C. The P-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 experimental conditions of the two aquaria were approximately identical and the changes in the P-32 activity of both aquaria followed a similar pattern, the P-32 activities of the polyps incubated for the same period of time in

Figure 16. The P-32 uptake by $\underline{F}$. $\underline{scutaria}$, expressed

as concentration ratio, as a function of time; $\underline{F}$.

$\underline{scutaria}$ was kept in sea water containing 8.9 μg-at.

P/1 of the initial phosphate concentration under

constant illumination (6,000 lux).

CONCENTRATION RATIO, $\frac{uc/ugat P/g \; wet}{uc/ugat P/ml} \times 100$

TIME,   hour
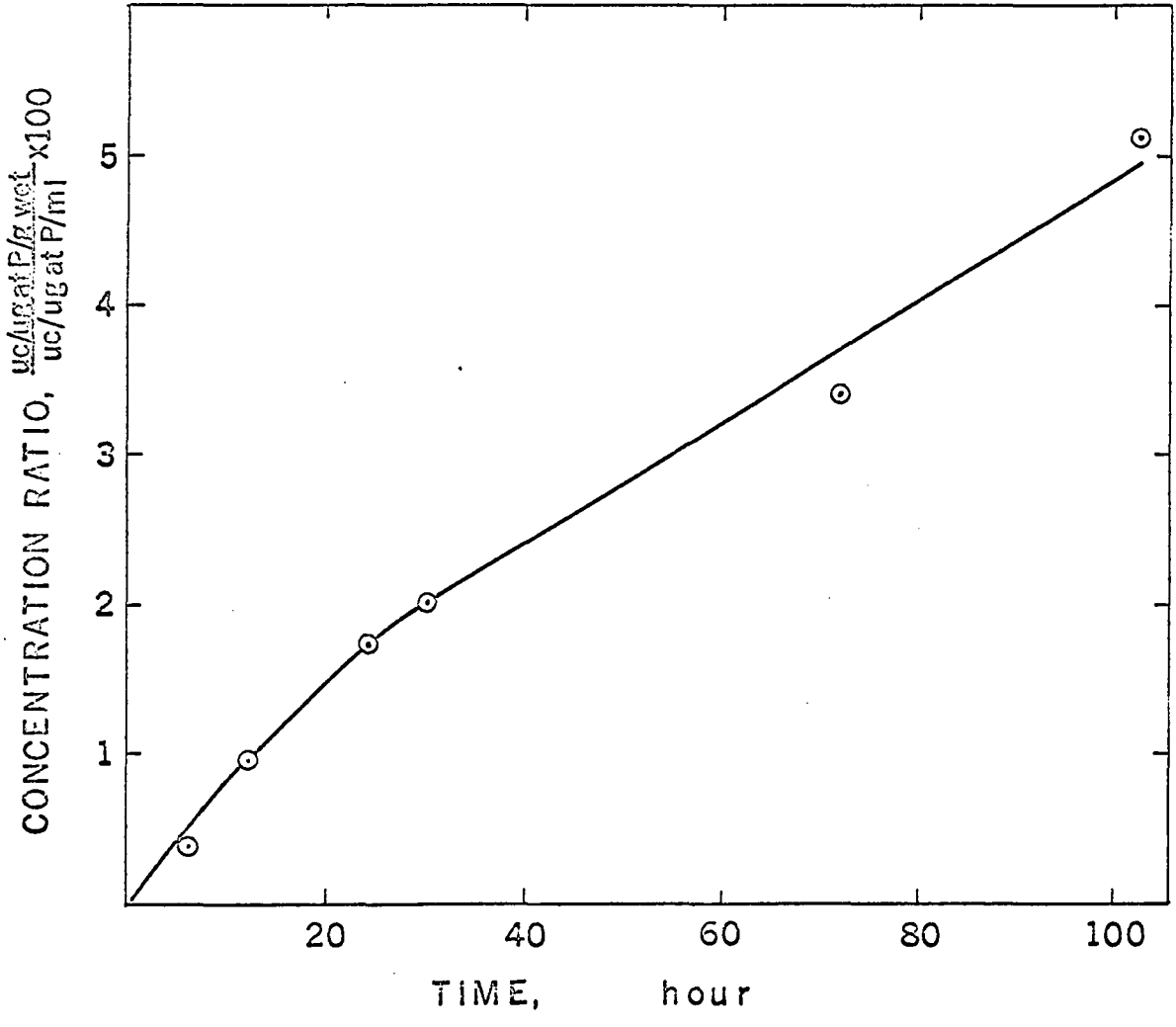
two aquaria were averaged. The concentration ratio (Equation 4, page 69) was calculated from these values and are presented in Figure 16. The graph shows that the concentration ratio increases at a constant rate from the 30th to 102nd hour. This indicates that the rate of phosphorus uptake by F. scutaria did not reach a steady state within 102 hours under the conditions of the experiment.

Distribution of P-32 in the Tissues of Fungia. - The distribution of P-32 in the tissues of F. scutaria was studied by radioautography on the polyps placed in sea water containing P-32 for 13 and 24 hours in the light and in the dark, using transparent and opaque containers, respectively.

Eight polyps of Fungia 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 sea water 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 100 ml filtered sea water containing 18.5 μc P-32 and 0.27 μg-at. P/l dissolved inorganic 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 1 g were cut off with 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-32 taken up. The incubating medium was analyzed for dissolved inorganic phosphate. The P-32 activity was determined in 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 (page 20).

The loss of P-32 from the tissues during the processes of histological preparation was estimated by counting two 0.2-ml aliquots from 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-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 proceses.

The concentration ratio as determined according to Equation 4 (page 69) was 0.00020 for a group incubated in the dark for 13 hours and 0.00018 for the remaining three

Figure 17. The phosphorus uptake and release by F. scutaria kept in sea water containing 0.27 μg-at. P/l of dissolved inorganic phosphate for 13 and 24 hours, and the changes in the specific activity of sea water.
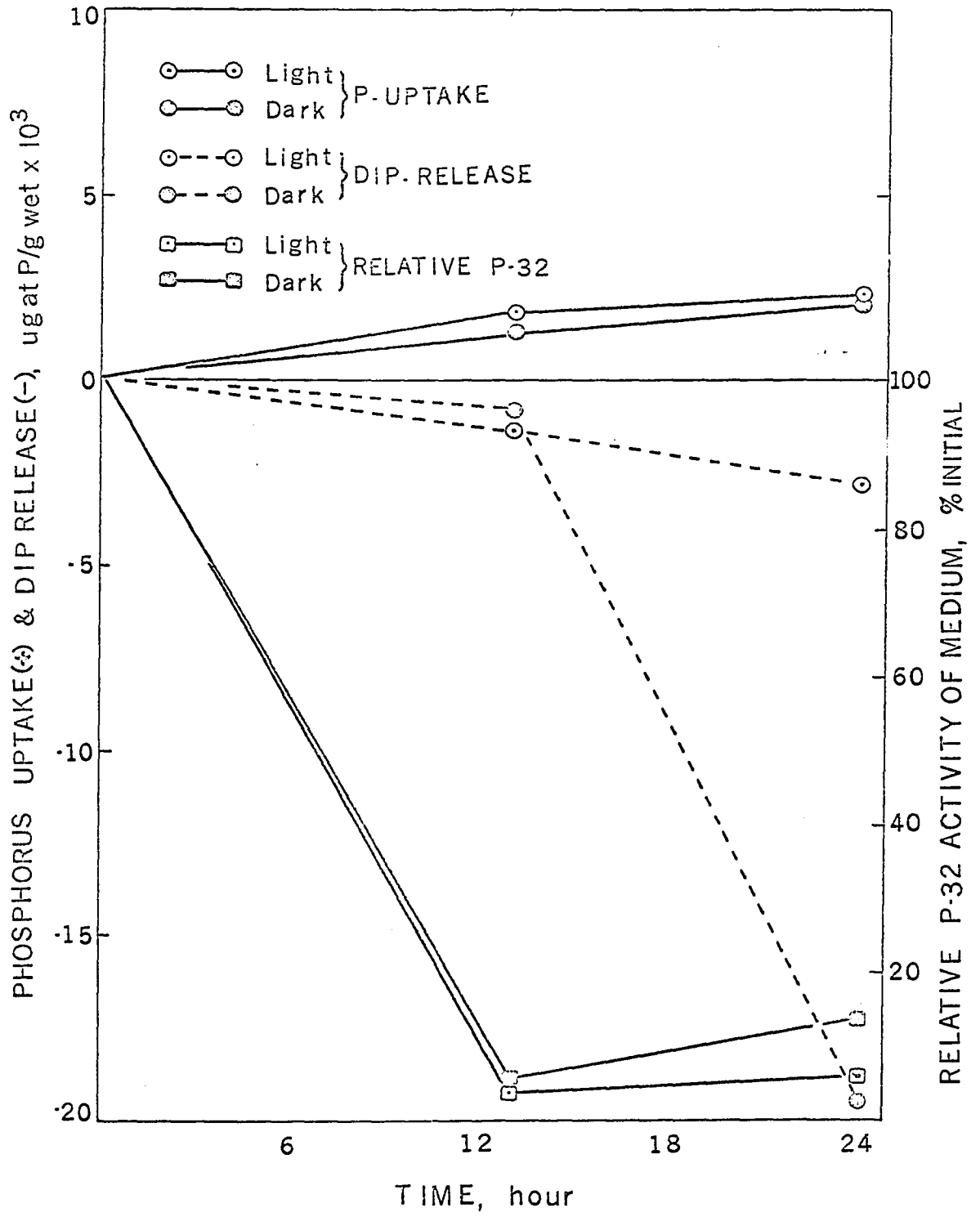
groups. This indicates that the exchange of P-32 between

the polyps and the media reached a steady state during the

first 13 hours. The changes in the P-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-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

P-32 activity of the ash samples, absolute quantities of

phosphorus taken up by the polyps were calculated according

to Equation 3, page 69 (Figure 17).

The results obtained by radioautography are illustrated

by the photomicrographs (Figures 18 and 19). 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 (Figure 18). 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

Figure 18. 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. (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; MG, mucous gland of the inner region of the mesenterial filament; IM, inner region of themesenterial filament; S, decalcified skeleton; Z, zooxanthellae)

Figure 19. 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. (Lettering same as in Figure 18.)

aboral body wall were not significantly positive.

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

The polyps subjected to the P-32 containing medium for 24 hours in the dark also exhibited a detectable concentration of P-32, and with similar pattern of distribution as described above. No P-32 accumulation was detectable in the polyps incubated with the radioactive medium for 13 hours in the light or in the dark (Figure 19). Because a large fraction of P-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-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-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-32 in the present experiment. However, the fact that the mucous glands and the gland cells were positive to P-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-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 isotopes P-32 and P-31. Because the specific activity of the incubating medium was high, it is expected that there was a rapid exchange between the P-32 of the medium and the P-31 present in zooxanthellae. Because there was no net uptake of DIP by the polyps, the possibility of the active absorption of the P-32 by zooxanthellae is weak.

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 µc of P-32 as previously described (page 76). 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 µg-at. P/l of dissolved inorganic phosphate. These containers were kept under constant illumination (approximately 6,000 lux) and at constant temperature ($23.4^{\circ} \pm 0.3^{\circ}$ C). After incubating for periods 1, 3, 7, 17 and 30 hours, four polyps from a "light" and four polyps from a "dark" container were removed, treated by the standard procedure (page 14), 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 μ. The filter was mounted on an aluminum planchette with a drop of acetate glue diluted with acetone. Each sample was then counted to determine PP-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 (page 15).

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 μc and the other (B) 1.0 μc 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 by filtering the control solutions averaged 3.68% of the total activity. This factor 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:

	DOP-32(A)	DOP-32(B)	DOP-32(C)
Control A	2.5%	0.12%	0.7%
Control B	2.7%	0.10%	1.2%
Mean	2.6%	0.11%	0.9%

The total P-32 activity recovered in the medium (TP-32/
200 ml) 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 20 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 surround-
ing 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-32 and PP-32 released between the light
and dark treated animals. The sharp difference was found in
the quantities of DIP-32 released; the main portion of P-32
released in the dark is attributable to DIP-32. The ratio
of DIP-32 to DOP-32 released was about 9:1 in the dark and
1:1 in the light of the 30th hour.

The change in the quantities of dissolved inorganic

Figure 20. The changes with time in the relative

quantities of DIP-32, DOP-32, and PP-32 in sea water

during incubation in the light (6,000 lux) and in the

dark with F. scutaria labelled with P-32. The P-32

fractions are expressed as percent initial activity of

the polyps of F. scutaria.

Figure 21. 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. scutaria labelled with P-32.
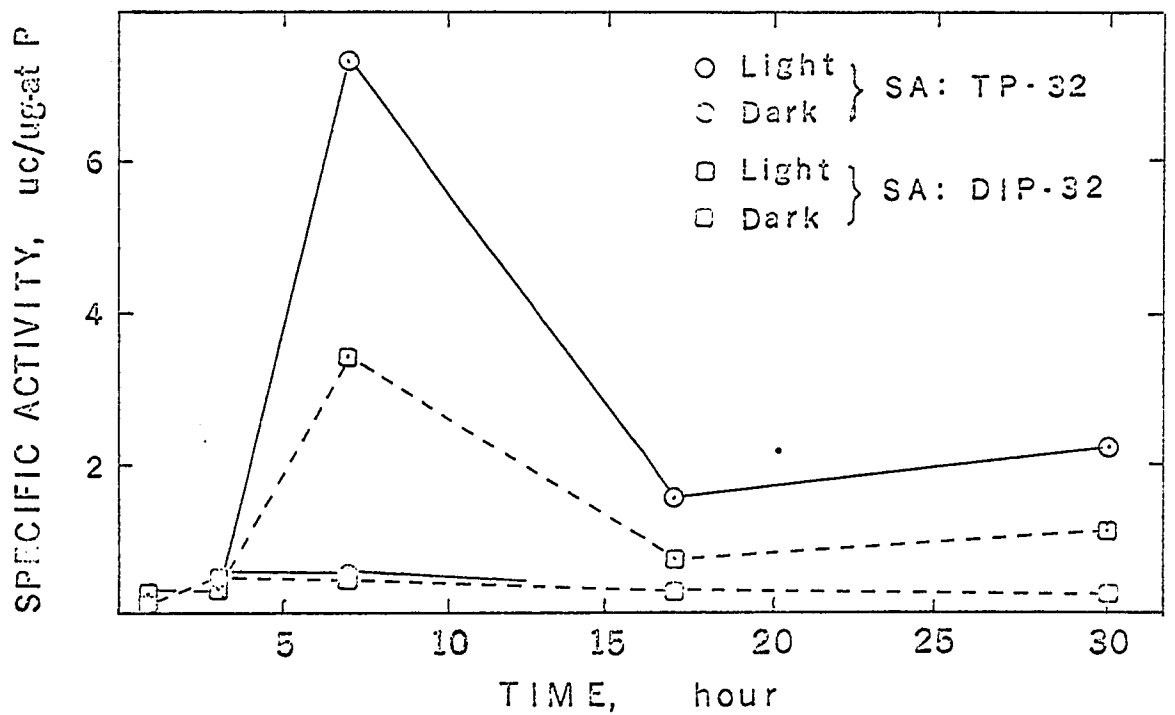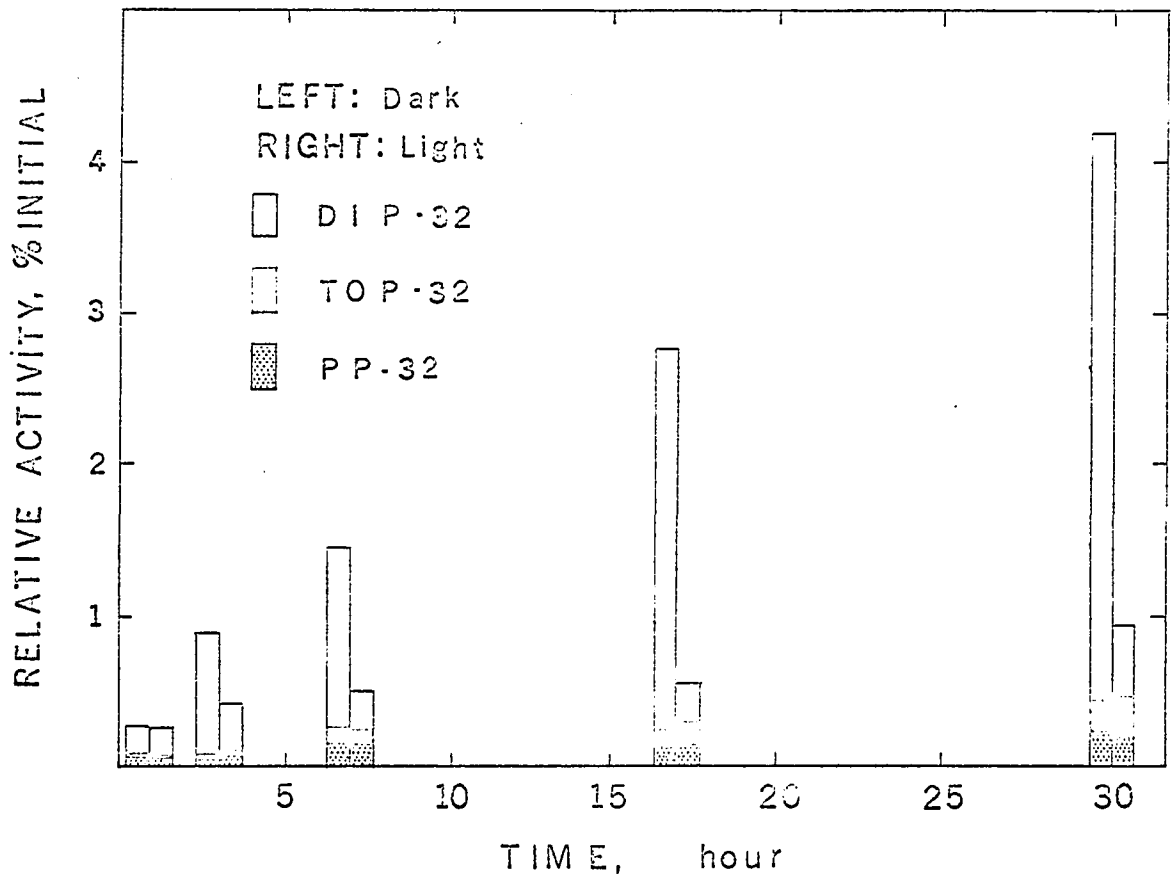
phosphate as determined spectrophotometrically roughly

paralleled that of DIP-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

21 as changes in the specific activities. Two values of the

specific activities were calculated as before (page 75),

SA:TP-32 (TP-32/DIP) and SA:DIP-32 (DIP-32/DIP). The unusual-

ly 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/l). 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.

This relationship is more clearly shown in Figure 22 as

Figure 22. The release of DIP-32 expressed as concen-

tration ratio $\left(\dfrac{\text{uc DIP-32 released/ug-at. P DIP released}}{\text{uc TP-32 in animal/ug-at. P TP in animal}}\right)$

by $\underline{F}$. $\underline{scutaria}$ in the light (6,000 lux) and in the dark

into sea water containing 0.14 ug-at. P/1 of DIP but no

P-32.

Figure 23. The release of DIP by $\underline{F}$. $\underline{scutaria}$ in the

light (6,000 lux) and in the dark as a function of

time.
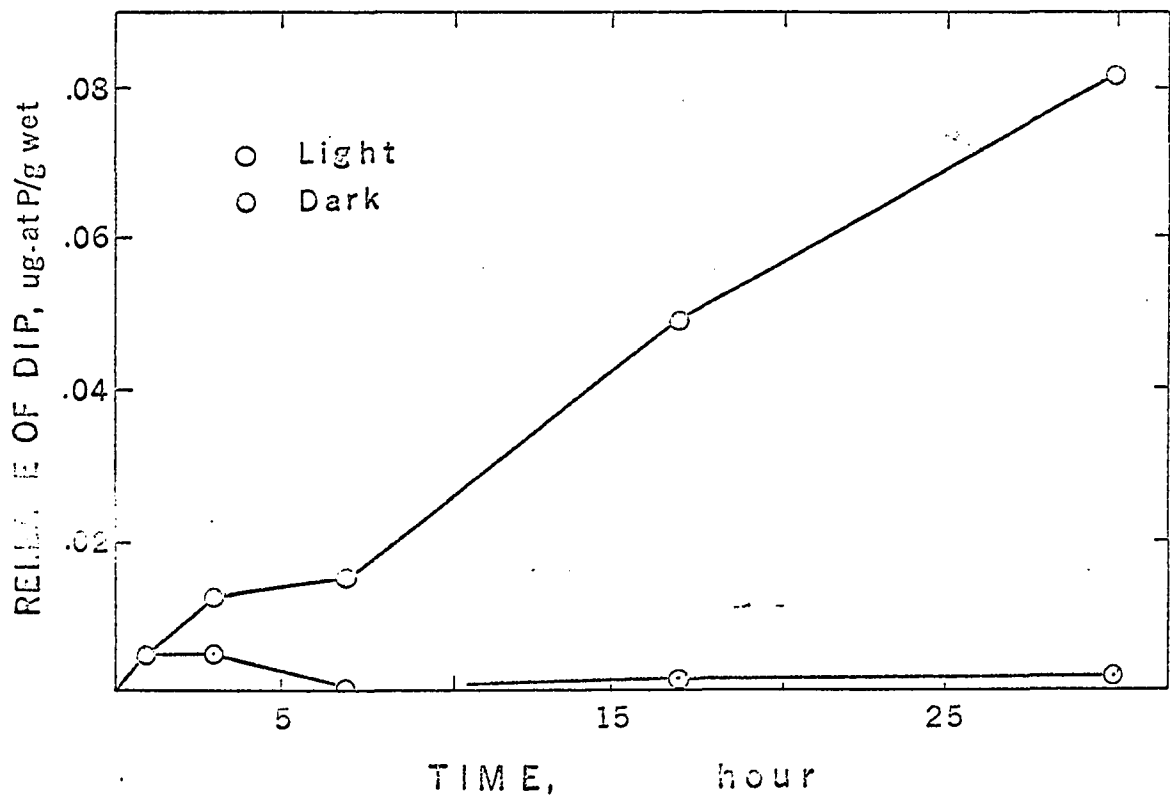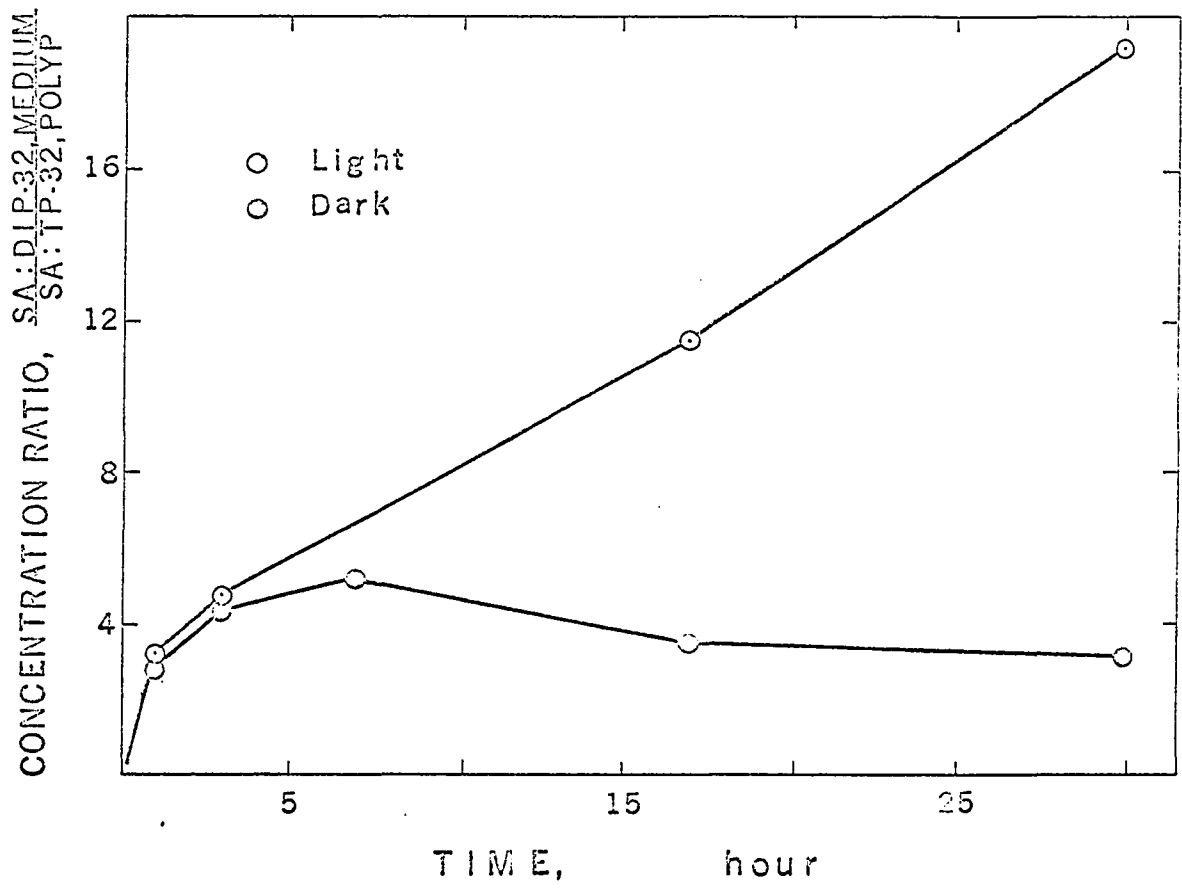
changes in the "concentration ratio":

$$\frac{\text{DIP-32 released/DIP released}}{\text{initial TP-32 in animal/initial TP in animal}}.$$

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, that the distribution of P-32 did not become homgeneous inside and outside the polyps by the exchange processes.

The absolute quantities of phosphorus released by corals are shown in Figure 23 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 introduced into the medium 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 μg-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 21 and 22, no significant net release of dissolved inorganic phosphate occurred in the light.

EFFECT OF TEMPERATURE ON PHOSPHORUS UPTAKE

The effect of temperature on phosphorus uptake by _Fungia 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 procedures for these experiments were already described (pages 21 to 26).

The initial activity of P-32 of the incubating medium was determined by counting three aliquots (0.2 ml each) placed and dried on aluminum planchettes. Upon removal of polyps, the incubating medium was filtered through an HA Millipore filter (pore size of 0.45 μ), 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

Figure 24. The rate of phosphorus uptake by $\underline{F}$. $\underline{scutaria}$

as a function of temperature in the light (3,300 lux) and

in the dark in sea water containing 11.45 μg-at. P/l (A)

and 2.70 μg-at. P/l (B) of dissolved inorganic phosphate.

The rate of phosphorus exchange by the dead corallum of

$\underline{F}$. $\underline{scutaria}$ under the same condition is also included in

the figure.
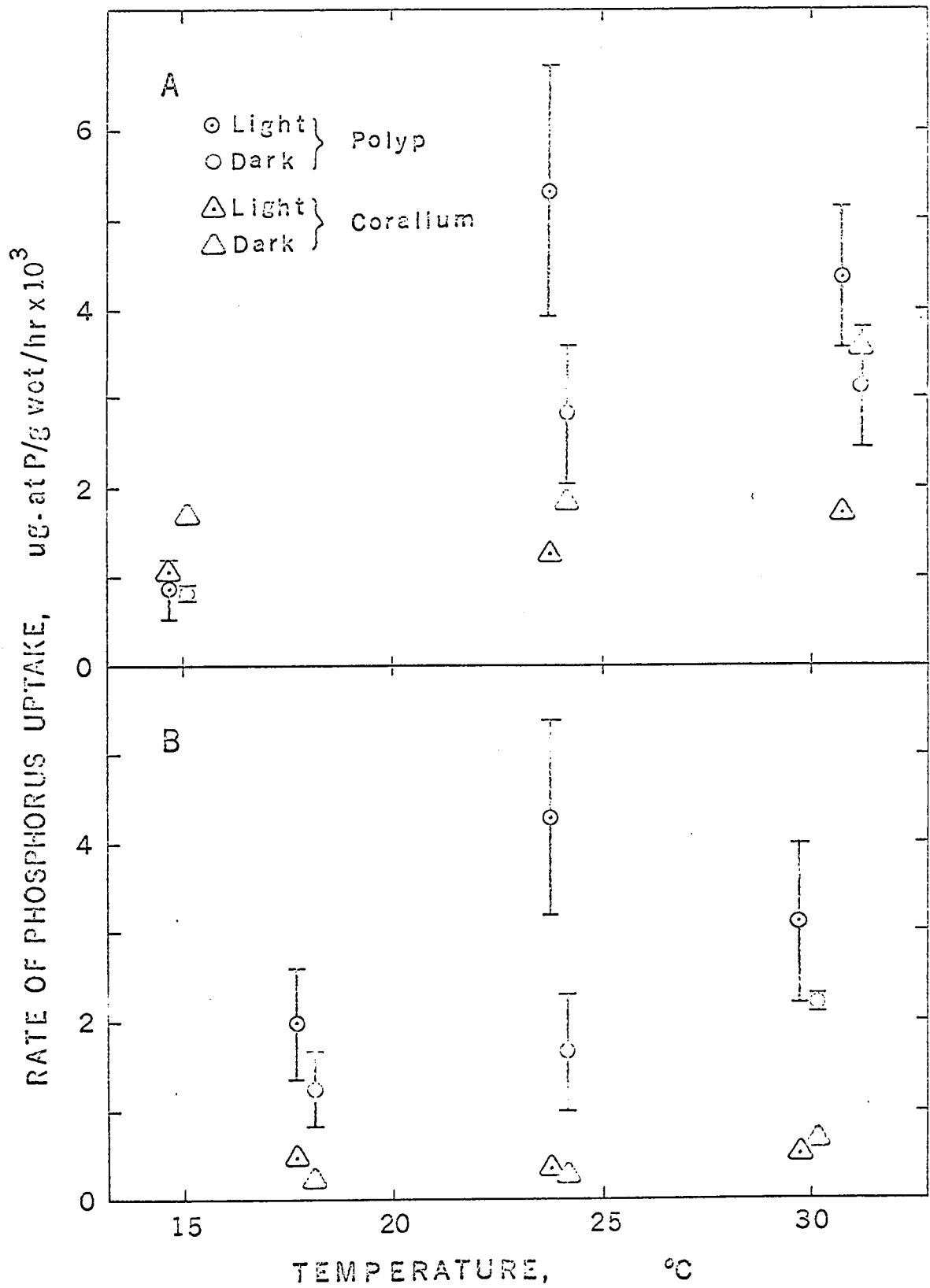
phosphate concentration of the medium used in the first series of experiments were 11.45 μg-at. P/1, and that in the second series 2.70 μg-at. P/1.

The absolute quantities of phosphorus taken up by the polyps were obtained from the data on the P-32 determinations for the ash samples by using Equation 3, page 69. Figure 24A shows the results of the experiment employing 11.45 μg-at. P/1, and Figure 24B those employing 2.70 μg-at. P/1. The low values for the rate of phosphorus uptake by the polyps kept at 15° C (Figure 24A) 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° C and lowest at 18° 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).

The results on the determination of dissolved inorganic phosphate (DIP) is shown in Figure 25. The changes in DIP

Figure 25.   The rate of uptake or release of dissolved

inorganic phosphate by $\underline{F}$. $\underline{scutaria}$ as a function of

temperature during 26-hour incubation in sea water

containing 11.45 μg-at. P/1 (High PO$_4$) and 2.70 μg-at.

P/1 (Low PO$_4$) of dissolved inorganic phosphate in the

light (3,300 lux) and in the dark.

Figure. Rate of DIP uptake (ugatP/gwet/hr ×10³) versus temperature (°C). UPTAKE and RELEASE regions. Legend: ⊙ Light, ○ Dark } High PO₄; ⊡ Light, ▢ Dark } Low PO₄.

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.

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 (pages 52 to 57) in which both normal polyps
and polyps from which zooxanthellae have been removed were
exposed to P-32 and Ca-45.

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 normal polyps. There was again no significant
difference in the light between 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 normal polyps. However, in the dark, normal polyps

Table 5. The effect of the removal of zooxanthellae from tissues on the rate of phosphorus uptake by $\underline{F}$. scutaria in light and darkness.

Treatments		Rate of Phosphorus Uptake* (ug-at. P/g wet/hr)	N/B	L/D
	Normal (N)	0.00937 ± 0.00246		1.77
Separate	Corallum	0.00105	8.22	
	Bleached (B)	0.00114 ± 0.00017		1.30
	Corallum	0.00111		
Light (L)				
	Normal	0.00879 ± 0.00028		2.03
Mixed	Bleached	0.00101 ± 0.00041	8.70	0.88
	Corallum	0.00076		
	Normal	0.00530 ± 0.00036		
Separate	Corallum	0.00152	6.02	
	Bleached	0.00088 ± 0.00017		
Darkness (D)	Corallum	0.00174		
	Normal	0.00432 ± 0.00050	3.72	
Mixed	Bleached	0.00116 ± 0.00007		
	Corallum	0.00098		

* Mean ± standard deviation.

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 normal polyps absorbed phosphorus at significantly higher rate than those kept separately (p = 0.025). In the dark, some interaction can be suspected between normal polyps and the 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 groups. 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 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 normal polyps absorbed phosphorus in the dark, at considerably higher rate than the bleached polyps. More significant is the fact that there was net absorption of DIP by these polyps whereas net release was observed for the bleached polyps under the same condition; the absorption rate for normal polyps was 0.0013 μg-at. P/g wet/hr, and the release rate for the bleached polyps was 0.0010 μg-at. P/g wet/hr. The comparable uptake rates of normal polyps and

Figure 26. Historadioautograph showing accumulation
of P-32 in the mucous gland of the oral epidermis 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. (C, coelente-
ron; E, epidermis of the oral body wall; G, gastro-
dermis of the oral body wall; GC, gastrodermis lining
calicoblastic layer; M, mesentery; MG, mucous gland;
S, decalcified skeleton; Z, zooxanthellae.)

Figure 27. Historadioautograph showing a failure to
accumulate P-32 in tissues of a polyp of F. scutaria from
which zooxanthellae were removed, kept for 24 hours in
the dark in sea water containing Ca-45 and P-32; 120x.
(Lettering same as Figure 26)

the bleached polyps in the light were 0.0065 and 0.0052 μg-at./g wet/hr, respectively.

The release rate of TOP-32 was also estimated by the method given on page 16. The following results were obtained: normal, light: 1.45%, bleached light: 0.66%, normal, dark: 1.19%, bleached dark: 1.09% of the initial P-32 activity of the incubating medium. These results show that more organic phosphorus was released by normal polyps 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 Figures 26 and 27. 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 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.

DISCUSSION

FACTORS CONTROLLING CALCIFICATION

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° 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 been previously 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 tempera-
tures 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 28A).

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 24 may be regarded as an index of photosynthesis,
because phosphorylation is an integral part of photosynthe-
sis. Because nearly parallel effects of light and temperature
were obtained both on the rates of calcium and phosphorus
uptake, both calcification in the animal body and photosyn-
thesis 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

Figure 28. The net effect of light on the rate of
calcium uptake by $\underline{F}$. scutaria (A — E) and $\underline{P}$. 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 available (corallum alone).
High PO_4 and low PO_4 in A stand for phosphate concen-
trations of medium, 11.45 μg-at. P/1 and 2.70 μg-at. P/1
respectively.

Y-axis (vertical label): DIFFERENCE IN RATE OF CALCIFICATION (ugCa/g/hr: LIGHT−DARK)

A — high PO4, low PO4; TEMPERATURE, °C

B — DINITROPHENOL, M

C — SALINITY, % SEA WATER

D — CALCIUM CONCN., MG/L

E — Fungia scutaria

F — Porites compressa

PHOSPHATE CONCENTRATION, M
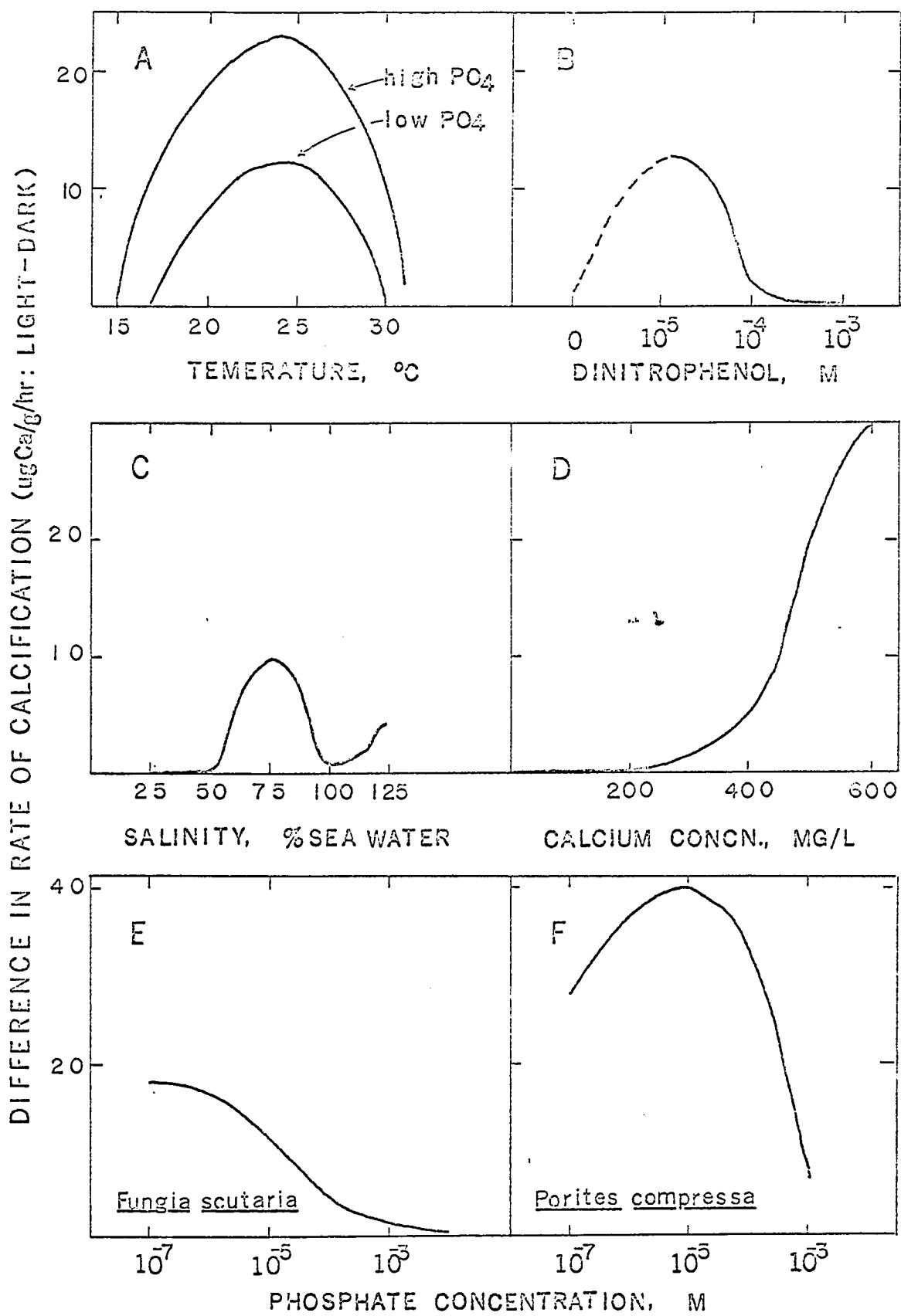
35° C radiocalcium uptake of his animal was lower than at the room temperature (ca. 25° C), and at 40° C the gastrula failed to develop further. This information suggests that the rate of calcium uptake by F. scutaria may eventually reach an optimum value with increase in temperature beyond 31° C in the dark.

Salinity. - The only available information on the effect of altered salinity on the growth of corals was obtained by Edmondson (1929). Leptastrea 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 provided

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.

Calcium Concentration. - By analogy to an enzyme-catalyzed 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, <u>Pedalion</u>, 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 a striking difference in the rate of calcification between

light and darkness related to the calcium concentration. 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 calcium

concentration (200 mg Ca/1) where a steady state was reached

in the dark. This difference can again be best attributed to

the presence of zooxanthellae in <u>F</u>. <u>scutaria</u>.

The sharp increase in the rate of calcium uptake between

the concentrations 400 and 600 mg Ca/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 change 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 a sea urchin larva as the decreased calcium concentration, is worth mentioning.

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, 1964a, 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} - 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 X 10^{-3}

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 chlo-

roplast, 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} M, and then 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

(1964a, b, c, see page 5), and if local accumulation of

phosphate is prevented by photosynthetic activity of zooxan-

thellae, 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 thephosphate concentration of about 10^{-3} M and then decrease beyond this concentration, and the rate of calcium uptake would decrease in the dark with 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 28F), the greatest differences were found in the intermediate phosphate concentrations (10^{-6} - 10^{-5} 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 28F 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 $\underline{F}$. $\underline{scutaria}$ (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}). The fact that in 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} M), while those of 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 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".

Other Factors. - 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 28. 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. The

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.)

Phosphate Concentration	Light	Photosyn-thesis	Inhibi-tion	Calcifica-tion
Low $(0-10^{-7}M)$	Yes	1	0	1
	No	0	0	0
Intermediate $(10^{-7}-10^{-4}M)$	Yes	3	1	2
	No	0	1	-1
High $(10^{-4}M-)$	Yes	2	2	0
	No	0	2	-2

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 some 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 th n phosphorus uptake by the presence of zooxanthellae. This is, then, 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 $\underline{F}$. 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 cali-

fication includes some reaction which is dependent upon

phosphorylation. The present study suggests that photo-

phosphorylation is less sensitive to dinitrophenol than

oxidative phosphorylation.

PHOSPHORUS METABOLISM OF $\underline{F}$. SCUTARIA 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/l 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 coral 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 the polyps of F. scutaria with zooxanthellae under conditions of the experiments 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 the polyps without zooxanthellae rather excreted phosphate in the dark into the medium supports this suggestion.

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

SUMMARY

1. The rate of calcium uptake of $\underline{F}$. 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 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 Ca/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 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 polyps with or without zooxanthellae placed in the same container in darkness; the rate of calcium uptake of normal polyps was lower when incubated together with polyps without zooxanthellae than when incubated in separate container, while the rate of polyps without zooxanthellae was higher when incubated together with normal polyps than when incubated in a separate container. This interaction was stronger for phosphorus uptake than for calcium uptake.

8. Phosphorus was absorbed by $\underline{F}$. $\underline{scutaria}$ in the dark from sea water supplemented with phosphate (2.7 μg-at. P/l). 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/l) net release (as DIP) rather than uptake was observed.

9. Polyps devoid of zooxanthellae as well as normal polyps incubated in the light also absorbed DIP (net uptake) from sea water with phosphate concentration 7.9 μg-at. P/l. 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 times more inorganic phosphorus was released by 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. 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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