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Carbon Flow Within the Colonial Radiolarian Microcosm

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Abstract

Colonial radiolaria (CR) are planktonic sarcodines generally found in the upper 30 m of oligotrophic oceanic waters. The CR feed on microplankters such as copepods and mollusc larvae which become entangled in the feeding rhizopodia of the floating colonies. In addition to their carnivorous nutrition, CR receive photosynthates from algal endosymbionts located within the colonies and even ingest some of these endosymbionts. This nutritional versatility may be the key to the exceptional success of colonial radiolaria in highly oligotrophic environments such as the central gyres of the Atlantic and Pacific Oceans. Although most of the small plankters that collide with CR are rapidly consumed, there are several small organisms associated with the colonies which apparently possess the means to avoid predation. These include hyperiid amphipods, dysterid ciliates, gyrodinioid dinoflagellates and *Cryptobia*-like bodonid flagellates. Colonial radiolaria also harbor large populations of bacteria on their outer surfaces. The flux of carbon through the colonial radiolarian microcosm was summarized in a model which includes estimates of the carbon flow attributed to the various associated organisms. In this model, the major sources of carbon entering the CR microcosm (all rates are normalized to a 24 hr day) are predation (estimated rate = 239 ng carbon/hr) and algal photosynthesis (measured rate = 9–53 ng carbon/hr), assuming an average colony has 100 cells and a biomass of 48 μg carbon. A colony of these proportions respires approximately 110 ng carbon/hr. In this model, associated bacteria take up between 0.04 and 10 ng

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carbon/hr (estimated by a "bacterial carbon demand equation"), juvenile amphipods consume 4.6–22 ng carbon/hr and adult amphipods consume 40–200 ng carbon/hr, while free-swimming gyrodinioid dinoflagellates incorporate between 0.002–30 ng inorganic carbon/hr. The carbon uptake rates of dysterid ciliates and *Cryptobia*-like bodonids were not determined and their trophic role in the CR microcosm is not clear. One of the unique characteristics of the radiolarian microcommunity is the fact that it is a self-supporting system, similar in many respects to coral reef communities. Carbon is incorporated by the radiolarian, either by predation or through endosymbiont activity, and some of this carbon is then utilized by associated organisms. Furthermore, these associated organisms may play an important role by recycling nutrients within the microcosm and possibly to the surrounding waters as well.

Keywords: microbial ecology, carbon budgets, planktonic sarcodines

1. Introduction

Some of the most widely encountered macroscopic organisms in the surface waters of warm tropical oceans are the colonial radiolaria (CR) (Swanberg, 1979). Abundances of this group of radiolaria are generally between 0.01 and 10 colonies per m^3 in the top 30 m of the water column (Swanberg, 1983; Angel, unpublished; McGillivary, 1988). However, much higher abundances, on the order of hundreds (Swanberg, 1983) and thousands of colonies per m^3, have been reported in the surface waters (probably the top meter) of the Davis Straits (Pavshitiks and Pan'Kova, 1966), in the Gulf of Aden (Khmeleva, 1967), and in the eastern North Atlantic Ocean, near Mauritania, Africa (Rico, 1977).

Radiolarian colonies consist of a few to several hundreds or thousands of cells that are identified by the cytoplasm-rich "central capsules" (cc) interconnected within a complex network of rhizopodia. These are often surrounded by a gelatinous matrix of sulfated mucopolysaccharides (Hollande and Hollande, 1975). Each of the central capsules maintains one or more algal symbionts which are enclosed within rhizopodial sheaths and provide organic photosynthates to the host (Anderson, 1978). In addition to the photosynthates provided by the algal endosymbionts, the colonial radiolaria consume endosymbionts at a steady rate (Anderson, 1976) and opportunistically feed on planktonic prey, such as copepods, tintinnids, mollusc larvae, ostracods, larvaceans and heteropods which become entrapped in the radiolarian rhizopodia (Swanberg, 1979). Thus, considerable trophic autonomy is available which may account for the widespread occurrence of CR in oligotrophic regions (Anderson, 1983; Swanberg, 1983).

Colonial radiolaria often serve as host to a consortium of microscopic organisms including hyperiid amphipods, dysterid ciliates, gyrodinioid dinoflagellates, *Cryptobia*-like bodonid flagellates and bacteria. Whereas the association between hyperiid amphipods and CR was observed more than a century ago (Brandt, 1885), the other members of the radiolarian microcosm were reported only recently (Angel, 1989) and the full nature of their association with the hosts is still not known. I consider the associated organisms "symbionts" of the radiolaria, using de Bary's original definition: "the living together of differently named organisms" (Smith and Douglas, 1987). This definition, however, does not necessarily imply a mutually beneficial relationship, and hence may include relationships others categorize as commensal. Because all of the associated organisms were observed on the outer surfaces of the host colonies, I have collectively named these "episymbionts" (see Taylor, 1982) of the CR.

While previous workers examined the various pathways in which carbon flows into the radiolarian microcosm (Anderson, 1978; Anderson, 1980; Anderson et al., 1983; Swanberg, 1979; Swanberg, 1983; Swanberg and Harbison, 1980), the goal of this study was to assess the role of associated organisms in the flow of carbon through (and possibly out of) the radiolarian microecosystem.

2. Materials and Methods

CR collections

Colonial radiolaria were hand-collected by SCUBA divers using 125 ml glass jars (for detailed diving protocol, see Hamner et al., 1975 and Anderson, 1980) during three oceanic cruises (R.V. *Oceanus* 176 (OC 176), R.V. *Oceanus* 177 (OC 177), and R.V. *Oceanus* 191 (OC 191) and three field excursions (Barbados, 1986, 1987; Curacao, 1988) from 1986 to 1988 (see Fig. 1). Most samples were collected during the spring and summer months (March–August). Radiolarian colonies were generally collected within the top 10 m of the water column.

Colonial radiolaria were examined within 1 to 3 hr of collection using a Nikon Diaphot inverted microscope (range of magnifications = 40× to 400×) and assigned to genus or species following the descriptions of Swanberg (1979), Haeckel (1887) and Strelkov and Reshetnyak (1971). Many of the CR did not match existing descriptions in the radiolarian literature, therefore they were placed in the "unidentified colonies" category.

In addition to identification of the radiolarian, the (a) shape and dimensions of CR, (b) number of central capsules (ccs) within CR, and (c) types

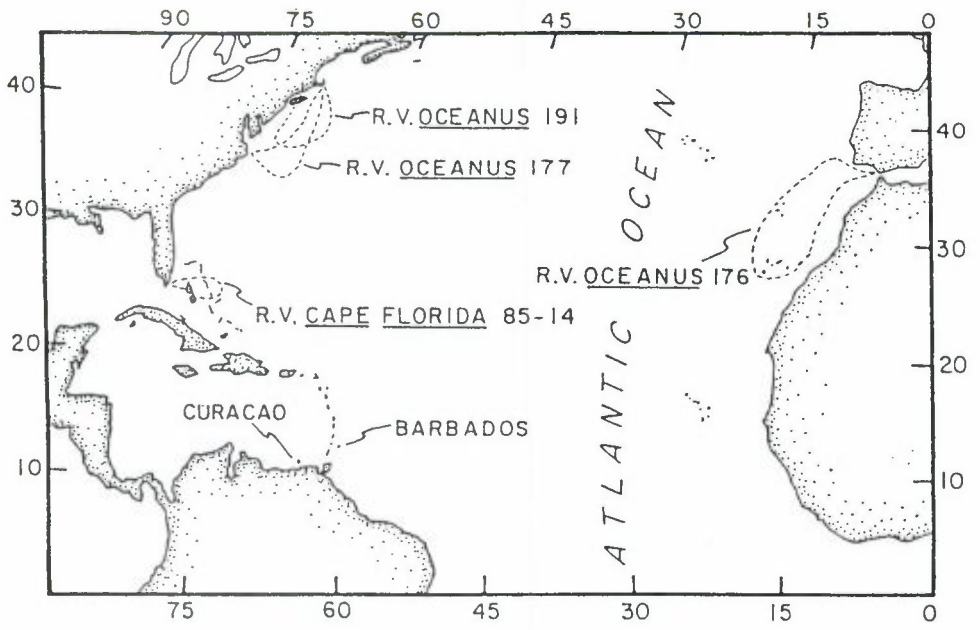

Figure 1. Locations of oceanic cruises and field stations in the North Atlantic Ocean. (CF 85-14: 10/21/85-11/13/85; OC 176: 5/21/86-6/18/86; OC 177: 7/28/86-8/25/86; OC 191: 7/14/87-7/30/87; Bellairs Research Institute, Barbados; Caribbean Marine Biological Institute, Curacao).

and abundances of protozoa and amphipods associated with the colony were recorded. Although other types of protozoa were occasionally found on the surfaces of colonial radiolaria, the vast majority of protists associated with CR were dysterid ciliates, *Gyrodinium* sp. and *Cryptobia*-like bodonid flagellates, therefore only these were recorded. CR were examined at a magnification of 400× to maximize the chances of (a) seeing infestations of even the smallest protozoa, and (b) properly identifying the protozoa and assigning these to one of the three "dominant" groups of associated protists. Notwithstanding these precautions, it is possible that a small fraction (less than 5%) of the associated protozoa were misidentified.

Estimating the carbon demand of CR-associated bacteria

The abundances and sizes of bacteria on the surfaces of CR were determined by staining radiolarian colonies with 4'6-diamidino-2-phenylindole (DAPI; Porter and Feig, 1980) and examining stained colonies with a Leitz Laborlux 11 epifluorescent microscope (total magnification 1000×). In addition to the total number of bacteria per unit surface area, I recorded the

proportion of cells that had recently divided or were in the process of dividing (Davis and Sieburth, 1984) in order to calculate bacterial growth rates.

The following equation was employed to estimate the "bacterial carbon demand" (BCD) of the populations of bacteria observed on CR surfaces.

$$BCD(\text{g carbon/CR/day}) = V \times N \times C \times GR \times (GE)^{-1} + (N \times MC) \quad (1)$$

$V =$ mean cell volume of single bacterium (μm^3)
$N =$ number of bacteria/CR
$C =$ 0.38 ± 0.05 pg carbon/μm^3 (conversion factor for cell volume to carbon (Bjornsen, 1986))
$GR =$ bacterial growth rate (doublings/day)
$MC =$ bacterial maintenance carbon (1.84 fg carbon/bacterium/day Marr et al., 1962))

For each CR included in this study, dimensions of 20 bacterial cells (of each morphological type) were measured by ocular micrometer (smallest interval: 0.4 μm; resolution 0.2 μm). Mean dimensions of the bacterial cells were used to calculate cell volumes (V). Volumes of rod-shaped cells were calculated by employing the equation for a cylinder with 2 hemispherical caps (Fuhrman, 1981).

The frequency of dividing or recently-divided bacteria (FDDC; Davis and Sieburth, 1984) observed on CR surfaces was recorded. "Dividing" and "recently-divided" bacteria included cells with a clear invagination of the cell wall (Hagstrom et al., 1979), yet not more than 0.2 μm separating 2 adjacent cells that were oriented in a manner indicating recent division. FDDC values were subsequently converted to bacterial growth rates (GR) by:

$$GR(\text{doublings/day}) = 0.0357 \times FDDC - 0.4219$$
$$R^2 = 0.948 \quad (P \ll 0.01) \quad (2)$$
(Davis and Sieburth, 1984)

Bacterial growth efficiencies (GE) estimated by various workers range from 20% (Hobbie et al., 1977; Bjornsen, 1986; Linley et al., 1983) to 80% (Williams, 1984). Therefore, I have assumed a median value, $GE = 0.5$ which was also used by Azam et al. (1983) for oceanic bacteria.

Measurement of inorganic carbon uptake by Gyrodinium *sp.*

Dinoflagellates were micropipetted off the surfaces of colonial radiolaria (mostly *Sphaerozoum* and unidentified colonies), deposited in a sterile seawater rinse and then transferred into 2-chambered Lab-Tek tissue culture slides filled with sterile seawater spiked with radioactively-labelled bicarbonate (specific activities ranged between 0.39 and 2.78 mCi/mmole).

Incubation chambers, for both light measurements and dark controls (incubated in chambers wrapped with aluminum foil), were placed 12.5 cm from a fluorescent white-light bank where the measured light intensity was 60 $\mu E/m^2/sec$ and the water temperature was maintained at 25°C (±1°C) by circulating the air around the jars with a small air fan. Durations of these experiments ranged from 2.5 to 6.5 hr; and the incubations were carried out during the daylight hours to avoid artifacts resulting from disruption of endogenous light-dark cycles. A control group containing glutaraldehyde-fixed cells was set up by arranging the same experimental system as used in the light and dark chambers; but in addition, glutaraldehyde (3%, final concentration) was added to check for passive adsorption of ^{14}C-bicarbonate to the cell surface.

Experiments were terminated by micropipetting individual dinoflagellates out of the chambers, through a non-radioactive seawater rinse and onto 25 mm dia, 0.45 μm Millipore filters. The number of *Gyrodinium* sp. on each filter ranged from 2 to 30 dinoflagellates. These filters were placed over concentrated HCl for 10 min to eliminate traces of $H^{14}CO_3$, oven dried for 10 min at 60°C and placed in mini-scintillation vials with 4 ml fluor Eco-Lite (West Chem Inc., CA) for liquid scintillation counting.

Amphipod carbon consumption

Amphipod carbon uptake was measured in two different sets of experiments described below.

A. Radio-isotopically Measured Carbon Uptake (RMCU)

Amphipod consumption of radioactively labelled carbon from within the colonial radiolarian was measured in the following manner. Amphipod-infested colonies were rinsed three times with sterile seawater to eliminate foreign microplankters before incubation in clear plastic flasks containing radioactively-labelled bicarbonate in sterile SW. The volumes of the incubation media ranged from 14 ml to 100 ml with the ratio of colony volume to

surrounding medium always set at 1:100 or more to minimize containment artifacts. The final concentrations of the ^{14}C-Na-bicarbonate used in these experiments ranged from 0.6 to 2.0 μCi/ml and the calculated specific activities ranged between 0.2 and 0.5 mCi/mmole. The flasks were maintained at room temperature (25°C) and illuminated by a "Cool White" fluorescent light bank which delivered 60 μE/m^2/sec with a light/dark cycle of 12/12. Control samples consisted of free amphipods (removed from the host colonies just prior to incubation) incubated under the same conditions as described above. These controls tested the ability of the animals to take up inorganic carbon from the water.

All flasks (controls and amphipod-infested colonies) were incubated for 24 to 26 hr, whereupon the amphipods were removed from the colonies and controls, placed on 0.45 μm Millipore HA filters and washed several times with filtered seawater. Next, the amphipods were acid fumed for 30 min over concentrated HCl, briefly oven-dried (60°C for 20 min) and transferred with the filters to mini-scintillation vials which were filled with 4 ml of Eco-Lite scintillation fluor. All samples were subsequently counted in a liquid scintillation counter.

B. Consumption of CR cells by amphipods

The rate at which amphipods consume CR cells was determined by observing the decrease in total number of intact central capsules over a period of 2 days. The amphipod-infested CR were observed 5 or 6 times during the 2 day period and after each examination, the colonies were returned to the seawater-filled glass jars in which they were originally collected. Between examinations, the jars were kept at room temperature (25°C) and placed in front of a fluorescent white-light bank which provided 60 μE/m^2/sec on a 12/12 light/dark cycle. Several amphipod-free colonies were observed for 2 to 4 days and the number of central capsules did not change, therefore, I concluded that the reduction in number of intact central capsules was the result of amphipod feeding.

The measured feeding rates were converted to carbon consumption rates by multiplying the carbon content of a central capsule by the number of cells consumed over a given period of time. The carbon content of a central capsule was measured in the following manner. Central capsules were separated from uninfested colonies by repeatedly passing the colonies through a clean pasteur pipet. Attached rhizopodia and extra-cellular "debris" were further removed from the cells by passing the isolated cells through successively smaller drawn-out pipets and finally, depositing the cells in filtered (0.45 μm Millipore) seawater. These cells were then collected on precombusted quartz-fiber-filters,

dried for 12 hr at 60°C and frozen desiccated until analyzed. Cells were analyzed for elemental carbon content using a Carlo Erba NA 1500 C and N elemental analyzer.

3. Results

Bacteria were found on all of the CR that were stained with DAPI. These bacteria were usually larger than the majority of free-living bacteria that were examined in the same waters. Most of the CR-associated bacteria were either straight or slightly curved rods with cell volumes in excess of 0.20 μm^3 (Table 1). On many colonies, there was more than one morphologically distinct type of bacterium. Some of the CR-associated bacteria that were morphologically distinct from the free living forms included "streptobacilli" (chains of rods) and extremely long filamentous forms. Planktonic bacteria in the surrounding waters consisted mostly of small cocci and rods, generally less than 0.10 μm^3. The total abundances of bacteria on CR surfaces ranged between 0.22 and 9.60 $\times$ 10^7 bacteria/ml CR for the Caribbean stations located near Barbados and Curacao, and between 0.33 and 14.10 $\times$ 10^7 bacteria/ml CR for the stations taken during the cruises in the northwestern Atlantic Ocean (OC 177 and OC 191). In comparison, abundances of free-living bacteria in the surrounding seawater ranged between 0.28 and 13.10 $\times$ 10^5 bacteria/ml seawater for the Caribbean stations located near Barbados and Curacao and between 1.90 and 13.20 $\times$ 10^5 bacteria/ml seawater for all the stations taken during the cruises in the northwestern Atlantic Ocean. Therefore, the bacteria on CR were not only larger than the free living bacteria; they were also more abundant when compared with the numbers of bacteria in an equivalent volume of surrounding seawater. The average bacterial production rate was 132 ng carbon/day (range = 1 to 523 ng carbon/day) for a mean CR-associated bacterial population of 1.49 $\times$ 10^6 cells/CR (range = 1.75 $\times$ 10^4 to 5.00 $\times$ 10^6 cells/CR).

The episymbiotic dinoflagellate, *Gyrodinium* sp. incorporated inorganic carbon in both illuminated and dark conditions (Table 2). Incorporation rates were generally higher in the light, however in two experiments (4/6/87 and 4/9/87), where both light and dark uptake rates were low, dark uptake rates were higher. Passive adsorption of radioactive bicarbonate by the dinoflagellates was ruled out after a "killed" control (dinoflagellates were treated with glutaraldehyde) showed no uptake.

Amphipods used in the ^{14}C uptake experiments (RMCU) were similar in size to one another (mean length = 0.74 mm, st. err. = 0.02). The Fisher-Behrans test for comparison between the means of samples with different variances

Table 1. Summary of the bacterial carbon demands (BCD) in 19 colonial radiolaria collected during Barbados 1987, Curacao 1988 and OC 191. The variables of the BCD equation (described in the Methods section) are presented below.

Radiolarian Species /Field Excursion	Total # Bacteria per CR ($\times 10^6$)	Mean bacterial cell Volume (μm^3)	μFDDC (doublings/day)	BCD (μg carbon) per day/CR)
Barbados 1987				
Sphaerozoum sp.	2.11	0.396	0.285	0.181
Sphaerozoum sp.	0.34	0.736	2.777	0.522
Collosphaera sp.	1.15	0.285	0.724	0.180
Unidentified colony	2.79	0.169	0.339	0.121
Unidentified colony	0.52	0.234	0.588	0.054
Unidentified colony	1.21	0.285	0.249	0.065
Unidentified colony	0.27	0.134	0.335	0.009
Unidentified colony	0.94	0.234	0.381	0.064
Unidentified colony	0.72	0.077	0.524	0.022
Unidentified colony	0.31	0.148	0.410	0.014
Curacao 1988				
Siphonosphaera sp.	5.00	0.113	0.781	0.335
Siphonosphaera sp.	3.24	0.169	0.103	0.043
Collosphaera sp.	0.02	0.084	0.696	0.001
Unidentified colony	0.86	0.523	0.563	0.193
Unidenified colony	1.07	0.159	0.385	0.050
Unidentified colony	0.15	0.268	0.131	0.004
OC 191				
Siphonosphaera sp.	0.59	0.339	0.585	0.089
Collozoum caudatum	3.92	0.109	0.488	0.158
Unidentified colony	1.98	0.791	0.246	0.293
mean ± standard error	**1.49±0.32**	**0.288±0.045**	**0.538±0.122**	**0.132±0.031**

(Campbell, 1974) was applied to the data to compare the gross carbon uptake rates of amphipods in radiolaria with those of carbon uptake rates by control amphipods. The differences between the experimental and control uptake rates were significant ($P<0.01$). Net carbon uptake rates were calculated by subtracting the average carbon uptake measured for living "control" amphipods from the gross uptake rates. Although the amphipods used in these experiments were of similar body lengths, there was considerable variability in net carbon uptake rates (see Table 3), from 35 to 310 ng carbon/day/amphipod. The mean net carbon uptake rate was 147 ng carbon/day/amphipod (st. err. = 54).

Consumption rates were calculated for three amphipod-infested colonies by recording the decrease in number of intact radiolarian central capsules (cc) at 5 intervals spaced over a period of 2 days. Average cc consumption rates presented in Table 4 were based on regression equations calculated from the plots of "number of consumed central capsules" versus time. The correlation coefficients of the regression equations were 0.83 for the amphipods in the *A. spinosa* and 0.96 and 0.97 for the amphipods in each of the *Collosphaera* sp. colonies. The average carbon uptake rate based on cc ingestion was 965 ng carbon/day/amphipod (st. err. = 174). All of the cc-consuming amphipods were large females, with fully-developed appendages (i.e. mature individuals), in comparison to the juvenile amphipods described above in the RMCU experiment.

Table 2. Summary of light and dark inorganic carbon intake rates by *Gyrodinium* sp. All rates are presented as picograms of carbon per dinoflagellate per hour.

Date of experiment	Carbon uptake rate in the light	Carbon uptake rate in the dark	Difference between light and dark uptake rates
3/28/87	10.8	7.6	3.2
3/29/87	22.8	18.7	4.1
4/1/87	2.5	0.1	2.4
4/6/87	3.6	4.7	−1.1
4/9/87	3.6	3.7	−0.1
4/17/87	15.6	14.5	1.1
8/14/87	3.6	0	3.6
mean ± st. err.	**8.9 2.9**	**7 2.7**	**1.9**

Table 3. Radioactive carbon uptake by amphipods embedded inside colonial radiolaria (RMCU). Radiolaria with amphipod infestations were incubated in illuminated (60 $\mu E/m^2/sec$) radioactively-labelled seawater for 24–26 hr (light/dark cycle = 12/12) to determine the rate of carbon uptake by the amphipods. (st. err. = standard error)

Field station date	Number of amphipods tested	Average amphipod length in mm (± st. err.)	μg Carbon fixed by CR symbionts	Amphipod carbon uptake (ng/day/amphipod) (± st. err.)
Barbados 4/16/87	3	0.78 (0.03)	1.72	310 (61)
Barbados 8/28/87	4	0.70 (0.00)	0.56	178 (16)
Curacao 4/1/88	5	0.79 (0.03)	1.28	35 (14)
Curacao 4/9/88	10	0.69 (0.02)	1.36	64 (8)

Mean amphipod carbon uptake = 147 ng/day/amphipod (st. err. = 54)
Mean amphipod length = 0.74 mm (st. err. = 0.02)
Mean amount of carbon fixed by CR symbionts = 1.25 μg carbon/CR (st. err. = 0.24)
Mean carbon uptake by control-amphipods (free-swimming amphipods) = 37 ng/day/amphipod (st. err. = 12)

Table 4. Amphipod consumption of radiolarian central capsules (cc). The rate of central capsule consumption was determined by observing the reduction in number of intact ccs over a period of 2 days. Carbon consumption rates were calculated by multiplying central capsule carbon content (ng) by the cc consumption rate. Carbon content of *Acrosphaera spinosa* = 91 ng/cc; carbon content of *Collosphaera* sp. = 30 ng/cc.

Field excursion	Colonial radiolarian	Number of amphipods per CR	Amphipod species	Average amphipod length (mm)	Number of central capsules consumed per amphipod/hr	Carbon uptake (ng carbon/day per amphipod)
R.V. *Cape Florida* 85–14 (Nov 1985)	*Acrosphaera spinosa*	3	*Hyperietta* sp.	1.4	0.6	1310
Curacao 1988 (Apr 1988)	*Collosphaera* sp.	1	*Hyperietta stephenseni*	1.8	1.4	1008
Curacao 1988 (Apr 1988)	*Collosphaera* sp.	1	*Hyperietta stephenseni*	1.6	0.8	576
Mean				**1.6**	**0.9**	**965**

4. Discussion

During the past 15 years there has been a growing interest in the microbial communities associated with amorphous particles in marine waters (e.g. Alldredge, 1976; Alldredge and Cox, 1982, Caron et al., 1982; Herndl, 1988; Silver and Alldredge, 1981; Silver et al., 1984). This interest has been due in part to the important role attributed to particle-associated microbes in the regeneration of nutrients in the plankton (e.g. Alldredge and Cohen, 1987; Newell, 1984; Shanks and Trent, 1979; Silver et al., 1978) and in part to the role of marine snow in biogeochemical cycles (e.g. Asper, 1986; Bishop et al., 1980; Fellows et al., 1981; Fowler and Knauer, 1986). Whereas most of the previous research involving surface-associated microbes has focussed on microorganisms in association with non-living surfaces (detrital matter or inanimate surfaces), this study deals with the microorganisms found on and in living colonial radiolaria, and their contribution to the flow of carbon through the radiolarian microecosystem.

The organisms associated with CR, hyperiid amphipods, dysterid ciliates, gyrodinioid dinoflagellates, *Cryptobia*-like bodonid flagellates and bacteria are unique in the sense that they are not susceptible to capture by the radiolarian feeding rhizopodia. Other nano- or micro-plankters that come in contact with the colonies are rapidly engulfed by the rhizopodia and consumed.

It is not clear what determines whether a given radiolarian colony will have associated microbial organisms. The rates of infestation (or association) of some microorganisms, e.g. *Cryptobia*-like bodonid flagellates, with the CR was higher in the more productive regions studied (i.e. in the Caribbean) than elsewhere (see Angel, 1989). Increased infestation rates may indicate that a large proportion of the colonies in the more eutrophic waters were unhealthy. For example, Swanberg (1979) found that radiolaria with heavy ciliate infestations died soon after being collected. However, I did not find a significant difference between the longevity of infested versus uninfested colonies when these were maintained in the laboratory.

All of the radiolaria examined, from both oceanic (oligotrophic) and Caribbean (productive) populations had large communities of bacteria associated with their surfaces. The estimated production rates of CR-associated bacteria were very high (1–522 ng carbon/CR/day) in comparison to the mean production rates attributed to the planktonic bacteria in an equivalent volume of surrounding seawater (0.05–50 ng carbon/day; Williams, 1984). However, the abundance of colonial radiolaria is low in most waters, therefore the contribution of the CR-bound bacterial production to total bacterial production in the pelagic environment is quite small.

Associated bacteria probably act as a major sink for the dissolved organic carbon released by the radiolarian. This may include photosynthetic exudates released by the algal endosymbionts, "waste" products excreted by the radiolarian central capsules and rhizopodial network, and organic compounds released by radiolarian sloppy feeding. If the protozoa associated with radiolaria are bacterivores, CR-associated bacteria would serve as a link between the dissolved organic matter pool and higher trophic levels within this microecosystem.

One of the protista associated with the CR that may be a bacterivore is *Gyrodinium* sp.. Although bacteria were observed inside this dinoflagellate, the prokaryotes were not found within digestive food vacuoles and therefore may be symbionts. *Gyrodinium* sp. also contains chloroplasts and is able to incorporate inorganic carbon in both light and dark conditions. In addition to the above, results of tracer experiments, and a fine structure study suggest that this naked dinoflagellate may take up dissolved organic matter by pinocytosis (Angel, 1989). If this is true, the dissolved organic carbon pool within the CR microcosm would be partitioned among bacteria and dinoflagellates.

Whereas bacterial and dinoflagellate nutrition does not seem to directly affect the physical integrity of CR, hyperiid amphipod feeding activity can be destructive to the colony. Juvenile amphipods are relatively inactive and do not appear to cause much visible damage to the radiolarian colony. The mean uptake of photosynthetically-fixed carbon from the radiolaria was 147 ng carbon/day/juvenile amphipod. In an average radiolarian colony of 48 μg carbon, this uptake would amount to the loss of less than 0.15% of the radiolarian carbon/day; a relatively small loss in light of the radiolarian's ability to regenerate biomass. However, adult amphipods are much more active and may consume the entire contents of the radiolarian central central capsules within a few days. Adult uptake rates based on central capsule consumption ranged from 580 to 1310 ng carbon/day/amphipod; almost an order of magnitude more than the juvenile uptake rate. In two heavily infested colonies (10 or more amphipods), the amphipods consumed all of the host's central capsules within only 2 or 3 days and transformed a healthy colony into an amorphous gelatinous aggregate, similar to marine snow. These examples indicate that the amphipods may be both parasites and predators of the CR, and that it is probably more accurate to describe their relationship with the host colonies as "parasitoids" (Laval, 1980).

I have constructed a model (see Fig. 2) using previously published data and results from this study, in order to summarize the contributions of associated organisms to the flow of carbon through the radiolarian microcosm, and to place these in the context of what we currently know about colonial

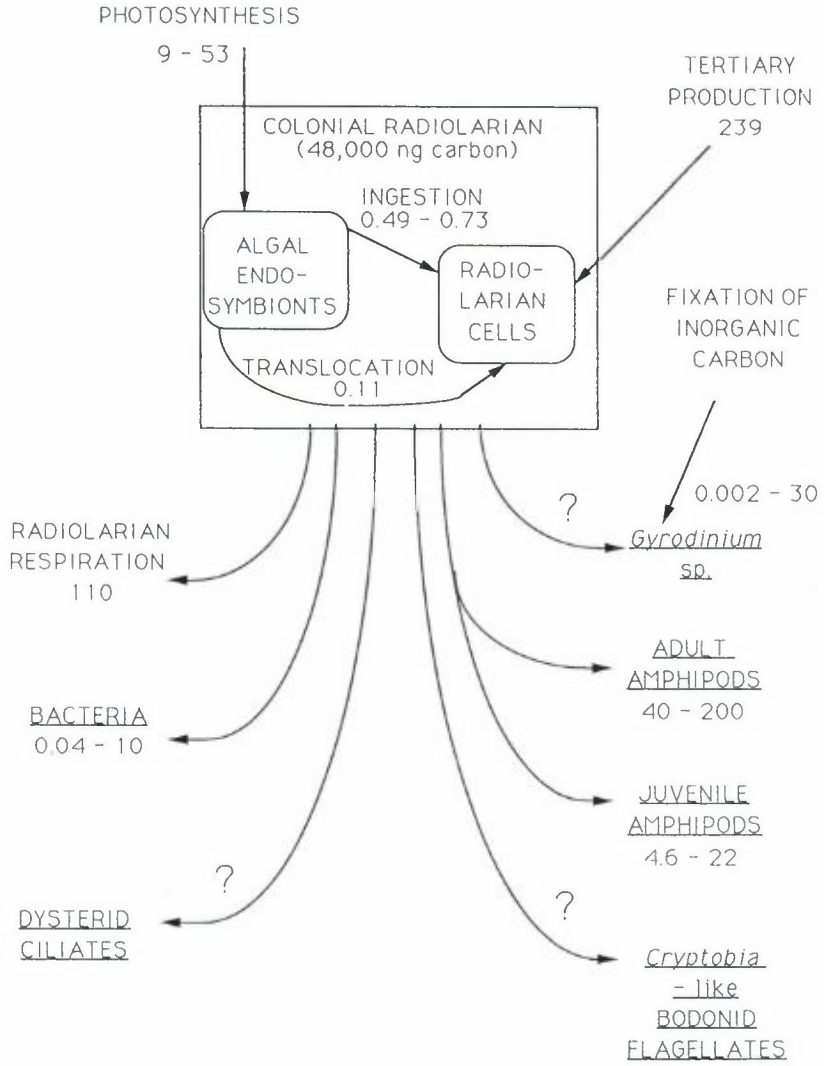

Figure 2. Carbon flow within the colonial radiolarian microcosm. The carbon content of an average *Acrosphaera spinosa* with 480 central capsules = 48 μg carbon (Swanberg, 1983). Underlined organisms are the recently described members of the CR microcosm and question marks indicate unverified pathways of carbon flow. the algal-endosymbiont photosynthesis rate (measured in *A. spinosa*) was taken from Swanberg (1979), and radiolarian tertiary production was taken from Swanberg (1983), assuming a steady state CR population of 2 colonies/m^2. The radiolarian respiration rate used here was taken from Swanberg (1983) assuming the theoretical *A. spinosa* colony in this model respires at the same rate as similarly-sized colonies of other species. Translocation of carbon from endosymbionts to host cells was based on the rate measured in *Collosphaera huxleyi* (Anderson et al., 1983) and endosymbiont-ingestion by the host was calculated from the rate of endosymbiont disappearance in *Collozoum inerme* (Anderson, 1983).

radiolarian production. Because the model includes production rates that were measured in several different species of radiolaria, it cannot be used as a formal carbon budget (since there are probably numerous differences in the physiology of different species) but instead, should be regarded as an approximation of processes within the CR microcosm. All production rates have been normalized to 24 hr and are expressed as ng carbon/hr. In this model, photosynthetic carbon uptake, tertiary production and respiration rates were adapted from Swanberg (1983). Swanberg (1979) found that an average *Acrosphaera spinosa* colony with 480 central capsules (mean central capsule carbon = 100 ng) photosynthetically incorporates from 9 to 53 ng carbon/hr at light intensities ranging from 700 to 2200 $\mu E/m^2/sec$. However, only a fraction of this photosynthetically-fixed carbon is translocated from algal endosymbionts to the radiolarian cells. Anderson et al. (1983) measured the rate of carbon translocation from the endosymbionts of *Collosphaera huxleyi* to the host cells and this translocation rate (0.11 ng carbon/hr) is presented here because translocation from endosymbionts to radiolarian cells has not been measured in *A. spinosa*.

In addition to the carbon gained in the translocated photosynthates, Anderson (1983) observed that *Collozoum inerme* cells may ingest (and digest) 4% of the algal endosymbionts each day. By farming a small part of the algal symbiont population, the radiolarian maintains the algae at a steady (manageable) state and also benefits nutritionally; a definite advantage in oligotrophic waters. The carbon content of an average algal endosymbiont was estimated as 48.6 pg carbon/endosymbiont, assuming its volume specific carbon content is the same as that of *Gonyaulax tamarensis* (Langdon, 1987). If there are 480 central capsules (ccs) per colony, and each central capsule has 20 endosymbionts (Anderson, 1976), digestion of 4% of this algal population would add 0.49 to 0.73 ng carbon/hr to the radiolarian cells.

Because there are no respiration data for *A. spinosa*, I have adopted the approach used by Swanberg (1983). He assumed the weight specific respiration rate of *A. spinosa* is similar to that of *Collozoum pelagicum* and *Sphaerozoum punctatum*, therefore a colony with 480 ccs would respire 110 ng carbon/hr. There are no direct measurements of colonial radiolarian tertiary production. However, Swanberg (1983) estimated that the radiolarian photosynthetic carbon incorporation rate measured at high light intensities was roughly equivalent to the respiration rate (when normalized to 24 hr, the ratio of photosynthesis/respiration is approximately 1/2), and suggested that the majority of radiolarian production must come from predation. The tertiary production rate he proposed, 4.2 mg $C/m^2/yr$, is based on the mean annual collosphaerid radiolarian production calculated from the flux data at Parflux station E (Takahashi

and Honjo, 1981). This production rate is equivalent to 239 ng C/hr/CR assuming there is a steady state population of 2 CR/m^2.

In Fig. 2, the five groups of organisms associated with the colonies and their carbon uptake rates have been underlined to indicate that these were the focus of this study. Question marks indicate that the designated path of carbon flow is possible, but has not been shown. The production rates of dysterid ciliates and *Cryptobia*-like bodonids were not measured in this study, however bacteria were observed within the cells of both of these protozoans and therefore, they may be bacterivores. Whereas it is not clear whether the intracellular bacteria within dysterids were symbionts or prey, there is evidence that these ciliates consume cells that contain chloroplasts. The partially digested chloroplasts have the ultrastructural features of dinoflagellate plastids, and it is possible that the dysterids graze the *Gyrodinium* sp. when these two types of associated organisms co-occur on the CR hosts.

The flow of carbon to associated bacteria (BCD) within the CR microcosm was calculated by estimating bacterial growth rates using the FDDC method (Davis and Sieburth, 1984). Bacterial production rates spanned 2.5 orders of magnitude (0.04–10 ng C/hr; see Fig. 2). This wide range of BCD values is mainly the result of variability in radiolarian surface area since neither abundance of bacteria per mm^2 radiolarian surface area (data not presented here), nor bacterial growth rates (0.10–2.78 doublings/day, Table 1) showed large variance (see the BCD equation in the Methods section). Bacterial production is a function of available dissolved organic matter (DOM), and these production rates may be an indirect indication of the rate of DOM release from the colonies. Because bacteria are an integral part of the radiolarian microcosm (bacteria have been observed on all of the colonies examined), there will be a flow of carbon from the CR to bacteria whenever DOM is released at the radiolarian surface. As stated above, the bacteria may serve as a DOM sink or a regenerative link if they are grazed by the resident protozoa, however, bacterivores have not been definitively identified in the microcosm.

The mean light-driven inorganic carbon uptake rate of *Gyrodinium* sp. (calculated by subtracting dark uptake controls from light uptake; the two "negative" uptake rates shown in Table 2 were excluded) was 2.88 pg carbon/cell/hr. Although the average abundance of *Gyrodinium* sp. in infested *A. spinosa* colonies, was 622 cells/CR, dinoflagellate infestations among other CR in the same size range as the CR in the model (48 μg carbon), sometimes exceeded 10,000 cells/CR (Angel, 1989). Therefore, the range of *Gyrodinium* sp. uptake rates in the model includes the uptake of a single cell and the total uptake rate calculated for 10,000 cells. At the upper end of the estimated range, dense populations of *Gyrodinium* sp. may fix carbon at rates that are equal

to the radiolarian endosymbiont production, however the consequences of this production to the CR microcosm are not clear.

I did not trace the source of the inorganic carbon taken up by *Gyrodinium* sp., but this may include CO_2 excreted by radiolarian respiration. This would serve as an example of carbon recycling within the CR microcosm. *Gyrodinium* sp. incorporated considerable amounts of inorganic carbon in the absence of light energy (dark uptake). Dark uptake rates ranged between 0 and 18.7 pg carbon/cell/hr, as compared to total (light + dark) uptake rates which ranged from 2.5 to 22.8 pg carbon/cell/hr. The mechanism driving the dark uptake of inorganic carbon is not known, however, I have excluded dark uptake rates from the model pending further experimentation and the rates shown represent the "light minus dark" inorganic carbon uptake.

Hyperiid amphipod production was assigned to two categories representing differences in trophic behavior on the part of juvenile and adult amphipods. Although the production rates of adult amphipods that do not consume central capsules were not measured, I assigned them the same production rates as juveniles until further data are available. Parasitized radiolaria contained either adult or juvenile amphipods; the two life stages were mutually exclusive (Swanberg, 1979; Angel, unpublished), therefore, the separate arrows in the model represent mutually exclusive production rates. Parasitized *A. spinosa* colonies contained from one to 5 amphipods per colony, while other CR species sometimes had 20 or more amphipods per colony. In comparison to the radiolarian production (248 to 292 ng carbon/hr, from the sum of symbiont photosynthesis and tertiary production), amphipod production rates (5 to 22 ng carbon/hr for juveniles and 40 to 200 ng carbon/hr for central capsule-consuming adults) are very high and amphipods are probably the largest consumers of radiolarian carbon in the microcommunity. Although their potential impact on radiolarian biomass is considerable, amphipod infestations were usually rare events (often less than 5% infestation among all colonies collected during a field excursion; see Angel, 1989), and hyperiid amphipod feeding is probably not a major influence in most radiolarian populations.

From what is currently known about the abundances and activities of the members of the CR microcosm, it is probably safe to conclude that unlike the hyperiid amphipods, the protozoan and bacterial episymbionts do not contribute significantly toward the demise of the colonial radiolaria. The production rates in Fig. 2 should be cited with caution since the model of carbon flow is fairly conceptual and is based on a small number of measurements. Additional measurements of associated protozoan and bacterial activities and of carbon flow within the CR microcosm are needed. Nevertheless, it would be naive to construct a carbon or energy budget for the radiolaria without

considering the contribution of associated organisms. In light of this study, it would probably be prudent to examine all aquatic plants and animals for associated microorganisms before measuring their production and metabolic rates. While associated microbes are generally small in comparison to their hosts, large populations of active cells may contribute substantially to overall production and should therefore be taken into consideration.

The model of carbon flow through the radiolarian microcosm demonstrates the complexity of even the smallest communities in the pelagic environment. One of the unique characteristics of the radiolarian microcommunity is the fact that it is a self supporting system. Carbon is incorporated by the radiolarian, either by predation, or through endosymbiont activity, and some of this carbon is then utilized by associated organisms. Goldman (1984) proposed that small flagellates may survive in oligotrophic waters by migrating between organic microaggregates (which contain "up to three orders of magnitude greater concentrations of microbial populations and nutrient concentrations") to fulfill their nutritional needs. The presence of the protozoa in the CR microcosm may be an example of this proposed survival mechanism since it is unlikely that the individual species would succeed in the surrounding waters in the absence of patches of elevated concentrations of nutrients or microbial prey. Goldman (1984a) also emphasized the significance of the dynamic microaggregate communities to recycling and regeneration of nutrients in pelagic environments. The activities of organisms associated with the CR may therefore play an active role in exporting nutrients to the surrounding waters.

Michaels et al. (1988) have found that planktonic sarcodines, including acantharia, foraminifera and radiolaria sometimes constitute the bulk of the particulate carbon in oceanic sediment trap samples. In light of this observation, and the growing interest in global oceanic fluxes, it is important to gain a better understanding of the microbial community associated with colonial radiolaria and its role in the flow of carbon through the living colonies, even before their ultimate sinking out of oceanic surface waters.

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