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Functional autotrophy of *Heteroxenia fuscescens* (Anthozoa: Alcyonaria): carbon assimilation and translocation of photosynthates from symbionts to host *

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Abstract

The association of the alcyonarian *Heteroxenia fuscescens* (Ehrb.) with its cytosymbiotic algae shows structural and physiological adaptations optimizing the living together of the two partners as one functional unit. To enhance the energetic contribution of the autotrophic partner, the organization of the heterotrophic partner bears typical plant-like imprints. Up to 20% of the inorganic C photosynthetically fixed was translocated to the host (=2 mg C mg d.w.$^{-1}$ d^{-1}). This net C gain by the host is used for anabolic purposes including the deposition of storage material. Especially the wax-esters and triglycerides of the host – in contrast to those of the symbionts – were intensively labelled. The *in-vivo* ^{14}C-fixation of zooxanthellae is more than double the *in-vitro* fixation. In both symbionts and host, the lipids show the highest relative ^{14}C-incorporation. In particular the polyol component was strongly labelled. After 120 min of continuous incubation, approximately 40 labelled intracellular metabolites were detectable in the ethanol/water soluble fractions of zooxanthellae. Glycerol is the main low-molecular weight carbohydrate being transferred. This is corroborated by the deacylation of lipids of the host.

Introduction

Many cnidarian species possess polytrophic feeding capacities: (1) by means of nematocysts, prey is captured and finally digested within food vacuoles of the gastrodermal cells; (2) organic material dissolved in the sea is utilized by direct absorption through the epidermis; (3) assimilates of endosymbiotic algae are used by the host metabolism. These three possibilities of nutrition support metabolism at different rates, which may depend either upon the availability of the various substrates or upon the degree of specialization of a given species. The evolutionary advantage of trophic flexibility is obvious. Metabolic demands can thus be supplied by three quite independent mechanisms (Schlichter, 1973, 1980, 1982 a).

It is difficult to calculate, for a particular species provided with all three possibilities, the benefits which can be drawn from one of them. With this in mind, the alcyonarian *Heteroxenia fuscescens* seems to be a particularly appropriate organism to study the nutritive role, especially of zooxanthellae. Under experimental conditions *H. fuscescens* does not ingest any particulate food. Nematocysts, structures for capturing prey, are minute and very scarce. The internal surface enlargement of the septa by mesenteric filaments is reduced, i.e. the capacity of the gastrodermis for secretion of enzymes and for the endocytosis of food particles is distinctly diminished (Schlichter, 1982 b). *H. fuscescens* therefore seems to obtain energy exclusively from photosynthates of its endocytobionts and by the uptake of dissolved organic material (DOM). The importance of DOM uptake has already been studied and the results published elsewhere (Schlichter, 1982 a).

The structural features of *Heteroxenia fuscescens* mentioned above give the impression that particulate food is of minor or no importance for the nutrition of this alcyonarian. Organic substances synthesized by the autotrophic algal partners – the primary producers – of the symbiosis play the major role in the energetic supplementation of the heterotrophic host – the primary consumer. The presumable total energetic dependence of the host on its symbionts was a main reason for conducting further studies in this field, although many cnidarian species have already been investigated (for review see e.g. Trench, 1979). Another reason of some importance for the present studies was to evaluate whether or not *H. fuscescens* possesses physiological patterns similar to hermatypic corals or sea anemones, which have already been analyzed biochemically.

* A second paper is concerned with the utilization of external acetate by *Heteroxenia fuscescens*

In this paper quantitative data are given that are particularly concerned with the uptake and utilization of inorganic carbon, the labelling pattern of photosynthates, and the unidirectional transfer of assimilates to the host. Results of *in-vivo* and *in-vitro* experiments are compared.

Material and methods

Collection and maintenance of colonies

On the inner side of Kenyan reefs, colonies of *Heteroxenia fuscescens* (Ehrb.) (Anthozoa: Alcyonaria) were collected, avoiding any damage, put individually in plastic bags, and brought back in tanks with running natural seawater.

The colonies of *Heteroxenia fuscescens* are composed of a fleshy, mushroom-shaped syndete and two types of polyps: large, flower-like autozooids and tiny siphonozooids (Fig. 1 a and b). For taxonomical, biological, and ecological details the papers of Gohar (1940 a, b) may be consulted.

Conditions of maintenance were a natural light cycle (of which 2–4 h were full sunshine), a temperature of 27 °C ± 2 C°, and a salinity approximating 35‰ S.

Experimental procedures

Amputation and incubation of autozooids

The autozooids were amputated from the syndete and transferred into sterile filtered natural seawater (SNW) (Sartorius membrane filter, 0.45 μm-pore width). The SNW was changed several times. After about 20–40 min, the tentacles of the isolated autozooids again began to pulsate rhythmically (about 60 beats min^{-1}), just as they do as colony members. For each incubation period (10, 30, 60, 120 min), 200 autozooids were placed in a glass vessel containing 100-ml SNW to which $NaH^{14}CO_3$ (37 KBq) ml^{-1}, specific activity 37 mBq $mmol^{-1}$) (purchased from Amersham) was added. The temperature was 27 °C ± 2 C°. The water in the glass beakers was stirred by a gentle air stream so that the pulsating autozooids floated slowly in the containers. With the same operation the added ^{14}C-radioactivity was homogenously mixed, and it was ensured that all autozooids were permanently illuminated during the experiments. Furthermore, the pH remained constant.

At the end of each incubation period the autozooids were divided into two portions: 100 individuals were instantly fixed in MCF (methanol – chloroform – 6 N formic acid = 12 : 5 : 1) (v/v/v). This portion constitutes the total ^{14}C-incorporation (host plus endosymbionts). The other portion was transferred into chilled SNW and minced in a Waring blender (1 min at high speed) to separate host tissue from symbiotic zooxanthellae according to Trench (1971 a). The supernatants and pellets of 4 washings, centrifugation and resuspension steps were collected. The samples belonging together were pooled and also fixed in MCF. Separation of previously fixed autozooids into zooxanthellae and host tissue was not successful. The loss of organic material during separation averaged 10% of the initial weight on a protein basis.

For final extraction of ^{14}C-labelled compounds, the autozooids fixed *in toto* (see above), and the separated host tissue and algal symbiont samples were homogenized (Porter) in MCF and centrifuged. The pellets were resuspended in 70% ethanol (EtOH) and extracted for 24 h on a shaker, followed by a second centrifugation. The supernatants from the first and second extraction were combined and mixed with chloroform. By the whole procedure,

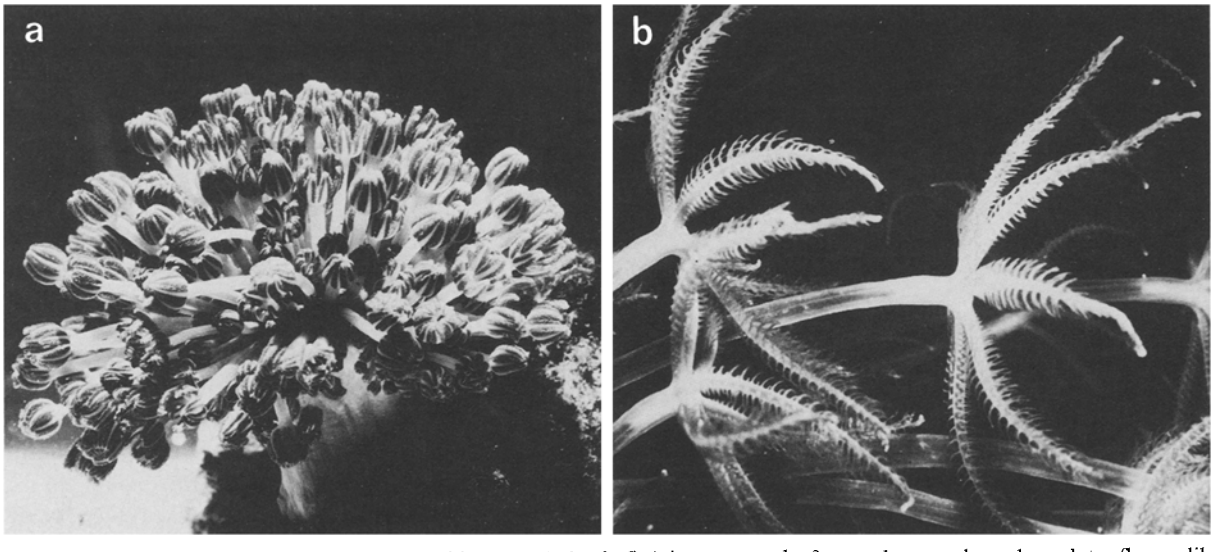


Fig. 1. *Heteroxenia fuscescens.* (a) A colony (big as an infant's fist) is composed of a mushroom-shaped syndete, flower-like autozooids and tiny siphonozooids (not visible). (b) Close-up of autozooids of a Xeniid. From the central part of the tentacles up to 8 rows of pinnules protrude, thus large well-illuminated plant- like structures are formed in which the host may 'farm' much more symbiotic algae as compared to non-pinnuled tentacles. Approximate size of the extended tentacle crown up to 2 cm

three different extracts were obtained: (1) an insoluble fraction (polymeric compounds), (2) a chloroform soluble fraction (lipids), and (3) an EtOH/water soluble fraction (organic acids, amino acids, sugars, ureids, other neutral compounds). The last fraction was desalted on a Sephadex G 10. All fractions were dried, weighed, and subsequently redissolved either in chloroform (lipids) or in 20% EtOH. Prior to thin-layer chromatography (TLC), the EtOH fractions were (in some cases) prefractionated on Dowex 1×8 and Dowex 50 W×8 (Splittstoesser, 1969). The insoluble fractions, predominantly proteins, were decomposed with Soluene 350 (Packard) before counting by LSC-radiospectrometry. From each sample type (complete autozooids, host tissue, algal symbionts), the extracts mentioned above were prepared.

Preparation of zooxanthellae for in-vitro experiments

The method of Trench (1971 a) was used. A total of 200 autozooids were minced in SNW (Waring blender). The homogenate was first filtered through synthetic filter wool. The filtrate was washed 4 times, centrifuged and resuspended in SNW. Aliquots of the last resuspended pellet were added to SNW to give a final volume of 25 ml, containing 37 KBq ml^{-1} $NaH^{14}CO_3$. After 10, 30, 60 and 120 min of incubation under light or dark conditions (see *in-vivo* experiments), the samples were fixed in MCF. Subsequently the samples were extracted as described above. The data obtained hence represent intracellular products of the endosymbionts exclusively.

Analytical procedures

Quantifications of protein and pigments were performed according to standard methods (Lowry *et al.*, 1951; Jeffrey and Humphrey, 1975). Deacylation of lipids was carried out with Na-methylate in 30% methanol for 6 h at room temperature. The two resulting fractions were separated, dried and redissolved in 20% EtOH or chloroform, respectively. Aliquots were pipetted onto glassfiber filters, dried and counted in a standard scintillation cocktail.

Chromatography

EtOH extracts were separated by two-dimensional TLC on cellulose plates (MN 300, Macherey and Nagel) using the solvent systems of Feige *et al.* (1969). The position of ^{14}C-labelled compounds were visualized by contact autoradiography. Identification was established according to procedures published earlier (Kremer, 1978). $CHCl_3$-extracts were separated on silica gel plates (Merck) according to Glasl and Pohl (1974).

Quantitative ^{14}C-analysis of thin-layer chromatogram was performed in the case of one-dimensional TLC ($CHCl_3$-extracts) with a scanner (Berthold), followed by planimetrical quantification. Individual spots of two-dimensional TLC (EtOH-extracts), localized by X-ray fingerprinting, were counted separately with a wide-window methane flow counter coupled with a linear rate meter. The values were corrected for background.

Results

Association of *Heteroxenia fuscescens* and their symbiotic zooxanthellae

The large majority of zooxanthellae of *Heteroxenia fuscescens* is located in the autozooids. The structural organization of the tentacles of the autozooids show a leaf-like intensive surface enlargement. A maximum of 8 rows of pinnules (on the average 22 pinnules/row) protrude from the central part (Fig. 1 b). The formation of pinnules not only enlarges the external surface, but also much more internal epithelium is produced in which a high number of zooxanthellae can be 'farmed' under well illuminated conditions (Schlichter, 1982 a, b).

The intracellular placement of the algae, the dinoflagellate *Gymnodinium microadriaticum* (Freudenthal), within vacuoles of the endodermal host cells is in accordance with conditions described for other cnidarian associations (Taylor, 1973; Trench, 1979).

The fresh weight of experimental colonies averaged 15–65 g; the syndete together with the siphonozooids accounting for 75%, the autozooids for 25%. Dry weight (d.w.) of the whole colonies was 3.5–6.0% of the fresh weight. The average number of autozooids of experimental colonies was 400–500. Protein content of complete colonies was 0.35 mg mg^{-1} d.w. and that of autozooids 0.8 mg mg^{-1} d.w. The distribution of protein between autozooids and rest-colony was 55:45. From the protein of the autozooids 65% was host tissue and 35% zooxanthellae.

Chlorophyll was determined for complete colonies from different depths. The data obtained are: 1.9; 1.5; 1.7 and 1.8 mg chlorophyll *a* g^{-1} d.w. for individuals from 1, 5, 10 and 20 m water depth, respectively (Svoboda and Machan, in preparation). In contrast to hermatypic coral species, in *Heteroxenia fuscescens* there obviously exists no depth-dependent increase of pigment content. The chlorophyll content of autozooids is seven times higher when compared to the rest-colony.

Uptake of inorganic carbon from the incubation medium

After the incubation of whole colonies as well as of amputated autozooids alone in the light, the amounts of ^{14}C-bicarbonate contained in the incubation medium steadily decreased (Fig. 2). In evaluating the time-course of the resulting uptake curve, one can distinguish between a linear part at the beginning of the incubation, when the concentration of the tracer is still high, and an exponential (hyperbolic) part, the latter being due to the experimental

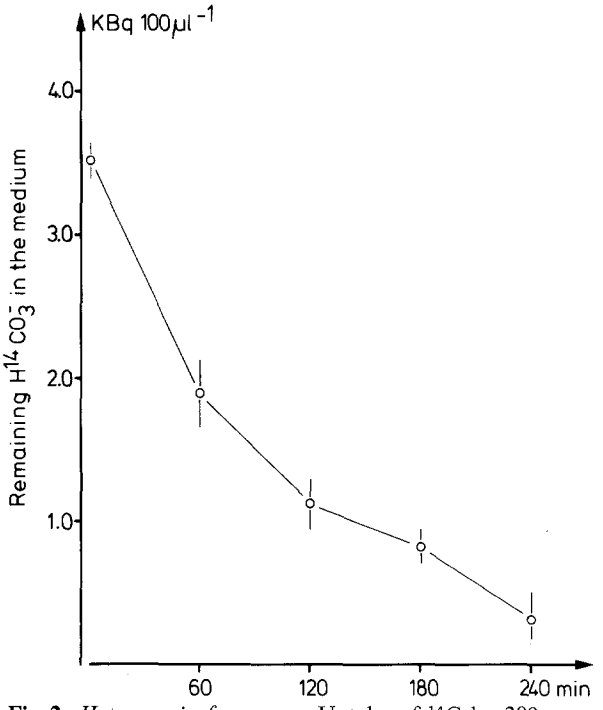


Fig. 2. *Heteroxenia fuscescens.* Uptake of ^{14}C by 200 amputated autozooids in the light. $n = 3$; ± min. and max. values

situation. After 60 min the concentration of labelled bicarbonate had dropped to such a level that the ^{14}C portion of the total C-uptake decreased.

Time course of inorganic carbon incorporation by autozooids

The amounts of the total ^{14}C incorporated from the incubation medium in the light and in the dark and their distribution among the three fractions of assimilates (insoluble, EtOH/water-soluble and $CHCl_3$-soluble assimilates) are combined in Fig. 3. The proportions of the total ^{14}C recovered from the symbionts and from the host tissue, respectively, is represented by the thick central columns (upper part: symbionts; lower part: host tissue). After 10-min incubation, 91% of the total incorporated ^{14}C was located within the symbionts. This amount decreased to 83% after 120 min. In contrast, the quantity of fixed ^{14}C in the host tissue increased from 9 to 17% during the same period. Dark carbon fixation averaged 6% after 10 min and 2% of the total uptake after 120-min photosynthesis (Fig. 3). Taking the total ^{14}C content of the symbionts or the host as 100%, the dashed portion of the thick central columns and the black arrows with white numerals indicate the proportions of ^{14}C dark incorporation, each calculated in % of the light-dependent carbon uptake. Absolute values of ^{14}C-incorporation are shown in Fig. 4. Based on a total HCO_3^--content of seawater of 230 μM on the average, the total inorganic C-incorporation by complete autozooids accounted for 21 $\mu g\ C\ mg^{-1}$ d.w. h^{-1}. The data contained in Fig. 4 are neither corrected for the loss of labelled compounds by leakage to the water, nor for the ^{14}C-loss by dark respiration, nor for isotopic exchange.

The distribution of incorporated ^{14}C among the three major fractions of assimilates (insoluble, EtOH/water-soluble and $CHCl_3$-soluble) is also shown in Figs. 3 and 4. The thin white columns in Fig. 3 on the right side of the thick ones give the ^{14}C-content of the symbionts and the host tissue under light conditions (photosynthesis). The thin grey columns on the left side indicate the ^{14}C-distribution among the assimilate fractions under dark conditions. In the light the ^{14}C-labelling of the $CHCl_3$-soluble fractions increased most rapidly in the symbionts as well as in the host tissue (top section of the thin columns in Fig. 3). The labelling of the EtOH/water-soluble fraction continuously decreased and averaged 13% after 120 min in the symbionts and 6% in the tissue as based on the total ^{14}C-uptake (middle part of the thin columns). The percentage of labelled compounds confined to the insoluble fraction showed an increasing tendency as well (bottom part of the thin columns in Fig. 3). An average of 15 to 25% of the detectable ^{14}C incorporated occurred as insoluble polymeric material in the symbionts and in the host tissue, respectively. These results, expressed in terms

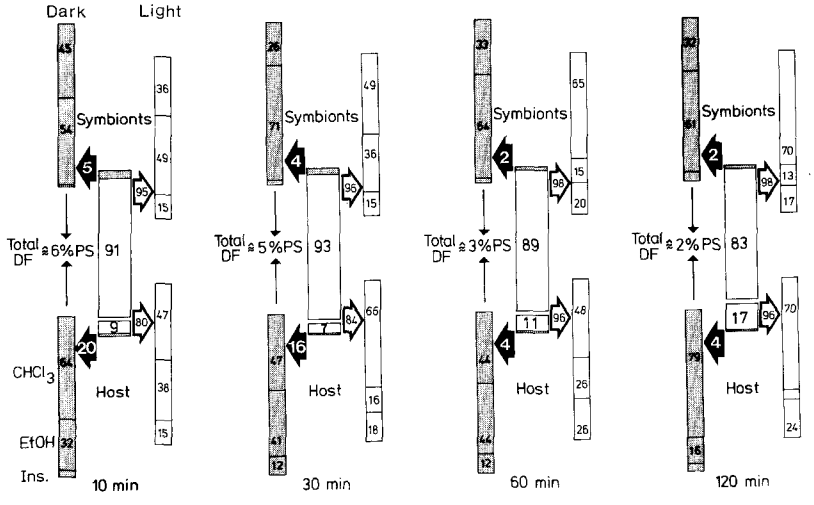


Fig. 3. *Heteroxenia fuscescens.* Time-dependent incorporation of inorganic ^{14}C by amputated autozooids under dark (dashed columns, black arrows) and light conditions (open columns, white arrows): all values expressed as % of ^{14}C uptake. Thick columns: total ^{14}C-incorporation into autozooids; top: ^{14}C-content of the symbionts, bottom: ^{14}C-content of host tissue. The thin columns show the distribution of ^{14}C among three major fractions of assimilates (Ins. = insoluble, EtOH = EtOH/water-soluble, $CHCl_3$ = $CHCl_3$-soluble fraction). Total DF = incorporation of ^{14}C in the dark expressed in % of photosynthesis. $n = 3$

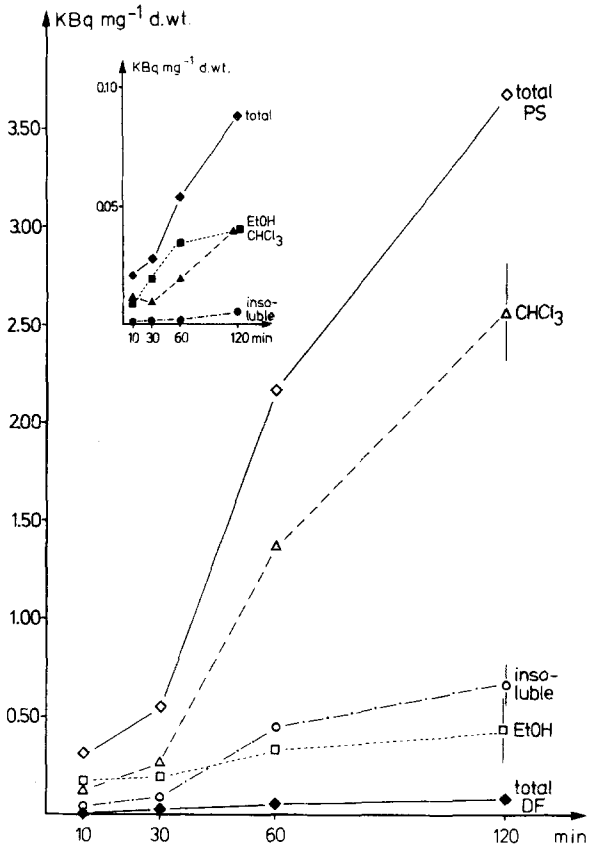


Fig. 4. *Heteroxenia fuscescens.* Incorporation of ^{14}C into intact autozooids and distribution among different assimilate fractions characterized by their solubility. Large graph: photosynthesis. Insert: dark incubation. Abbreviations: total PS = total ^{14}C incorporation in the light, total DF = total ^{14}C incorporation in the dark. $n = 3$. For further details see "Material and methods"

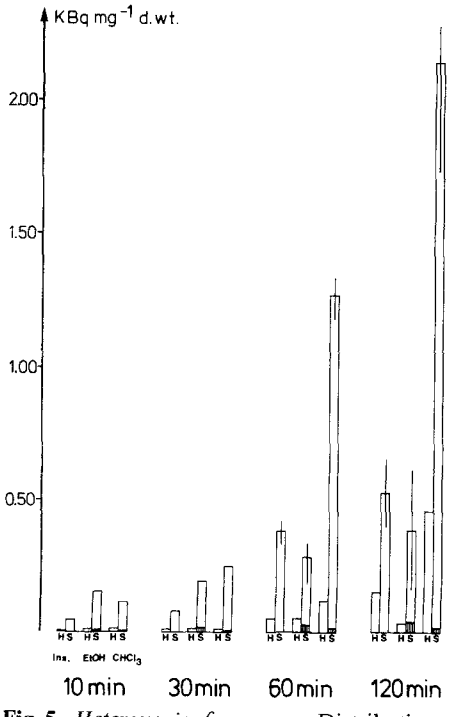


Fig. 5. *Heteroxenia fuscescens.* Distribution of incorporated ^{14}C among symbionts (S) and host (H) after incubation of autozooids under dark (dashed columns) and light conditions (open columns). Abbreviations: see Fig. 3

of absolute ^{14}C-activity, are shown in Fig. 5. Under dark conditions or DCMU-treatment [DCMU = 3-(3.4-dichlorophenyl)-1.1-dimethylurea], the ^{14}C-uptake was low and achieved 2 to 6% of the incorporation encountered during photosynthesis (cf. Figs. 3 and 4). In contrast to the incubation in the light, the EtOH/water-soluble fraction of the symbionts remained most strongly labelled in the dark. The $CHCl_3$-soluble fraction is labelled to a smaller extent, and the transfer of fixed ^{14}C into the insoluble fraction thus appears to be extremely low.

Time course of CO_2-fixation by isolated zooxanthellae

The results of total ^{14}C-uptake and the incorporation into the three major fractions of assimilates of *in-vitro* incubated, isolated zooxanthellae corresponded well with the results obtained from *in-vivo* experiments. In both cases, only intracellular ^{14}C-labelled products were taken into consideration. Again, the bulk of photosynthetically assimilated radiocarbon accumulated in the $CHCl_3$-soluble fraction representing lipid constituents (Fig. 6). Despite

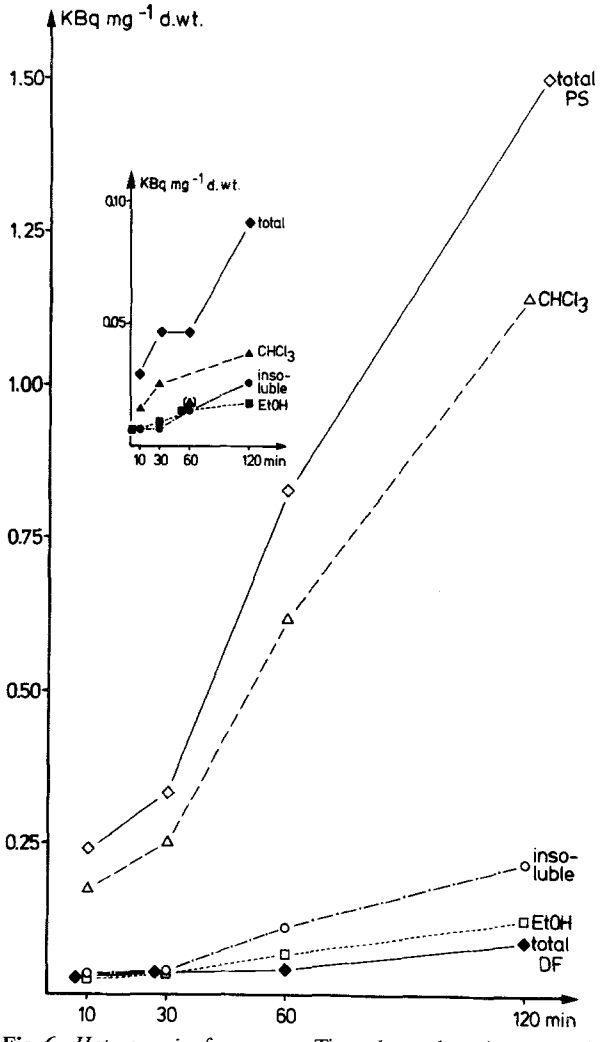


Fig. 6. *Heteroxenia fuscescens.* Time-dependent incorporation of ^{14}C by isolated zooxanthellae under light and dark conditions and distribution of radiocarbon among different assimilate fractions. For further details see Figs. 3 and 4

the similarity in a qualitative sense, there is a striking quantitative difference between *in-vitro* and *in-vivo* experiments: The carbon assimilation of zooxanthellae within the host tissue averaged 29 μg C mg^{-1} d.w. h^{-1}, whereas on the average the isolated zooxanthellae only fixed 12 μg C on the same reference basis. Possible reasons for these divergent rates of carbon assimilation will be discussed below.

Labelled metabolites of *in-vivo* and *in-vitro* experiments

The autoradiographs of TLC separated extracts of EtOH/water-soluble fractions of *in-vitro* experiments with isolated zooxanthellae are shown in Fig. 7. The initials and numerals, respectively, indicate the substances involved (abbreviations are explained in Table 1) as well as the percentage of ^{14}C-content of the particular separation illustrated.

Table 1. *Heteroxenia fuscescens.* Survey of ^{14}C-labelled compounds recovered from the EtOH/water-soluble fraction of assimilates after 120-min incubation in the light

Compound	Abbreviation used in Fig. 7
Anionic	
3-Phosphoglycerate	A 1
Fructose-1,6-bisphosphate	A 2
Fructose-6-phosphate	A 3
Glucose-6-phosphate	A 4
Phosphoenolpyruvate	A 5
Unknown sugar phosphates	A 6 – 12
Citrate	O 1
Malate	O 2
Fumarate	O 3
2-Oxoglutarate	O 4
Lactate	O 5
Succinate	O 6
Glycolate	O 7
Unknown organic acids	O 8 – 9
Cationic	
Aspartate	C 1
Asparagine	C 2
Glutamate	C 3
Glutamine	C 4
Alanine	C 5
Threonine	C 6
Glycine	C 7
Serine	C 8
Histidine	C 9
Leucine/Isoleucine	C 10
Phenylalanine	C 11
Cysteine	C 12
Unknown amino acids	C 13 – 16
Neutral	
Oligo-/polyglucane	N 1
Glyceraldehyde (?)	N 2
Glycerol	N 3
Glucose	N 4
Mannose	N 5
Unknown carbohydrate	N 6

After 120 min of continuous exposure to $H^{14}CO_3^-$, about 40 metabolites were ^{14}C-labelled. A critical comparison of the labelling patterns of *in-vivo* and *in-vitro* incubated zooxanthellae provided evidence that the patterns of assimilates are essentially similar. It is noteworthy that certain amino acids, such as alanine, aspartate, glycine, serine, and threonine, usually accounted for more than 30% of ^{14}C confined to the EtOH/water-soluble fraction of assimilates. This appears to be a typical feature of brown pigmented unicellular algae as the same pattern is found with free-living dinoflagellates and diatoms. A further 30–40% of radiocarbon was located in intermediates of the tricarboxylic acid cycle of which more than 30% was in citric acid, which is remarkably high. Glycerol was the only ^{14}C-labelled low-molecular weight carbohydrate recovered besides small amounts of glucose and a compound tentatively identified as glyceraldehyde. The occurrence of glycerol and glucose is consistent with the usual pattern of soluble carbohydrates encountered with the dinoflagellates, although it should be noted that the percentage of ^{14}C does not exceed 10%. Hence, both compounds were obviously not accumulated in a free state.

The ^{14}C-incorporation into $CHCl_3$-soluble compounds is shown in Fig. 8. Separation of *in-vivo* and *in-vitro* incubations are presented, including TLC separations of all sample types experimentally analyzed. Comparing the autoradiographs of the different extracts originating from four different sample types, the most striking results concern the difference of the ^{14}C-incorporation into triglycerides and wax esters of symbionts and host. The radiochromatographs of extracts containing host tissue (Fig. 8 a and 8 c) clearly show an appreciable ^{14}C-labelling of triglycerides after 10-min incorporation and of wax esters after 30-min ^{14}C-feeding. In contrast, in extracts containing only algal material (Fig. 8 b and 8 d), the wax esters are lacking after relatively shorter incubation periods, some label appearing after longer incubation. To explain this finding, one may assume that photosynthates were transferred to the host tissue and there subsequently converted into storage material at a noticeable rate. This explanation agrees well with the ^{14}C-labelling patterns of the components of lipids after deacylation (Figs. 9 and 10).

Deacylation of ^{14}C-labelled lipids

The results of deacylation (Fig. 9) clearly show that, even after longer incubation, the fatty acid chains became only weakly labelled in the light, in contrast to the polyol component which showed appreciable ^{14}C-labelling. Considering the fact that the labelling of the EtOH/water-soluble fraction of autozooids slowly increased on an absolute scale as compared to the total incorporation, whereas the labelling of the $CHCl_3$-soluble material increased rather rapidly, one may speculate that labelled compounds are quickly converted from one pool of assimilates into the other. In the EtOH/water-soluble fraction obtained upon deacylation, only one labelled com-

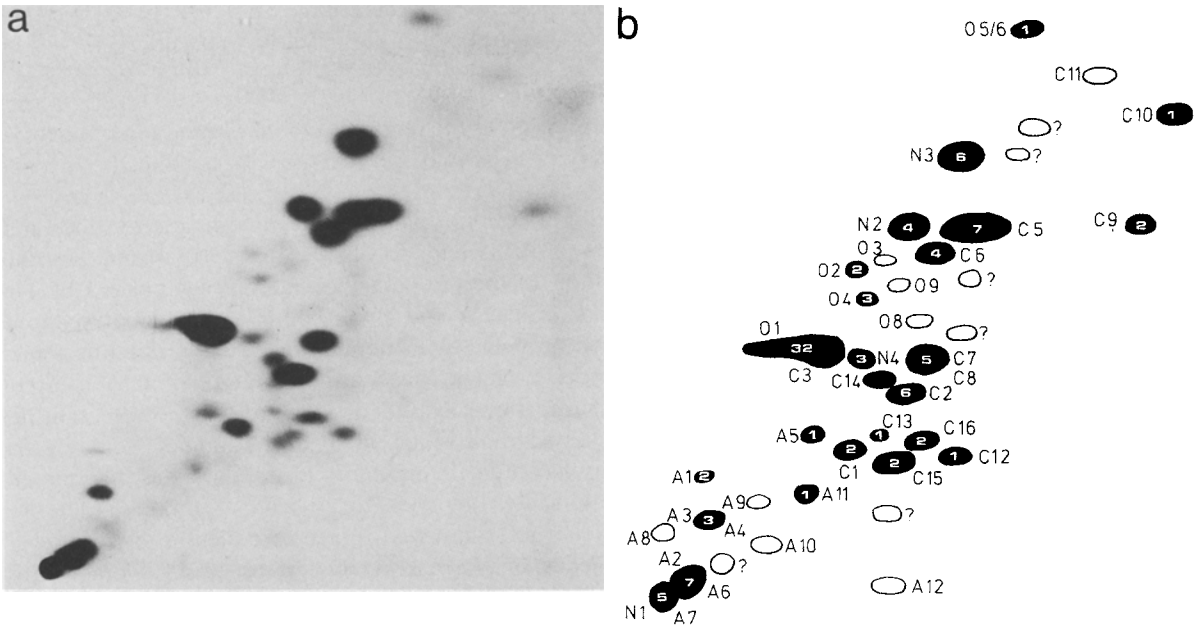
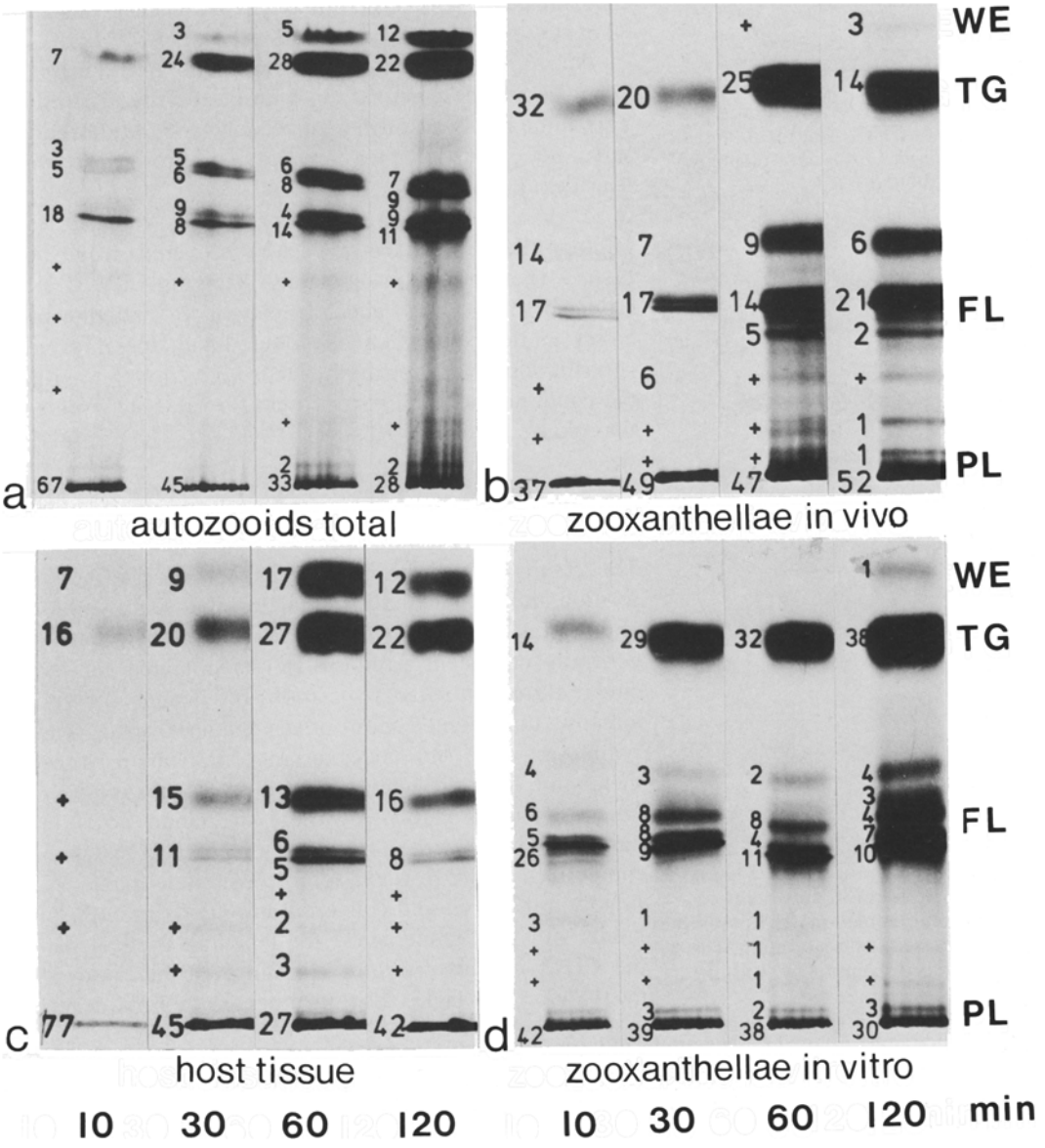


Fig. 7. *Heteroxenia fuscescens.* Autoradiograph of a two-dimensional TLC separation of EtOH/water-soluble fraction originating from isolated zooxanthellae after 120-min incubation in the light. (a) Original autoradiograph; (b) redrawn copy including identification (initials) of the labelled compounds and the amount of labelling expressed as % of total ^{14}C on the TLC plate. For abbreviations see Table 1



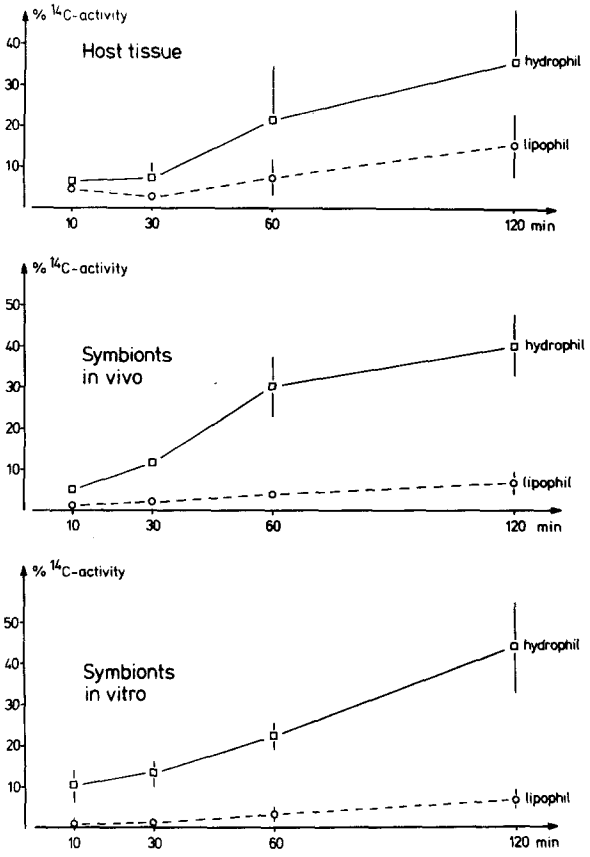


Fig. 9. *Heteroxenia fuscescens.* The portion of ^{14}C in the lipophil and hydrophil phase after deacylation of $CHCl_3$-soluble material. The extracts originated either from in *in-vivo* or *in-vitro* incubated complete autozooids or isolated zooxanthellae. $n=3$

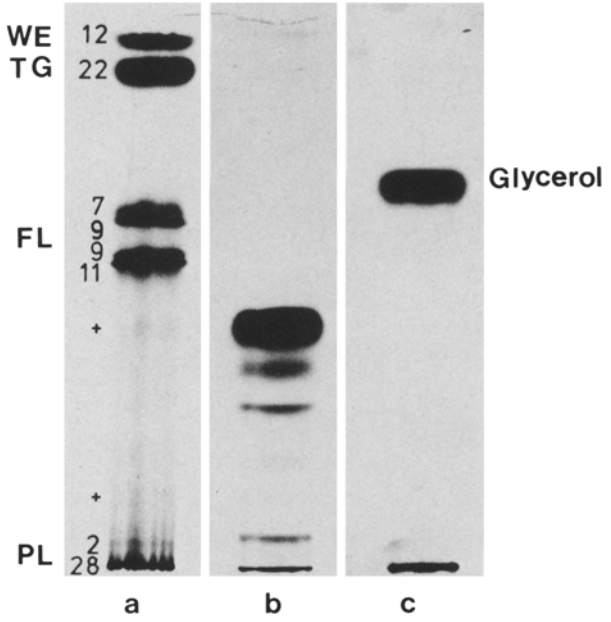


Fig. 10. *Heteroxenia fuscescens.* Autoradiographs from TLC separation of $CHCl_3$-soluble fractions before (a) and after deacylation (b, c). The separated extracts originate from autozooids being incubated for 120 min. (a) Total $CHCl_3$-soluble fraction, (b) lipophil phase after deacylation, (c) hydrophil phase after deacylation. The comparison clearly shows that the glycerol component was the strongest ^{14}C-labelled. For abbreviations see Fig. 8

pound, glycerol, was detectable (Fig. 10a–c) as revealed by TLC in n-butanol–water = 9:1 (v/v) on silicagel.

Contribution of zooxanthellae to the carbon requirement of *Heteroxenia fuscescens*

Bearing in mind that *Heteroxenia fuscescens* does not ingest particulate food, it is reasonable to assume that the nutrition of the individual is solely accomplished by the uptake of DOM and by a continuous flow of reduced carbon provided by its population of endocytobiotic algae. In order to get a rough approximation of the extent to which the translocation of algal photosynthates satisfies the daily carbon requirement of the animal (colony), the gas exchange in the light and in the dark was determined oxymetrically.

Net photosynthetic oxygen production by the zooxanthellae of *Heteroxenia fuscescens* on an average subtidal light day of 10 h amounts to 111 mg O_2 mg^{-1} d.w. d^{-1} as derived from *in-situ* measurements in 1- and 5-m water depth, respectively. The measurements were performed in April under suboptimal light conditions. During the same time, the entire colonies (animal and algal cells) consumed 83 mg O_2 mg^{-1} d.w. d^{-1}.

On a molar basis, a photosynthetic net release of 28 mg O_2 means a net surplus gain of 10.5 mg reduced C. This value needs no further correction for respiratory loss, since net yields of the daily oxygen production were considered.

As has been shown above (cf. Fig. 3), about 20% of photosynthetically fixed ^{14}C are recovered from host tissue. Therefore, approximately 2 mg mg^{-1} d.w. d^{-1} of reduced, translocated 'algal' C remain for anabolic processes in the colony. On the other hand, the metabolic contribution by the zooxanthellae is high enough to satisfy the continuous carbon requirement for catabolic events in the colony.

Discussion

The results dealt with in this paper concern the ^{14}C-fixation and the metabolic interactions between symbionts and host tissue in *Heteroxenia fuscescens.* The data are generally consistent with the results obtained for other cnidaria/zooxanthellae associations (cf. Trench, 1979). As already documented for other cnidaria/algae endosymbioses (also for alcyonarians), the main transfer metabolite from the symbionts to the host cells is glycerol. Glucose and probably a variety of amino acids may participate in the carbon translocation as well. ^{14}C-labelled compounds of EtOH/water-soluble assimilate fractions are rapidly transferred and converted into lipids in both symbionts and host tissue, since the labelling particularly of the $CHCl_3$-soluble fractions continuously increased with incubation. This fact is also documented by lipid deacyla-

◀ **Fig. 8.** *Heteroxenia fuscescens.* Autoradiographs from TLC separations of $CHCl_3$-soluble fractions of origin as indicated (separation in n-hexene). Time dependent the labelling patterns become more complex. The comparison of Fig. 8a, c with Fig. 8b and even 8d clearly demonstrates the supplementation of the host's storage pool (triglycerides, wax esters) with photosynthates. Abbreviations: TG = triglycerides; WE = wax esters; FL = further lipids, PL = phospholipids

tion, which shows that a larger amount of ^{14}C-labelled products can be determined in the water-soluble fraction upon deacylation. Especially in the light, this pathway operates rather intensively. The conversion of soluble photosynthates by the host metabolism into lipids (triglycerides and wax esters) seems to be meaningful from an energetic point of view. Lipogenesis confers upon an animal (for algae too) the advantage of storing eight times more Joules per unit weight than energy stored in the form of carbohydrates (Allen, 1976).

In his review, Trench (1979) listed the transfer ratio of various cnidaria/*Gymnodinium* spp. associations, the values varying between 20 and 58% of total ^{14}C fixed by the algae during photosynthesis. According to the data contained in Fig. 3, *H. fuscescens* must be located on the lower end of the scale: after 2-h feeding ^{14}C in the light, an average of 17% of total assimilated radiocarbon is recovered from the host tissue. This value is neither corrected for respiration nor for the loss of photosynthates to the sea, which is significantly high (Schlichter, 1982 b).

The transfer rates between symbionts and host listed by Trench (1979) and those found for *Heteroxenia fuscescens* are similar in dimension compared to the amount of photoassimilates 'voluntarily' excreted to the surrounding medium by free-living phytoplankton populations, which eventually release up to 30% of the total organic ^{14}C-incorporation (Mague *et al.*, 1980).

The rates of light-dependent ^{14}C-fixation under *in-vivo* and *in-vitro* conditions of the algal symbionts differ considerably. Taking the *in-vitro* fixation as 100%, the total *in-vivo* fixation by autozooids (host tissue plus zooxanthellae) averaged 180%, and the pure *in-vivo* fixation by zooxanthellae alone achieved 245%. All of these values are related to the same weight units and incubation periods. The data strongly suggest an enhanced fixation capacity for the symbionts under *in-vivo* conditions. The same phenomenon has already been described by Muscatine and Cernichiari (1969), Trench (1971 b), Muscatine *et al.* (1972), Kremer (1980), and Hofmann and Kremer (1981).

A higher primary productivity of symbiotic algae seems to be an inherent feature of these particularly mutualistic associations. Scott and Jitts (1977) studied the productivity of zooxanthellae in corals and free-living algae on the same reef. In fact, the fixation of the cytosymbionts was three times higher. Reasons to explain the amplified *in-vivo* fixation potentials of cytosymbionts have also been given by the authors mentioned above: the physicochemical living conditions for the algal cytosymbionts within the host cells are more suitable in comparison to the free water. Their overall higher productivity may clearly be ascribed to the stimulating effects of useless or toxic end products of the metabolism of their host organism (the primary consumer). C-, N-, P-, and S-components are likely to be utilized by the algae. In the open sea, these compounds are usually depleted to growth limiting factors.

As shown above, it is in the structural and physiological organization of unicellular algae to be leaky, i.e. these organisms continuously release photosynthates. Due to the continuous respiratory activities of their host cells, the algal endosymbionts live in a microenvironment particularly rich in CO_2. On the other hand, higher concentrations and availability of inorganic carbon is generally regarded as affecting the rates of carbon input as well. Therefore, the endosymbiotic dinoflagellates may benefit from some kind of a greenhouse CO_2-effect. In this case, the inorganic C-fixation rate calculations are even an underestimate of the actual *in-vivo* photosynthetic performance of the endosymbionts. Farming those cells within vacuoles of their own (host) cytoplasm, the host cells can easily use such energy-rich compounds. In the cytosymbiosis the primary consumer utilizes the primary producer very carefully and the transfer of energy, taking place within a single cell, is optimal. The localization of the symbionts within the host cells may stimulate the release of assimilates to a certain degree: caused by the continuous utilization of the excreted photosynthates, a steep concentration gradient is established thus driving the efflux ('vacuum cleaner effect'). By this effect, even the reactions of the photosynthesis could be stimulated, since the synthesizing steps are no longer controlled or inhibited by end products.

Besides the pure 'diffusion effect', there seems to exist a symbiosis immanent host factor, which allows the host to 'milk' the symbionts in a preserving manner. Studies by Trench (1971 b) and Muscatine *et al.* (1972) clearly showed that a host factor, not yet identified, stimulates zooxanthellae *in-vitro* to an enhanced release of assimilates. No figures in the present paper, however, allow for a further characterization of this factor.

Another reason to explain the diverging fixation capacities between *in-vivo* and *in-vitro* incubations is trivial: during the separation process and later on during incubation in sterile seawater, the potential for C-fixation could be negatively influenced. Under those conditions not only the milieu of the surrounding medium is altered, but also the illumination differs in intensity and spectral composition. Furthermore, an autotoxication of the isolated zooxanthellae by an elevated oxygen concentration could possibly occur.

A preparation effect might be expected in a common sense: In such highly mutualistic and complex associations regulating mechanisms exist which react sensitively not only to improved, but also to adverse exogenous conditions.

Muscatine and Porter (1977) have (to the authors' knowledge for the first time) calculated the total contribution of the algal symbionts to the metabolism of the hermatypic coral *Pocillopora damicornis*. A rough estimation of oxygen production by *Heteroxenia fuscescens* and a conversion of these values into terms of carbon have shown that, in fact, the complete daily carbon requirement of the animal colony can be satisfied by photosynthesis of the zooxanthellae. Moreover, a noticeable surplus gain of reduced carbon remains for anabolic processes. The amount of photoassimilates transferred to the host may be

controlled either by the actual demand of the heterotrophic partner (suction effect) or the excess production of the autotrophic partner.

The metabolic contribution of the zooxanthellae to the nutrition of the double organism *Heteroxenia fuscescens* is not the only way to supplement the carbon needs. A further pathway encompasses the absorption of DOM (Schlichter, 1982a, b). Even if the uptake of 12 amino acids along with glucose is regarded, the overall gain of organic material is sufficient to compensate for 80% of the respiratory loss. This calculation is based on free water concentrations of DOM and neglects the fact that concentrations of DOM near the surface of the colony are surely higher, and thus additionally affect the rates of uptake. The concentrations of DOM are enhanced by photoassimilates that exude from the coelenteron.

With regard to its nutrition and maintenance, *Heteroxenia fuscescens* has given up the typically animal feature of capturing prey. The alteration of its nutritional strategy (uptake of DOM, associations with endosymbiotic algae) as well as a variety of structural modifications emphasize this evolutionary trend. In *H. fuscescens* the photoautotrophic algae and the heterotrophic animal tissue are mutually integrated to a functionally autotrophic 'plant-animal', adapted to be particularly successful in an environment which is poor in particulate food, but must be shared with a variety of motile predators. Therefore the establishment of enlarged body surface to harbour dense populations of photosynthesizing algae and the thin, translucent epidermis are a direct functional analogy to a leaf, in particular of aquatic plants with their colourless epidermis and a mesophyll fully packed with photosynthesizing units.

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