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RESEARCH ARTICLE

Autotrophic carbon budget in coral tissue: a new ^{13}C-based model of photosynthate translocation

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SUMMARY

Corals live in symbiosis with dinoflagellates of the genus *Symbiodinum*. These dinoflagellates translocate a large part of the photosynthetically fixed carbon to the host, which in turn uses it for its own needs. Assessing the carbon budget in coral tissue is a central question in reef studies that still vexes ecophysiologists. The amount of carbon fixed by the symbiotic association can be determined by measuring the rate of photosynthesis, but the amount of carbon translocated by the symbionts to the host and the fate of this carbon are more difficult to assess. In the present study, we propose a novel approach to calculate the budget of autotrophic carbon in the tissue of scleractinian corals, based on a new model and measurements made with the stable isotope ^{13}C. Colonies of the scleractinian coral *Stylophora pistillata* were incubated in $H^{13}CO_3^-$-enriched seawater, after which the fate of ^{13}C was followed in the symbionts, the coral tissue and the released particulate organic carbon (i.e. mucus). Results obtained showed that after 15 min, ca. 60% of the carbon fixed was already translocated to the host, and after 48 h, this value reached 78%. However, ca. 48% of the photosynthetically fixed carbon was respired by the symbiotic association, and 28% was released as dissolved organic carbon. This is different from other coral species, where <1% of the total organic carbon released is from newly fixed carbon. Only 23% of the initially fixed carbon was retained in the symbionts and coral tissue after 48 h. Results show that our ^{13}C-based model could successfully trace the carbon flow from the symbionts to the host, and the photosynthetically acquired carbon lost from the symbiotic association.

Key words: coral, autotrophy, photosynthate translocation, ^{13}C.

INTRODUCTION

Symbiotic dinoflagellates of the genus *Symbiodinium*, commonly called zooxanthellae, live in the tissue of many scleractinian corals and are the major contributors to the primary productivity of reefs. Dinoflagellates fix inorganic carbon into organic molecules, partly use the photosynthetic products (photosynthates) for their own respiration and growth, and translocate a large fraction of their daily photosynthate production to their coral host. The latter uses it for its own needs such as growth, respiration and reproduction (Muscatine et al., 1983; Davies, 1984; Davy and Cook, 2001). A fraction of the carbon fixed is lost from the symbiotic association as dissolved and particulate organic carbon (DOC and POC, respectively) (Crossland et al., 1980), the latter being in the form of mucus. Photosynthates are a cocktail of glycerol, glucose, amino acids and lipids (Muscatine et al., 1994; Ishikura et al., 1999; Davy and Cook, 2001; Treignier et al., 2008). Because isolated dinoflagellates release less than 5% of the fixed carbon to the surrounding seawater as DOC and POC, it has been suggested that the release of photosynthates from the symbionts is induced by the host animal *via* chemical stimuli known as host release factors (Muscatine, 1967; Grant et al., 1998; Davy and Cook, 2001).

Numerous studies have investigated the budget of autotrophic carbon of tropical cnidarian–algal symbioses (Muscatine et al., 1984; Davies, 1991; McCloskey et al., 1994; Davy et al., 1996) and have suggested that symbionts living with shallow-water corals translocate more than 90% of their photosynthates to the animal host. This nutritional interaction is one of the key reasons for the success of corals and other symbiotic invertebrates in nutrient-poor tropical waters. As habitat depth increases, however, photosynthetic production decreases because of light limitation, and some coral species rely on zooplankton grazing to sustain their metabolism (Palardy et al., 2005; Palardy et al., 2008). Photosynthetic production is also severely decreased during bleaching events (loss of symbionts and/or photosynthetic pigments) following stressful conditions, which impact the ability of corals to survive (Hoegh-Guldberg, 1999). In order to predict the health of corals under future climate scenarios, it is crucial to accurately determine the amount of photosynthetically fixed carbon that is translocated from the symbionts to the host animal, the daily contribution of these photosynthates to the animal respiration, and the allocation of the remaining carbon to the various components of the symbiotic association.

Assessing the carbon budget of corals is therefore a central question in reef studies, and calculating the amount of photosynthetically fixed carbon that is translocated from the symbionts to the host coral is a problem that still vexes ecophysiologists. Indeed, it is currently assumed that a reduction in total carbon translocated to the host coincides, in general, with a

decrease in the rate of photosynthesis. However, it has been shown, mainly in sea anemones, that the amount of carbon translocated can vary with the symbiont species (Engebretson and Muller-Parker, 1999) or genotype (Loram et al., 2007), the host species (Davy et al., 1996), or the host nutritional status and symbiont density (Davy and Cook, 2001). Sachs and Wilcox (Sachs and Wilcox, 2006) even showed that *Symbiodinium* sp. in symbiosis with a jellyfish can adopt a parasitic existence. Such parasitism has also been suggested in temperate symbioses during the cold winter season (Ferrier-Pagès et al., 2011), when the coral host mostly relies on heterotrophy to sustain its metabolism. Developing an approach to construct budgets of autotrophic carbon in corals, for different species and under different environmental conditions, is therefore a key and still largely unresolved issue in coral ecophysiological research.

In symbiotic sea anemones, the fate of the photosynthetically fixed carbon can be assessed directly using the ^{14}C-bicarbonate radiotracer technique, because the organisms do not harbour a skeleton and the two partners of the symbiosis can easily be separated into two components (Bachar et al., 2007; Loram et al., 2007). This is not the case for scleractinian corals, where both the separation of tissues from the hard skeleton, which leads to radioactive projections, and the loss of respiratory ^{14}C to the atmosphere create health hazards for the investigators. The radioactive technique is also restricted to experiments with relatively short incubation times (less than 24 h). For many scleractinian corals, translocation has been estimated in the past using the 'growth rate method' and the CZAR model (contribution of zooxanthellae acquired carbon to daily animal respiration), initially described by Muscatine et al. (Muscatine et al., 1981; Muscatine et al., 1983; Muscatine et al., 1984) and modified by McCloskey et al. and Verde and McCloskey (McCloskey et al., 1994; Verde and McCloskey, 1996). Muscatine et al. (Muscatine et al., 1984) compared four methods to estimate total daily translocation: (1) a short-term *in vitro* technique, in which symbionts freshly isolated from coral tissue were incubated with ^{14}C-bicarbonate, and the release of ^{14}C in seawater was followed during 1 h of incubation; (2) a short-term *in vivo* technique, in which the whole coral colony was incubated with ^{14}C-bicarbonate, and the appearance of ^{14}C was followed in the coral tissue; (3) the 'diel' technique, in which the coral colony was incubated with ^{14}C-bicarbonate, and translocation was estimated from the difference between total fixed ^{14}C and ^{14}C remaining in the symbionts; and (4) the 'growth rate method', which assumed that translocation could be estimated from the difference between the net carbon fixed by photosynthesis and the carbon devoted to the production of new algal cells and algal respiration (Wilkerson et al., 1983). Muscatine et al. (Muscatine et al., 1984) concluded that the fourth technique gave the best results, with the additional benefit of not using radioactivity, although it still presented several shortcomings. Indeed, the fourth technique does not allow researchers to follow the pattern of photosynthate translocation, and several components of the carbon budget equations cannot be measured directly or easily, such as the duration of cytokinesis of the algal symbionts, which affects the estimation of their growth rate (Verde and McCloskey, 1996). As a practical solution, a constant value of 11 h for the duration of cytokinesis has been applied to symbionts for a variety of host species (McCloskey and Muscatine, 1984; Muscatine et al., 1984; Davy et al., 1996). Other models have subsequently been developed to estimate the budget of autotrophic energy in symbiotic associations (Davies, 1984; Davies, 1991; Edmunds and Davies, 1986; Edmunds and Davies, 1988), taking into account the rate of photosynthesis of the algal symbionts, the rates of respiration of the symbionts and the host, and the rates of growth of the animal tissue, the symbionts and the coral skeleton.

In the present paper, we develop and implement a new model based on measurements made with the 'clean' stable carbon isotope ^{13}C as an alternative to the approaches and models cited above. To the best of our knowledge, the ^{13}C-technique has been applied only once to trace the fate of photosynthetically fixed carbon in corals (Hughes et al., 2010), but without estimation of the rate of photosynthate translocation. In the present study, we combine the ^{13}C-enrichment values of the symbionts, host tissue and mucus together with measurements of photosynthesis and respiration rates in a model that quantifies the amount of photosynthate translocation and the fate of this autotrophic carbon in the tissue of the scleractinian coral *Stylophora pistillata* (Esper 1797). As our study was dedicated to autotrophic carbon, it did not consider the parallel intake of carbon by coral heterotrophy.

MATERIALS AND METHODS

Preparation of experiments

For the present study, we used eight colonies of the scleractinian coral *S. pistillata* (Pocilloporidae), originating from the Red Sea and maintained in the aquaria of the Centre Scientifique de Monaco. A total of 64 nubbins (eight nubbins per colony) were prepared by cutting the apical branches of the colonies, and each nubbin was suspended with a nylon thread on a transparent Plexiglas® bar after being identified individually according to the experimental setup detailed in Fig. 1. This system allowed us to sample nubbins from different colonies when measuring each physiological parameter. The nubbins were equally divided (i.e. four nubbins from each colony) in two tanks (20 l), and kept for 4 weeks until tissue entirely covered the skeleton. During this period, the nubbins were fed three times a week with *Artemia salina* nauplii (2000 nauplii per tank, in total). The tanks were kept under an irradiance of 250 μmol photons m^{-2} s^{-1} (12 h:12 h light:dark photoperiod) in an open flow system (renewal rate of 10 l h^{-1}) at a temperature of 25±0.5°C using heaters connected to electronic controllers. The seawater contained low levels of inorganic and organic nutrients (Ferrier-Pagès et al., 1998). After nubbin healing, feeding was stopped and the nubbins were kept under the same conditions as detailed above for 8 weeks. Several parameters were measured, as detailed below.

Rates of calcification, respiration and photosynthesis

The calcification rates of the same six nubbins (three per tank, from six different colonies; Fig. 1) were determined after 4 and 8 weeks of incubation using the buoyant weight technique (Jokiel et al., 1978; Davies, 1989). The amount of carbon used in the calcification process (C_C) was calculated according to the molar masses as follows:

$$C_C = M_{Sk}\,(12/100), \quad (1)$$

where M_{Sk} is the μg $CaCO_3$ produced and 12/100 is the ratio of the molecular masses of C (12) and $CaCO_3$ (100). All data were normalized to the skeletal surface area, which was assessed using the wax technique (Stimson and Kinzie, 1991).

Rates of respiration (R) and net photosynthesis (P_n) of 12 nubbins (six per tank, from the eight colonies; Fig. 1) were determined at 0 and 250 μmol photons m^{-2} s^{-1}, respectively, after 4 weeks of incubation using respirometry according to Hoogenboom et al. (Hoogenboom et al., 2010), except that temperature in the chambers was set to 25°C instead of 18°C. Rates of gross photosynthesis (P_g) were calculated by adding R to P_n. Samples were then frozen for determination of the chlorophyll (chl) content. For this, each sample was extracted twice during 24 h in 99% acetone. The extracts were

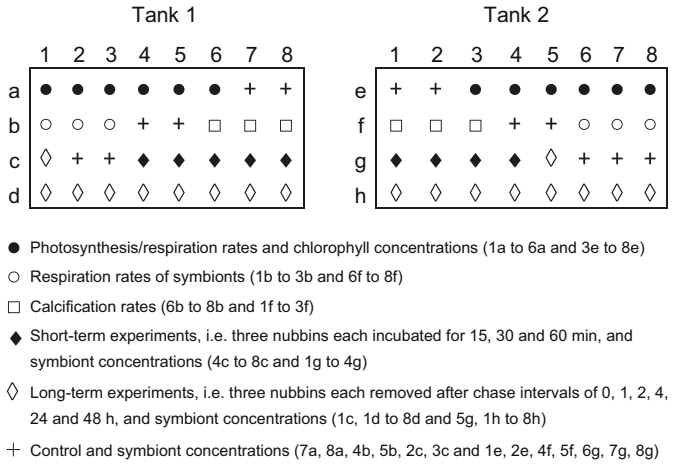

Fig. 1. Experimental setup of nubbins of the scleractinian coral *Stylophora pistillata* in two tanks. There are eight colonies, numbered from 1 to 8, and eight nubbins per colony, numbered from a to d (tank 1) and e to h (tank 2), for a total of 64 nubbins. Each nubbin was used for one type of measurement, as indicated under the figure.

centrifuged at 11,000 ***g*** for 15 min at 4°C and chl *a* and c_2 were determined according to the method of Jeffrey and Humphrey (Jeffrey and Humphrey, 1975) using a UVmc^2 spectrophotometer (Safas, Monaco).

In addition to the above measurements of total colony respiration, we also determined the respiration rates of freshly isolated symbionts from six different nubbins (three per tank, from six different colonies; Fig. 1). Symbionts were extracted in 35 ml of 0.45 µm-filtered seawater (FSW) using an air brush. The slurry was homogenized using a potter tissue grinder, and centrifuged at 850 ***g*** for 10 min to pellet the symbionts. The supernatant was discarded, and the symbionts were resuspended in FSW and counted according to Rodolfo-Metalpa et al. (Rodolfo-Metalpa et al., 2006). Respiration rates were measured using the same technique as for the colonies.

Oxygen fluxes were converted to carbon equivalents based on molar weights according to Anthony and Fabricius (Anthony and Fabricius, 2000):

$$P_C = P_g\,(12/PQ) \quad (2)$$

$$R_C = R\,(12RQ)\ , \quad (3)$$

where P_C is the amount of carbon acquired through photosynthesis; P_g is the µmol O_2 produced; 12 is the molecular mass of C; PQ is the photosynthetic quotient, equal to 1.1 mol O_2:mol C (Muscatine et al., 1981); R_C is the amount of carbon consumed by respiration; R is the µmol O_2 consumed; and RQ is the respiratory quotient, equal to 0.8 mol C:mol O_2 (Muscatine et al., 1981).

$H^{13}CO_3$ labelling experiments

Incubations and sample treatment

Corals were placed, during both short- and long-term incubations, in $H^{13}CO_3$-enriched FSW, and ^{13}C enrichment was measured at the end of the incubation in the symbionts, the coral tissue and the POC released by the colonies. For the two types of experiment, control nubbins were incubated in non-enriched FSW (i.e. without $H^{13}CO_3$ addition). The percentage ^{13}C enrichment of the incubation medium and the incubation time needed to enrich the symbionts and coral tissue was determined in preliminary experiments.

In the short-term experiments, three nubbins were incubated in the light for 15, 30 and 60 min (N=9; Fig. 1) in individual beakers filled with 200 ml of FSW and enriched with a concentration of 2 mmol l^{-1} $NaH^{13}CO_3$ (98 atom% ^{13}C, #372382, Sigma-Aldrich, St Louis, MO, USA), giving a final 50% ^{13}C enrichment of the incubation medium. For all incubations, the size of the nubbins was less than 2 cm^3 (2 ml). Hence, the incubation medium was fully sufficient in $H^{13}CO_3$ to satisfy the needs of one nubbin, as tested in previous experiments (Tambutté et al., 1995). In the present study, we calculated that corals used less than 4% of the total bicarbonate. There were also three control nubbins (one per incubation time, incubated in non-enriched FSW; 1e, 7a and 8a in Fig. 1). The beakers were left open, the medium being continuously stirred with a magnetic bar (to avoid any re-use of respired ^{13}C), and they were maintained at 25°C using a water bath. The levels of oxygen and pH were recorded at the beginning and the end of incubations. The fact that pH did not change was a good indicator that respired CO_2 did not accumulate in the beaker. At the end of the incubation, nubbins were immediately frozen at –20°C until analysis.

In the long-term experiments, beakers were prepared and maintained as described above, except that FSW was enriched with a concentration of 0.6 mmol l^{-1} $NaH^{13}CO_3$, corresponding to a final 23% ^{13}C enrichment of the incubation medium. Eighteen nubbins (Fig. 1) were incubated for 5 h in the light in the ^{13}C-enriched medium, after which they were transferred into 200 ml non-enriched FSW. Three nubbins each from three different colonies were removed from the incubation medium after chase intervals of 0, 1, 2, 4, 24 and 48 h (12 h:12 h light:dark photoperiod), and immediately frozen at –20°C, and the 200 ml of water containing the released POC was filtered onto 25 mm pre-combusted GF/F glass microfiber filters (#1825-025, Whatman, Dassel, Germany). The filters were treated with 10% HCl, rinsed with distilled water, and dried at 60°C, as in Tremblay et al. (Tremblay et al., 2011). Six control nubbins (from six different colonies; 2e, 4b, 5b, 6c, 7c and 8c in Fig. 1) were incubated for 5 h in the light and in 200 ml of non-enriched FSW, and sampled at the different chase intervals in the same way as the experimental nubbins. Four additional control nubbins (from four different colonies; 2c, 3c, 4f and 5f in Fig. 1) were incubated for 5 h in the dark and sampled after 24 h.

For ^{13}C measurements on coral nubbins, both frozen control and experimental nubbins were thawed, and tissue was detached from the skeleton using an air brush and 10 ml FSW. The slurry was homogenized using a potter tissue grinder, and a subsample (500 µl) was taken for determination of symbiont concentration (Rodolfo-Metalpa et al., 2006). The animal and symbiont fractions were then separated by centrifugation (1328 ***g*** for 5 min), and the symbionts were rinsed twice in 2.5 ml FSW to avoid animal tissue contamination (visually checked under the microscope). Samples were flash-frozen in liquid nitrogen and freeze-dried until analysis. The ^{13}C enrichment and carbon content in the animal tissue, the symbionts and the POC were determined with a mass spectrometer (Delta Plus, Thermo Fisher Scientific, Bremen, Germany) coupled *via* a type III interface with a C/N analyzer (Flash EA, Thermo Fisher Scientific), and compared with $^{13}C/^{12}C$ in the control nubbins. The efficiency of the same separation technique had previously been proven in determining isotope enrichments of the symbionts and host fractions (Grover et al., 2002; Grover et al., 2003; Grover et al., 2006), with no damage to the symbiont cell membrane that could have led to contamination of the host component. Indeed, this membrane is very tough, and cannot be easily broken without the use of a French press or a chemical agent (Wilkerson and Trench, 1985; Leggat et al., 2000; Kazandjian et al., 2008).

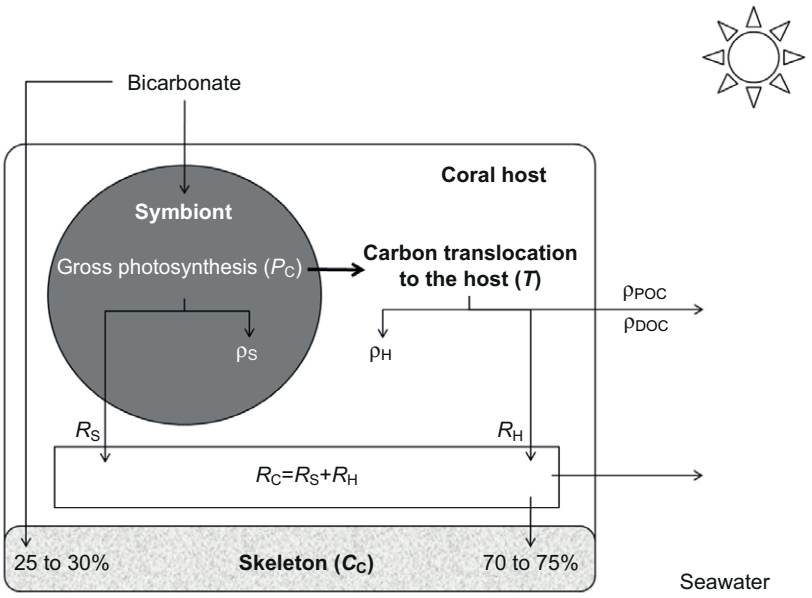

Fig. 2. Model of photosynthate translocation and carbon budget in *Stylophora pistillata*. Symbols are defined in the text, and a list of symbols and definitions is given in Table 1.

Model of carbon translocation between the host and the symbionts

Carbon incorporation rates

To quantify the carbon fluxes between the symbionts and the host (Fig. 2), we first calculated the carbon incorporation rates (ρ) in the symbionts (ρ_S), animal tissue (ρ_H) and released POC (ρ_{POC}) using the equation of Grover et al. (Grover et al., 2002; Grover et al., 2003; Grover et al., 2006) for nitrogen, adapted to corals from the equation of Dugdale and Wilkerson (Dugdale and Wilkerson, 1986):

$$\rho = \frac{\left(C_{meas} - C_{nat}\right) \times M_{sample} \times M_C}{\left(C_{inc} - C_{meas}\right) \times \left(t_{pulse} + t_{chase}\right) \times S}, \quad (4)$$

where ρ is the carbon incorporation rate ($\mu g\,C\,cm^{-2}\,h^{-1}$) into the symbionts ($\rho_S$), animal tissue ($\rho_H$) or released POC ($\rho_{POC}$); C_{meas} and C_{nat} are the percentages of ^{13}C measured on the samples (symbionts, host tissue or POC) from ^{13}C-enriched experiments and controls, respectively; C_{inc} is the percent ^{13}C enrichment of the incubation medium; M_{sample} is the mass of the freeze-dried sample (mg); M_C is the mass of carbon per milligram of symbiont or host tissue ($\mu g\,mg^{-1}$) or released POC (μg); S is the nubbin surface area (cm^2); and t_{pulse} and t_{chase} are the incubation times (h) of the nubbins in the light in the enriched and non-enriched incubation media, respectively. C_{inc} varied during the pulse-chase, i.e. before and after the period of chase, and was calculated as:

$$C_{inc} = \frac{\left(C_{pulse} \times t_{pulse}\right) + \left(C_{chase} \times t_{chase}\right)}{\left(t_{pulse} + t_{chase}\right)}, \quad (5)$$

where C_{pulse} and C_{chase} are the percent ^{13}C enrichment of the enriched and non-enriched incubation media, respectively (C_{chase}=1.1%). See Table 1 for a list of symbols and their definitions.

Percentage of fixed carbon remaining in symbionts, host tissue and POC

The percentage of fixed carbon remaining (C_R) in symbionts, host tissue and POC represents the amount of carbon fixed by photosynthesis that had been incorporated into the body masses of symbionts and the host or lost as POC. It is obtained by dividing the carbon incorporation rate of symbionts, animal or POC (ρ_S, ρ_H or ρ_{POC}, respectively) by the rate of gross photosynthesis (P_C, which represents the total amount of fixed carbon), and multiplying the result by 100:

$$C_R = (\rho_S \text{ or } \rho_H \text{ or } \rho_{POC} / P_C) \times 100. \quad (6)$$

Autotrophic carbon budget

The carbon acquired through photosynthesis (P_C) may have different fates (Fig. 2), i.e. some of that carbon is respired by the coral assemblage (R_C), and other fractions are incorporated in the biomass of symbionts (ρ_S) and host (ρ_H) and lost to the surrounding water as POC (ρ_{POC}) and DOC (ρ_{DOC}). The carbon budget equation is:

$$C_R = R_C + \rho_S + \rho_H + \rho_{POC} + \rho_{DOC}. \quad (7)$$

R_C includes two components, i.e. respiration by the symbionts (R_S) and by the host (R_H); hence, $R_S+R_H=R_C$.

The above budget equation does not consider the carbon incorporated in the skeleton by calcification because it has been shown that 25–30% of this carbon comes directly from the external medium as dissolved inorganic carbon and 70–75% comes from internal respiration R_C (Erez, 1978; Furla et al., 2000); the first component is external to the equation, and the second component has already been taken into account in the equation.

Amount of carbon lost as respiration and DOC

It follows from Eqn 7 that the amount of carbon lost as combined R_C and ρ_{DOC} (C_L) is:

$$C_L = R_C + \rho_{DOC} = P_C - \rho_S - \rho_H - \rho_{POC}, \quad (8)$$

where all parameters are expressed in $\mu g\,C\,cm^{-2}\,h^{-1}$. The percentage of carbon lost is obtained by dividing C_L by P_C, and multiplying the result by 100.

Amount of photosynthesized carbon translocated to the host

There are two ways to calculate the amount of carbon translocated by symbionts to the host (T_S and T_H; T_S=T_H). In the first calculation, T_S corresponds to the total amount of carbon gained by

Table 1. List of symbols, definition and units

Symbol	Definition
C	Carbon
C_C	C used by calcification ($\mu g\,C\,cm^{-2}\,h^{-1}$)
C_{chase}	^{13}C enrichment of the non-enriched incubation medium (equal to 1.1%)
C_{inc}	^{13}C enrichment of the incubation medium (%)
C_L	Amount of C lost ($\mu g\,C\,cm^{-2}\,h^{-1}$ or %)
C_{meas}	^{13}C measured in the sample (%)
C_{nat}	Natural abundance in ^{13}C in control nubbins (%)
C_{pulse}	^{13}C enrichment of the enriched incubation medium (%)
C_R	Percentage of fixed C remaining in symbionts, host tissue and POC (%)
M_C	Mass of C per milligram of tissue or symbionts ($\mu g\,mg^{-1}$) or released mucus (μg)
M_{sample}	Mass of the freeze-dried sample (mg)
M_{Sk}	$CaCO_3$ produced by calcification ($\mu g\,CaCO_3\,cm^{-2}\,h^{-1}$)
P_C	Gross C fixed photosynthetically by symbionts ($\mu g\,C\,cm^{-2}\,h^{-1}$)
P_g	Oxygen produced by gross photosynthesis ($\mu mol\,O_2\,cm^{-2}\,h^{-1}$)
P_n	Oxygen produced by net photosynthesis ($\mu mol\,O_2\,cm^{-2}\,h^{-1}$)
PQ	Photosynthetic quotient (equal to 1.1 mol O_2:mol C)
R	Oxygen consumed by respiration of holobiont ($\mu mol\,O_2\,cm^{-2}\,h^{-1}$)
R_C	C respired by holobiont ($\mu g\,C\,cm^{-2}\,h^{-1}$)
R_H	C respired by coral host ($\mu g\,C\,cm^{-2}\,h^{-1}$)
RQ	Respiratory quotient (equal to 0.8 mol C:mol O_2)
R_S	C respired by symbionts ($\mu g\,C\,cm^{-2}\,h^{-1}$)
S	Nubbin surface area (cm^2)
T_H	Amount of C translocated, calculated from the host C rate ($\mu g\,C\,cm^{-2}\,h^{-1}$ or %)
T'_H	Sum of ρ_H, R_H and ρ_{POC} ($\mu g\,C\,cm^{-2}\,h^{-1}$ or %)
T_S	Amount of C translocated, calculated from the symbiont C rate ($\mu g\,C\,cm^{-2}\,h^{-1}$ or %)
t_{chase}	Incubation time of the nubbins in the non-enriched incubation medium in the light (h)
t_{pulse}	Incubation time of the nubbins in the enriched incubation medium (h)
ρ_{DOC}	C incorporation rate in release dissolved organic carbon (not measured)
ρ_H	C incorporation rate in coral tissue ($\mu g\,C\,cm^{-2}\,h^{-1}$)
ρ_{POC}	C incorporation rate in released particulate organic carbon ($\mu g\,C\,cm^{-2}\,h^{-1}$)
ρ_S	C incorporation rate in symbionts ($\mu g\,C\,cm^{-2}\,h^{-1}$)

photosynthesis (P_C) minus the sum of the carbon retained in symbionts (ρ_S) and respired by them (R_S):

$$T_S = P_C - \rho_S - R_S, \quad (9)$$

where all parameters are expressed in $\mu g\,C\,cm^{-2}\,h^{-1}$.

In the second calculation, T_H corresponds to the organic carbon fixed by the host (ρ_H) plus the carbon lost by the host during the incubation as respiration (R_H), POC (ρ_{POC}) and DOC (ρ_{DOC}), because this lost carbon originated from symbiont translocation:

$$T_H = \rho_H + R_H + \rho_{POC} + \rho_{DOC}, \quad (10)$$

where R_H is the amount of carbon lost by the host respiration ($\mu g\,C\,cm^{-2}\,h^{-1}$), which is obtained by subtracting R_S from R_C.

In the present study, ρ_{DOC} was not measured, and we calculated T'_H from measured ρ_H, R_H and ρ_{POC}:

$$T'_H = \rho_H + R_H + \rho_{POC}. \quad (11)$$

The difference between T_S (=T_H) and T'_H provided an estimate of ρ_{DOC}:

$$\rho_{DOC} = T_S - T'_H = P_C - R_C - \rho_S - \rho_H - \rho_{POC}. \quad (12)$$

The percentage of translocated carbon was obtained by dividing T_S, T_H or T'_H by P_C, and multiplying the result by 100.

Statistical analysis

The characteristics of coral colonies and calculations from equations are reported as means ± s.e.m. As two tanks were used to maintain the coral nubbins, the 'tank effect' was tested using a *t*-test on the incorporation rates pooled for the different sampling times and fractions. The *t*-test showed that there was no 'tank effect' (short-term incubation, t=0.10, d.f.=16, P=0.9209; long-term incubation, t=0.99, d.f.=34, P=0.3281). Moreover, only three nubbins from three different colonies were used at each interval during the pulse-chase experiment. Because carbon incorporation rates can significantly vary among colonies, we tested whether changes in incorporation rates over time were indeed due to time, and not to differences among colonies. If colonies had been very different in their incorporation rates, not using all colonies at all chase intervals would have indeed resulted in erratic values for the mean and variance of incorporation rates between times, depending on which colony was included. In our case, variance was not different between chase intervals in either the symbionts or the coral tissue (Bartlett's test, d.f.=5, K^2=5.6, P=0.34 for symbionts, and K^2=10, P=0.075 for corals). Despite high variance, mean values were different between chase intervals (ANOVA, F=13.5, P=0.002 for symbionts, and F=6.16, P=0.02 for corals) and these differences were systematic, not erratic. For the symbionts, pairwise tests showed that concentrations at 48 h were significantly different from those at most of the other chase intervals (P<0.016), concentrations at 24 h were significantly different from those at the previous chase intervals (P<0.05), and so on. When looking at the results by colony, the trend (initial increase followed by a slow decrease) was also similar for all colonies. Therefore, the temporal trend observed was a strong and real effect.

The effects of fractions (symbionts and coral tissue) and time on carbon incorporation rates were tested using factorial ANOVA. Differences in the amount of carbon lost as combined respiration and DOC calculated with Eqn 8 (C_L) and total respiration rates of the nubbins (R_C) were tested using a *t*-test with 19 degrees of freedom (d.f.=n_{CL}+n_{RC}–2=9+12–2) for both short- and long-term experiments. Differences in the amount of carbon that symbionts

had translocated to the host obtained with Eqn 9 (T_S) and 11 (T'_H) were tested using a paired *t*-test with eight degrees of freedom (d.f.=*n*–1=9–1) for both short- and long-term experiments. Data were checked for normality using a Kolmogorov–Smirnov test with Lilliefors correction. Differences between factors were considered significant for *P*-values <0.05. Statistics were performed using SYSTAT 13 (Systat Software, Chicago, IL, USA).

RESULTS

Characteristics of the corals used in this experiment are given in Table 2. After the incubations, the corals were significantly enriched in ^{13}C compared with control corals incubated either in the light or the dark (atom% ^{13}C enrichment ranged between 0.25 and 0.18% in the host tissue, and between 1.25 and 0.45% in symbionts from 0 to 48 h in the long-term incubation). In the following text we generally provide values of carbon fixed, translocated and lost in terms of percentage, whereas Figs 3–5 show values in µg carbon $cm^{-2} h^{-1}$ (summarized in Fig. 5, values after 48 h of chase incubations).

In the short-term incubations, carbon incorporation rates in symbionts and coral host tissue ranged from 2.5 to 4.1 µg C $cm^{-2} h^{-1}$ (Fig. 3A), and although they seemed to be slightly higher in the symbionts than in the host tissue, the rates were not significantly different in the two fractions (Table 3). Similarly, the incubation time did not have a significant effect on carbon incorporation rates per hour, which remained equivalent (Table 3). The percentage of fixed carbon remaining in symbionts and host tissue was equal to ca. 25 and 20%, respectively (Fig. 3A). This result indicates that the symbiotic association lost ca. 55% of the carbon fixed by symbionts (Fig. 3B), according to Eqn 8 (C_L). The C_L value was not significantly different (*t*-test, *t*=1.18, d.f.=19, *P*=0.2527) from the total respiration rates of the nubbins (R_C) measured with the classical respirometric method (Table 2), indicating that most of the carbon lost in short-term incubations was due to respiration and not DOC release. Translocation of carbon from symbionts to host, calculated according to Eqns 9 (T_S) and 11 (T'_H), is shown in Fig. 3C. There was no significant difference between the two ways of calculating translocation (paired *t*-test, *t*=2.26, d.f.=8, *P*=0.0538), again indicating that, in the short term, carbon losses in the form of DOC did not account for a significant amount of the translocated carbon. After 15 to 60 min of incubation, ca. 60% of the fixed carbon had been translocated from symbionts to the host (Fig. 3C).

Concerning the long-term incubations, carbon incorporation rates were also not significantly different between symbionts and host tissue (Table 3, Fig. 4A). The amount of carbon retained in symbionts rapidly decreased between 1 and 4 h of pulse-chase incubation, and

Table 2. Characteristics of the coral colonies used in the experiments

Parameter	*N*	Mean ± s.e.m.
Symbiont concentration (10^6 cells cm^{-2})	31	1.40±0.07
Chlorophyll concentration (µg chl $a + c_2$ cm^{-2})	12	2.56±0.44
Growth rate (µg $CaCO_3$ $cm^{-2} h^{-1}$)	6	59.52±2.17
Growth rate (C_C; µg C $cm^{-2} h^{-1}$)	6	7.14±0.26
Gross photosynthesis (P_g; µmol O_2 $cm^{-2} h^{-1}$)	12	1.39±0.14
Gross photosynthesis (P_C; µg C $cm^{-2} h^{-1}$)	12	15.15±1.51
Respiration of holobiont (R; µmol O_2 $cm^{-2} h^{-1}$)	12	0.76±0.07
Respiration of holobiont (R_C; µg C $cm^{-2} h^{-1}$)	12	7.32±0.67
Respiration of symbionts (10^{-7} µmol O_2 $cell^{-1} h^{-1}$)	6	1.57±0.24
Respiration of symbionts (10^{-7} µg C $cell^{-1} h^{-1}$)	6	15.09±2.26
Respiration of symbionts (R_S; µg C $cm^{-2} h^{-1}$)	6	2.13±0.12
Respiration of coral host (R_H; µg C $cm^{-2} h^{-1}$)	12	5.19±0.67

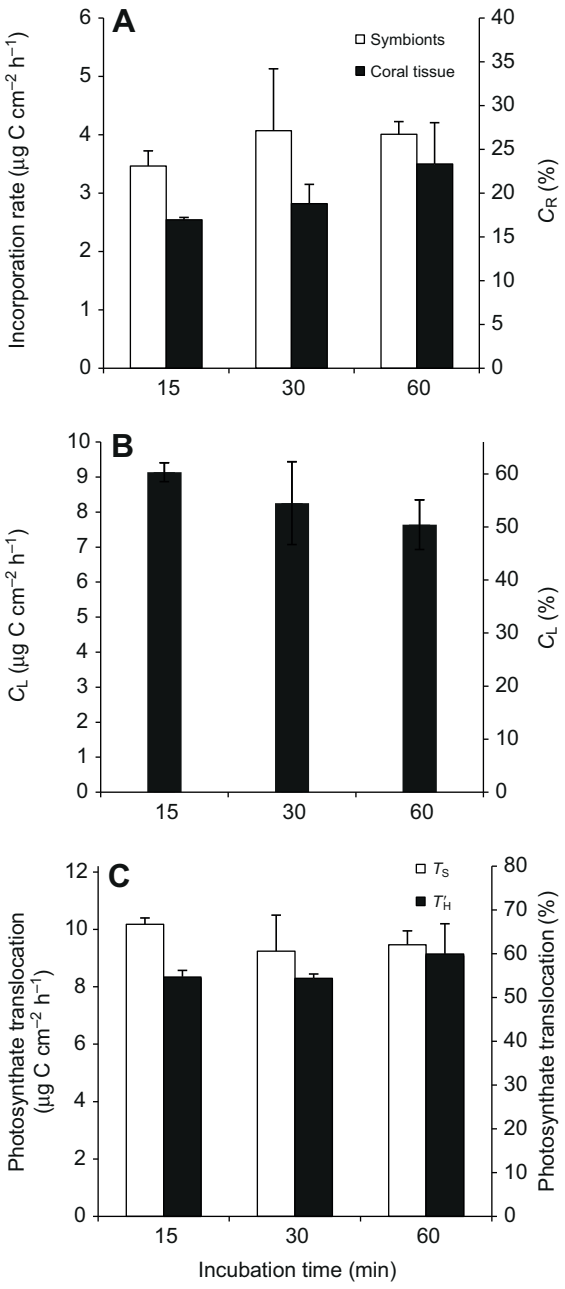

Fig. 3. Short-term incubations. (A) Carbon incorporation rates (ρ) and percentage of fixed carbon (C_R) in symbionts and coral tissue, (B) amount of carbon lost as combined respiration and dissolved organic carbon (C_L), and (C) amount of photosynthesized carbon translocated to the host by symbionts, calculated with Eqns 9 (T_S) and 11 (T_H). Data are means ± s.e.m. of triplicate measurements.

carbon retained in the host fraction increased during the same period (Fig. 4A). From 4 to 48 h, there was a decrease in the carbon retained by the symbiotic association, corresponding to losses of ^{13}C as respiration and DOC release (Fig. 4A). Incorporation rates into POC (i.e. loss of ^{13}C in the mucus) were negligible (<0.2% of the total carbon taken up; Fig. 4B).

At the beginning of the long-term incubations, the percentage of fixed carbon that remained in the symbionts and host fractions was 22 and 19%, respectively (Fig. 4A). After 48 h, this percentage decreased to 9% in symbionts and 14% in host tissue (Fig. 4A), and

Table 3. Results of factorial ANOVA testing the effect of time and coral fraction (symbionts and coral tissue) on the incorporation rates of carbon per hour in short- and long-term experiments

Factor	d.f.	*P*	*F*
Short-term experiments			
Fraction	1	0.0714	3.91
Time	2	0.4233	0.92
Fraction×Time	2	0.8012	0.23
Error	12	–	–
Long-term experiments			
Fraction (without POC)	1	0.7754	0.08
Time	5	0.0022	5.20
Fraction×Time	5	0.0751	2.32
Error	24	–	–

fixed carbon released as POC decreased from <0.2 to <0.1% (Fig. 4B). The carbon loss calculated with Eqn 8 (C_L) was comparable to the total respiration rates of the nubbins (R_C) for the first 2 h of pulse-chase (*t*-test, t=0.92, d.f.=19, P=0.3698), and was equal to 50 to 60% of the fixed carbon (Fig. 4C), which was equivalent to the results obtained in the short-term incubations. After 48 h, the carbon loss calculated with Eqn 8 increased to 77% of the fixed carbon, and significantly differed from the respiration rates of the nubbins (R_C) between 4 and 48 h (*t*-test, t=3.04, d.f.=19, P=0.0068).

In terms of carbon translocation (Fig. 4D), results obtained with Eqns 9 and 11 were comparable during the first 2 h of pulse-chase (paired *t*-test, t=1.82, d.f.=8, P=0.1065), giving a translocation of 55–65% of the carbon fixed by photosynthesis, which was equivalent to the rates obtained in the short-term incubations. After this period, the translocation rate computed with Eqn 9 (T_S) increased up to 78% of the fixed carbon, whereas it slightly decreased using Eqn 11 (T'_H) (paired *t*-test, t=4.97, d.f.=8, P=0.0011), the difference between the two equations being 28% of the fixed carbon after 48 h.

DISCUSSION

We described above a new model to estimate carbon fluxes within the dinoflagellate–coral symbiosis, and especially the percent carbon translocation from symbionts to host. The latter value has generally eluded estimation in previous work, where researchers have generally used the value of 90% translocation proposed by Muscatine and Cernichiari (Muscatine and Cernichiari, 1969) and Muscatine et al. (Muscatine et al., 1981).

Using our model, we were able to assess the rates of photosynthate translocation on time scales ranging from a few minutes to several days. The rates estimated for the first 2 h were comparable for the short- and long-term incubations, showing that ca. 60% of the carbon fixed by symbionts was rapidly (within 15 min) translocated to the coral host, *S. pistillata*. This is consistent with previous work that has indicated a rapid translocation of photosynthetic carbon products (Battey and Patton, 1984; Hughes et al., 2010). Our value is very close to previous estimates (from 60 to 65%) reported for the same species after a few hours of incubation with ^{14}C-bicarbonate (Muscatine et al., 1984; Gattuso et al., 1993). Using long-term incubations with ^{13}C-bicarbonate, we were able to follow the kinetics of carbon translocation and showed that the rates (T_S, calculated with Eqn 9) increased from 60% after 15 min (Fig. 3C) to 69 and 78% after 24 and 48 h, respectively (Fig. 4D). This high translocation rate is slightly lower, but in general agreement, with previous estimates based on the growth rate method, which showed translocation >90% (Davies, 1984; Falkowski et al., 1984; Muscatine et al., 1984; Edmunds and Davies, 1986). Our translocation values of 60 to 78% indicate that using a fixed value of 90%, as often done until now (see above), may lead to significant errors in the calculations of other rates. The translocation rate measured in the present study corresponds to 144 µg C cm^{-2} d^{-1}, which is similar to the previous estimate of 122 µg C cm^{-2} d^{-1} obtained by Muscatine et al. (Muscatine et al.,

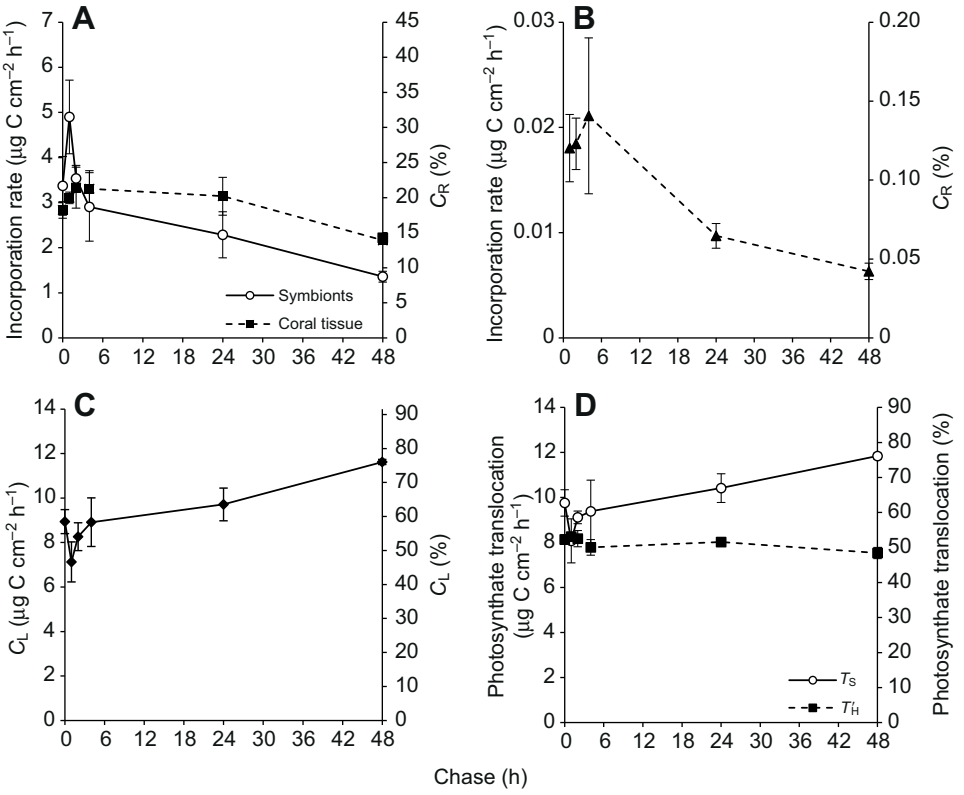

Fig. 4. Long-term incubations. Carbon incorporation rates (ρ) and percentage of fixed carbon (C_R) as a function of chase time in (A) symbionts and coral tissue, and (B) released particulate organic carbon (POC). (C) Amount of carbon lost as combined respiration and dissolved organic carbon (C_L). (D) Amount of photosynthesized carbon translocated to the host by symbionts, calculated with Eqns 9 (T_S) and 11 (T'_H). Data are means ± s.e.m. of triplicate measurements.

1984) using the growth rate method for the same species kept under high light. This shows the validity of our method for assessing photosynthate translocation.

The two equations we used to calculate the rates of translocation (i.e. Eqns 9 and 11) gave similar results during the first 2 h, with a progressively widening difference during the following hours (Fig. 4D), ending at 78 and 50% translocation after 48 h calculated with Eqns 9 and 11, respectively. This increasing difference is consistent with the results of Muscatine et al. (Muscatine et al., 1984), i.e. 40% radioactivity recovered in the animal fraction in the short term *versus* >90% translocation calculated with the growth rate method in the long term. We propose that this difference is mainly due to losses in the form of DOC (ρ_{DOC}). The difference was negligible in the short term, but increased with the length of pulse-chase incubation. We explained above (following Eqn 7) why the loss of ^{13}C to skeleton during the calcification process could not explain the difference between translocation calculated with Eqns 9 and 11. We did not measure the ^{13}C enrichment of the skeleton because of the low amount of $CaCO_3$ deposited during the incubation (a few nanograms only deposited during 5 h of incubation in the enriched medium), but our view is that the ^{13}C losses to calcification were accounted for by respiration, considering that almost 70 to 75% of the carbon used for calcification comes from internal respiration and the remaining 25 to 30% comes from external seawater carbon (Erez, 1978; Furla et al., 2000; Hughes et al., 2010). Indeed, given the calcification rate of 7.1 μg C cm^{-2} h^{-1} (Table 2) and considering that 70 to 75% of the skeleton carbon came from respiration, the product of the two numbers (result: 5.0 to 5.4 μg C cm^{-2} h^{-1}) corresponds to the host respiration calculated as the difference between respiration of the total symbiotic association and respiration of the freshly isolated symbionts (result: 5.2 μg C cm^{-2} h^{-1}; Table 2). The fact that the carbon from host respiration accounts for the carbon used for calcification is consistent with our view that there is no need to include in our equations a separate term for ^{13}C allocation to the skeleton. Hence, the carbon losses corresponding to the difference between Eqns 9 and 11 are essentially due to DOC release (ρ_{DOC}, Eqn 12).

It has long been known that the loss of carbon as DOC and POC is an important process in scleractinian corals (Crossland et al., 1980; Crossland, 1987; Ferrier-Pagès et al., 1998; Tanaka et al., 2009). The released organic matter (DOC and POC) functions as an energy carrier in reef ecosystems (Wild et al., 2004), as it is used by bacteria or ingested by organisms such as fish (Ferrier-Pagès et al., 2000). It often corresponds to an excess of photosynthetic carbon that cannot be used for new cell formation by the symbiotic association because of a lack of enough phosphorus and/or nitrogen (Dubinsky and Berman-Frank, 2001). Our results indicate that although POC release is low, DOC excretion was ca. 11, 16 and 28% after 4, 24 and 48 h, respectively, or 4.3 μg C cm^{-2} h^{-1} after 48 h. These values are in the range of estimates of total DOC release by some other scleractinian corals (Tanaka et al., 2009; Tanaka et al., 2010; Naumann et al., 2010; Wild et al., 2010), and within the range of 6–40% of total fixed carbon measured after a few hours (Muscatine et al., 1984) and up to 24 h (Crossland et al., 1980; Davies, 1984; Davies, 1991; Edmunds and Davies, 1986). Our results also indicate that in the branching species *S. pistillata*, a high amount of newly fixed carbon can be rapidly transferred (within 2 days) to the surrounding medium. Muscatine et al. (Muscatine et al., 1984), who worked on the same species using ^{14}C, came the same conclusion, i.e. depending on the light level, between 6 and 50% of the newly fixed carbon was lost within 24 h as DOC. This shows the validity of our method for assessing the release of carbon. However, opposite results were reported by Tanaka et al. (Tanaka et al., 2008) for other coral species such as the massive *Porites cylindrica* and the tabular *Acropora pulchra*, which released less than 1% of newly fixed carbon as DOC. Tanaka et al. (Tanaka et al., 2008) concluded that most of the released DOC was derived from old-synthesized organic carbon. This difference may reflect species-specific differences in tissue biomass or mucocyte density (Tanaka et al., 2008), metabolic functioning, nutrient acquisition or DOC origin.

We combined all the results obtained with our model to construct a mass-balance carbon budget for the tissue of *S. pistillata* (Fig. 5). This budget shows that after 48 h, 78% of the photosynthetically derived carbon was translocated to the host and 14% was respired by the symbionts, leaving 9% (or 16 μg C cm^{-2} d^{-1}) in the symbionts. Among the translocated carbon, ca. 64% was lost as respiration and DOC release, leaving 14% (or 26 μg C cm^{-2} d^{-1}) in the coral tissue. According to the carbon content of the symbionts and host tissue measured in this study (309±59 and 1402±270 μg C cm^{-2}, respectively), the coral would need at least 54 days to build 1 cm^2

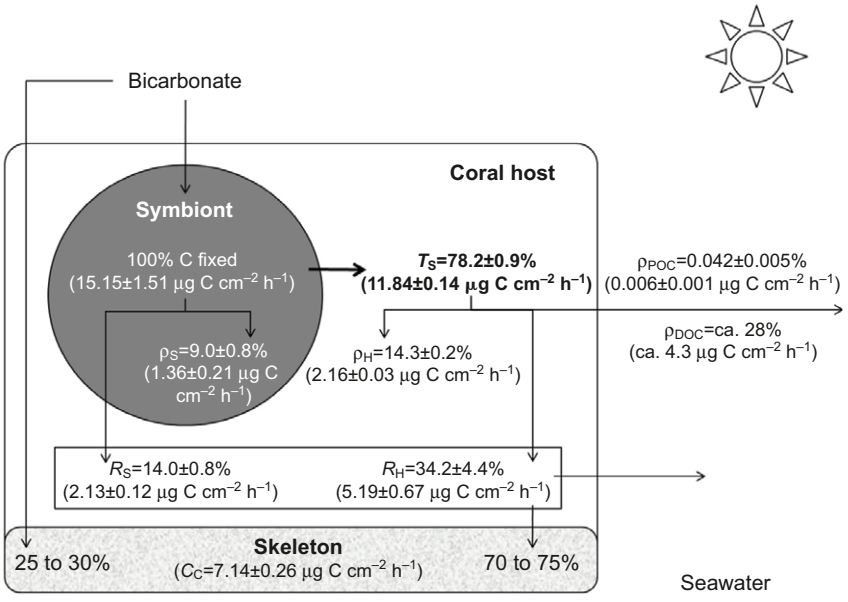

Fig. 5. Mass-balanced results of photosynthate translocation and carbon budget in *Stylophora pistillata* based on ^{13}C experiments after 48 h. Symbols are defined in the text, and a list of symbols and definition is given in Table 1. Data are means ± s.e.m. of triplicate measurements.

of tissue under our culture conditions. As previously observed for sea anemones and recently for corals (Dubinsky et al., 1984; Falkowski et al., 1993; Bachar et al., 2007; Hughes et al., 2010), a large fraction of the fixed carbon was respired shortly after its production (48% in the present study), showing the preferential use by the coral of autotrophic carbon for respiration as opposed to longer-term storage. The translocated organic compounds have been called 'junk food' by Falkowski et al. (Falkowski et al., 1984) because little of their carbon is incorporated into new biomass. Our percent value of respiration (48%) is in agreement with the previous estimates for *Pocillopora eydouxi* (51%) (Davies, 1984) and *Porites porites* (48%) (Edmunds and Davies, 1986). This establishes the validity of our method for assessing respiration.

Because the carbon budgets of different scleractinian corals may be markedly different under different conditions, researchers must assess coral ecophysiological processes on a large number of species and under a wide range of conditions. Hence, coral ecophysiologists need a general approach that can provide rates rapidly, precisely and safely for the investigators. Our approach, which combines ^{13}C measurements and a specifically developed model, can be used to investigate a wide variety of species and situations. For example, research on sea anemones has shown that different symbiont species or genotypes could have major effects on carbon translocation, which may also happen in scleractinian corals. Indeed, Engebretson and Muller-Parker (Engebretson and Muller-Parker, 1999) have shown that, in sea anemones, zoochlorella translocated less carbon than *Symbiodinium*, and Loram et al. (Loram et al., 2007) have made the same observation on two different genotypes of *Symbiodinium*. Also, Davy et al. (Davy et al., 1996) observed that the percent translocation depended on the host species, as it was higher in a zoanthid than in two sea anemones when the three species were maintained under the same environmental conditions. Finally, translocation has been shown to depend on the concentration of symbionts in the host tissue, which is lower in unfed individuals. Davy and Cook showed that translocation was higher for symbionts in symbiosis with unfed sea anemones than with fed sea anemones (Davy and Cook, 2001). Another example, this time about the effect of environmental conditions, is the major environmental difference experienced by tropical and temperate corals, the first living in deep, stable environments, and the second being subjected to low temperature and low irradiance in winter. There is debate about whether symbionts, in winter, provide the host with photosynthates or become parasitic. The construction of carbon budgets for tropical and temperate corals in different seasons is a tool for assessing the importance of autotrophy *versus* heterotrophy in corals.

Our laboratory study validated our approach by showing that our model produced rates that were similar to those obtained using other, well-established methods for the same coral species and under comparable experimental conditions. We have shown this to be true for the rates of photosynthate translocation, DOC release and respiration. Our study also showed that our new approach had the following advantages over other methods: the use of safe ^{13}C instead of risky ^{14}C, and the possibility of conducting both short- and long-term incubations using the same technique.

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