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RESEARCH ARTICLE

Trophic dynamics of scleractinian corals: stable isotope evidence

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ABSTRACT

Reef-building corals form symbioses with dinoflagellates from the diverse genus *Symbiodinium*. This symbiotic association has developed adaptations to acquire and share nutrients, which are essential for its survival and growth in nutrient-poor tropical waters. The host is thus able to prey on a wide range of organic food sources (heterotrophic nutrition) whereas the symbionts acquire most of the inorganic nutrients (autotrophic nutrition). However, nutrient fluxes between the two partners remain unclear, especially concerning heterotrophically acquired carbon and nitrogen. We combined physiological measurements and pulse-chase isotopic labeling of heterotrophic carbon and nitrogen, as well as autotrophic carbon to track nutrient fluxes in two coral species, *Stylophora pistillata* and *Turbinaria reniformis*, in symbiosis with *Symbiodinium* clades A, and C, D respectively. We showed a rapid acquisition, exchange and a long-term retention of heterotrophic nutrients within the symbiosis, whereas autotrophic nutrients were rapidly used to meet immediate metabolic needs. In addition, there was a higher retention of heterotrophic nitrogen compared with carbon, in agreement with the idea that tropical corals are nitrogen-limited. Finally, a coupling between auto- and heterotrophy was observed in the species *S. pistillata*, with a higher acquisition and retention of heterotrophic nutrients under low irradiance to compensate for a 50% reduction in autotrophic nutrient acquisition and translocation. Conversely, *T. reniformis* conserved an equivalent heterotrophic nutrient acquisition at both light levels because this coral species did not significantly reduce its rates of gross photosynthesis and autotrophic carbon acquisition between the two irradiances. These experiments advance the current understanding of the nutrient exchanges between the two partners of a symbiotic association, providing evidence of the complexity of the host–symbiont relationship.

KEY WORDS: Autotrophy, Carbon, Heterotrophy, Irradiance, Isotopic labeling, Nitrogen, *Stylophora pistillata*, *Symbiodinium*, Symbiosis, *Turbinaria reniformis*

INTRODUCTION

Nitrogen is an essential structural and functional component of all living organisms (Lehninger et al., 1993). It is one of the building blocks of amino acids and proteins, which are the main components of the tissues, enzymes and hormones essential for cell metabolism. Scleractinian symbiotic corals, which are the main reef builders in the oceans, thrive in oligotrophic tropical waters where nutrients, and nitrogen sources in particular, are scarce (D'Elia and Wiebe, 1990; Szmant, 1997). Corals have thus developed adaptations to this poor environment, by living in symbiosis with dinoflagellates of the genus *Symbiodinium* sp. On one hand, symbionts translocate to their host most of their carbon-rich photosynthetic products (photosynthates), which are mainly used by the host for respiration (Falkowski et al., 1984; Tremblay et al., 2012). On the other hand, symbionts enable corals to take up dissolved inorganic nitrogen and phosphorus from seawater (Muscatine and D'Elia, 1978; Grover et al., 2002; Godinot et al., 2009), in addition to recycling the nitrogenous waste products of the coral host (Cook, 1983; Smith and Douglas, 1987). Corals are also animals, and can acquire nutrients through the capture of a whole range of prey (Sebens et al., 1996; Houlbrèque et al., 2004; Lai et al., 2013; Leal et al., 2014).

In tropical reefs, concentrations of inorganic nutrients are usually in the nanomolar range (D'Elia and Wiebe, 1990; Davis and Jones, 1997; Shyka and Sebens, 2000) and coral symbionts cannot always exploit them (D'Elia and Webb, 1977; Shyka, 2000). Prey capture by the host (i.e. planktivory or heterotrophy) can thus be an important source of nitrogen (Bythell, 1988; Anthony, 1999; Ferrier-Pagès et al., 2003) and carbon for the symbiosis, depending on the environmental conditions (Houlbrèque and Ferrier-Pagès, 2009). Heterotrophy was shown to enhance the metabolism of several coral species under non-stressful conditions (Houlbrèque and Ferrier-Pagès, 2009), and increase coral resilience and resistance to stress (Grottoli et al., 2004, 2006; Rodrigues and Grottoli, 2007; Ferrier-Pagès et al., 2010; Edmunds, 2011). Host nutritional state also affects symbiont metabolism as well as their acquisition and translocation of inorganic nutrients (D'Elia and Cook, 1988; Cook et al., 1992; McAuley and Cook, 1994; Davy and Cook, 2001; Morar et al., 2011). Although we now have a better knowledge of the importance of heterotrophy in corals (Houlbrèque and Ferrier-Pagès, 2009), we still know very little about the more complex aspects of metabolic interplay between cnidarians and their algal partners. The ultimate metabolic destinations of nutrients, as well as the flux of metabolites between the symbiotic partners are still unclear.

Among the open questions, the symbiont capacity for heterotrophic nutrient acquisition, i.e. the partitioning of these nutrients between the host and symbionts, are still a matter of debate. While particulate nitrogen was rapidly (within a few hours) observed both in the host and symbionts of the temperate corals *Oculina arbuscula* and *Oculina diffusa* (Piniak et al., 2003; Piniak and Lipschultz, 2004) or the sea anemone *Anthopleura aureoradiata* (Morar et al., 2011), no nitrogen enrichment from detritic particulate matter was measured in the symbionts of four other tropical scleractinian corals, *Siderastrea radians*, *Montastrea franksi*, *Diploria strigosa* and *Madracis mirabilis* (Mills et al., 2004). The traditional view of the nitrogen conservation hypothesis (Rees and Ellard, 1989) is that nitrogen is recycled between the host and the symbionts to limit losses (Rahav et al., 1989; Falkowski et al., 1993), but there is little direct evidence for such recycling. Also, the fate of heterotrophic carbon within the coral symbiosis has been studied only twice (Szmant-Froelich, 1981; Hughes et al., 2010), and needs to be further investigated in order to have a better knowledge of the heterotrophic nutrient requirements of each partner of the symbiosis.

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The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

List of symbols and abbreviations

AC	autotrophic carbon
C_C	amount of carbon used by calcification (μg C cm^{-2} h^{-1})
chl	chlorophyll
C_L	AC lost (μg AC cm^{-2} h^{-1} or %)
C_{meas}	^{13}C measured in the sample (%)
C_{nat}	natural abundance of ^{13}C in control nubbins (%)
C_{prey}	^{13}C enrichment in $^{13}C/^{15}N$-labeled *A. salina* (2.293%)
C_R	percentage of AC remaining in symbionts or host tissue (%)
HC	heterotrophic carbon
HN	heterotrophic nitrogen
M_C	mass of carbon per mg of tissue or symbionts (μg mg^{-1})
M_N	mass of nitrogen per mg of tissue or symbionts (μg mg^{-1})
M_{sample}	mass of the freeze-dried sample (mg)
M_{Sk}	amount of $CaCO_3$ produced by calcification (μg $CaCO_3$ cm^{-2} h^{-1})
N_{meas}	^{15}N measured in the sample (%)
N_{nat}	natural abundance in ^{15}N in control nubbins (%)
N_{prey}	^{15}N enrichment in $^{13}C/^{15}N$-labeled *A. salina* (14.4%)
P_C	amount of AC fixed by symbionts (μg AC cm^{-2} h^{-1})
P_g	oxygen produced by gross photosynthesis (μmol O_2 cm^{-2} h^{-1})
P_n	oxygen produced by net photosynthesis (μmol 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 (μmol O_2 cm^{-2} h^{-1})
R_C	amount of carbon respired by holobiont (μg C cm^{-2} h^{-1})
R_H	amount of carbon respired by coral host (μg C cm^{-2} h^{-1})
RQ	respiratory quotient (equal to 0.8 mol O^2:mol C)
R_S	amount of carbon respired by symbionts (μg AC cm^{-2} h^{-1})
S	nubbin surface area (cm^2)
T_S	amount of AC translocated from symbionts to their host (μg AC cm^{-2} h^{-1}or %)
t_{inc}	feeding length (5 or 17 h)
$^{AC}\rho_{DOC}$	amount of AC lost in released dissolved organic carbon (μg AC cm^{-2} h^{-1})
$^{AC}\rho_H$	amount of AC incorporated in coral host (μg AC cm^{-2} h^{-1})
$^{AC}\rho_{POC}$	amount of AC lost in released particulate organic carbon (μg AC cm^{-2} h^{-1})
$^{AC}\rho_S$	amount of AC incorporated in symbionts (μg AC cm^{-2} h^{-1})
$^{HC}\rho_H$	amount of HC incorporated in coral host (μg HC cm^{-2} h^{-1})
$^{HC}\rho_S$	amount of HC incorporated in symbionts (μg HC cm^{-2} h^{-1})
$^{HN}\rho_H$	amount of HN incorporated in coral host (μg HN cm^{-2} h^{-1})
$^{HN}\rho_S$	amount of HN incorporated in symbionts (μg HN cm^{-2} h^{-1})

Finally, it is still not known whether symbiont activity has any influence on the heterotrophic nutrient acquisition and partitioning, or in other words, whether the heterotrophic acquisition of carbon and nitrogen on the autotrophic acquistion of carbon by the symbionts. Heterotrophy is suggested to increase in few scleractinian coral species under low irradiance levels (deep or turbid waters) to compensate for the decrease in symbiont photosynthesis and autotrophic nutrient supply (Muscatine et al., 1989; Anthony and Fabricius, 2000; Tremblay et al., 2014). However, increased heterotrophy is strongly associated with a higher symbiont translocation rate in sea anemones (Leal et al., 2015). Therefore, how *Symbiodinium* activity influences the nutritional plasticity of a cnidarian–dinoflagellate symbiosis remains unclear.

Since organic feeding is an important nutritional source for corals, there is a need to expand our understanding on how corals use the heterotrophic nutrients depending on the environmental conditions or symbiont activity. In this study, we assessed the partitioning of heterotrophic nitrogen (HN) and carbon (HC) between the symbionts and host of the scleractinian branching coral *Stylophora pistillata* (Esper 1797) and the foliose coral *Turbinaria reniformis* Bernard 1896 under two irradiance levels. Photosynthetic performance and autotrophic carbon (AC) translocation by the symbionts to the host were studied in parallel, to link the two flows of energy within the symbiosis. The two coral species investigated present a long-term association with *Symbiodinium* clade A for *S. pistillata* and clades C and D for *T. reniformis*, suggesting that the partnership is the most efficient under our environmental conditions. They also have similar high grazing rates (Tremblay et al., 2011, 2014) and rely on heterotrophy in their natural environment, either because of turbid waters (*T. reniformis*; Veron, 2000; Anthony, 2006) or because it thrives in deep environments (*S. pistillata*; Muscatine et al., 1989; Mass et al., 2010). The final aims were to assess: (1) the fate of HC and HN within the symbiotic associations of *S. pistillata* and *T. reniformis* and the capacity of the symbionts to quickly acquire these nutrients; (2) whether rates of both auto- and heterotrophic nutrient acquisition is holobiont (host–symbiont) dependent; (3) whether the acquisition of heterotrophic nutrients is dependent on photosynthesis, or in other words, whether the acquisition depends on the autotrophic acquisition of carbon by the symbionts.

RESULTS

Physiological parameters

At each irradiance level, the areal concentrations of symbionts and chlorophyll were three times higher in *S. pistillata* than *T. reniformis* (Fig. 1A,B; supplementary material Table S1). *Stylophora pistillata* also had significantly higher calcification, gross photosynthesis and host respiration rates per skeletal surface area compared with *T. reniformis* (Fig. 1C–E; supplementary material Table S1). However, this latter species had a protein content twice as high (~5.0 mg proteins cm^{-2} at both irradiance levels) as *S. pistillata* (~2.5 mg proteins cm^{-2}; supplementary material Table S1), indicating a different use of resources. The individual rates of gross photosynthesis and respiration of symbiont cells were higher in *T. reniformis* than *S. pistillata*, although the chlorophyll concentration per symbiont cell was similar in both species (Table 1; supplementary material Table S2).

Under low irradiance, *S. pistillata* significantly increased symbiont concentration, chlorophyll concentration per skeletal surface area (Fig. 1A,B; supplementary material Table S1) and chlorophyll concentration per symbiont cell (Table 1; supplementary material Table S2) as well as the areal symbiont respiration rate (Fig. 1F; supplementary material Table S1). Conversely, *S. pistillata* decreased rates of gross photosynthesis per skeletal surface area (Fig. 1D; supplementary material Table S1) or symbiont cell (Table 1; supplementary material Table S2), symbiont respiration per symbiont cell (Table 1; supplementary material Table S2) and host respiration and calcification (Fig. 1C,E; supplementary material Table S1). In *T. reniformis*, chlorophyll concentration per skeletal surface area (Fig. 1B; supplementary material Table S1) and per symbiont cell (Table 1; supplementary material Table S2) was also increased, whereas calcification, host respiration (Fig. 1C,E; supplementary material Table S1), gross photosynthesis and symbiont respiration per symbiont cell (Table 1; supplementary material Table S2) were significantly lower.

Allocation of heterotrophic nitrogen and carbon

The heterotrophic carbon (HC) and nitrogen (HN) incorporation rates ($^{HC}\rho$ and $^{HN}\rho$, respectively) measured after 1 day of feeding show the short-term exchanges of heterotrophic nutrients between the host ($^{HC}\rho_H$ and $^{HN}\rho_H$, Fig. 2A,B) and

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

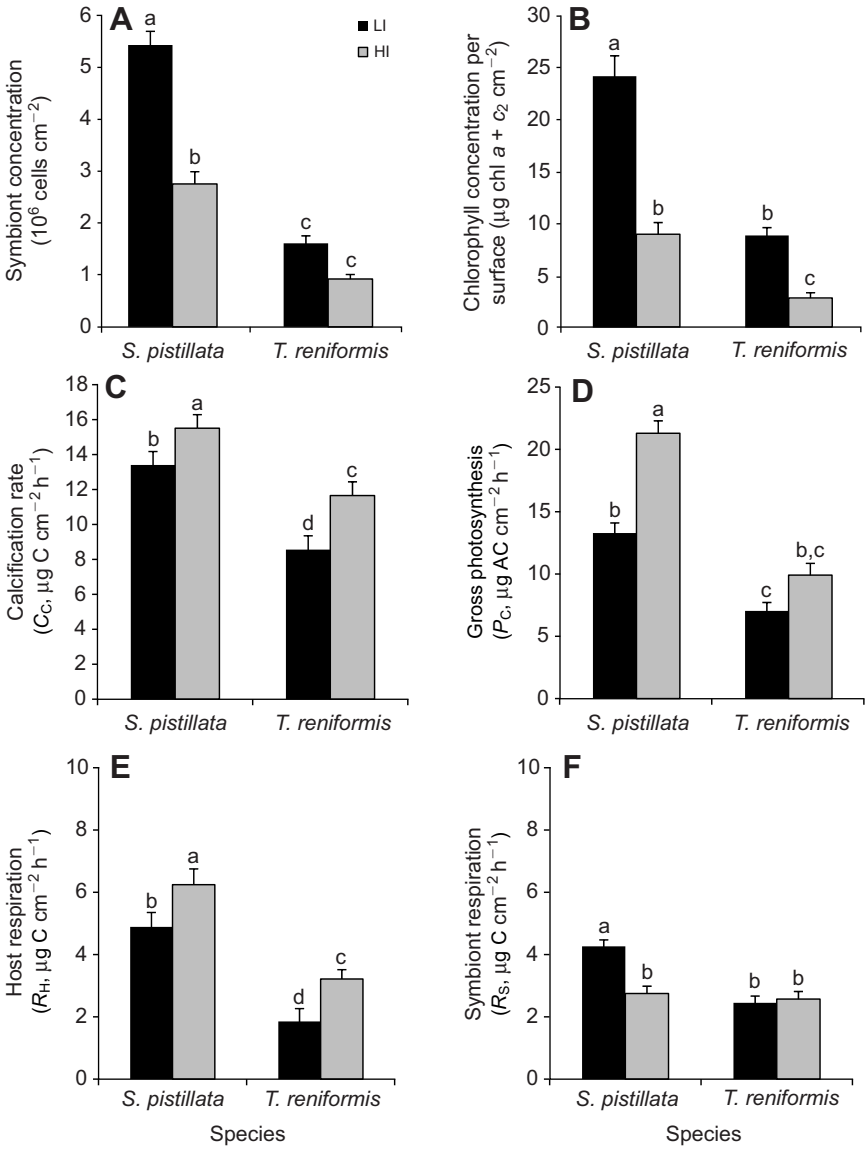

Fig. 1. Effect of irradiance on the main physiological parameters of two species of scleractinian coral. (A) Symbiont concentration, (B) chlorophyll concentration, (C) calcification rate, (D) gross photosynthesis (P_C),(E) host respiration (R_H) and (F) symbiont respiration (R_S) per skeletal surface for *Stylophora pistillata* and *Turbinaria reniformis* maintained at low (LI) and high (HI) irradiance levels. Data are means±s.e.m. of N=6 measurements. For each fraction, bars with different letters (a to d) are significantly different (P<0.05).

the symbionts ($^{HC}\rho_S$ and $^{HN}\rho_S$, Fig. 2C,D), and the loss of nutrients from the symbiotic association after 48 h. The incorporation rates measured after 1 week of feeding (Fig. 2E, F) rather show the long-term distribution of nutrients within the symbiotic association. Although the HC and HN incorporation rates were generally much higher in *S. pistillata* than *T. reniformis*, they followed the same patterns in both species (Fig. 2). The HC:HN ratios were equivalent in both species. They varied between 2.6 and 3.4 in the coral hosts and between 1.5 and 3.0 in the symbionts under low and high irradiance, respectively (Table 2). Moreover, HC and HN incorporation rates in the host tissue of *S. pistillata* and *T. reniformis* were proportional (R^2>0.8 for both nutrients) to the symbiont concentration (Fig. 3A,B), as well as to the incorporation rate

Table 1. Physiological data normalized per symbiont cell (10^{-6} µg chl a+c_2 per cell, 10^{-6} µg C or N per cell h^{-1})

	Stylophora pistillata		*Turbinaria reniformis*	
	Low irradiance	High irradiance	Low irradiance	High irradiance
Chlorophyll concentration	4.48±0.36^a	3.32±0.35^b	5.52±0.55^a	3.11±0.25^b
Gross photosynthesis (P_C)	2.46±0.12^a	8.04±0.87^b	4.45±0.38^c	10.82±0.48^d
Symbiont respiration (R_S)	0.78±0.10^a	1.00±0.04^b	1.52±0.11^c	2.79±0.42^d
HC incorporation ($^{HC}\rho_S$)	0.12±0.05^a	0.20±0.05^b	0.06±0.03^a	0.45±0.12^b
HN incorporation ($^{HN}\rho_S$)	0.08±0.02$^{n.s.}$	0.06±0.01$^{n.s.}$	0.07±0.02$^{n.s.}$	0.12±0.02$^{n.s.}$
AC incorporation ($^{AC}\rho_S$)	0.42±0.02^a	0.60±0.13^a	0.57±0.10^a	1.54±0.25^b
AC translocation (T_S)	1.24±0.02^a	6.13±0.13^b	2.28±0.10^c	6.41±0.25^b

Values were measured after 48 h for *Stylophora pistillata* and *Turbinaria reniformis* maintained at low and high irradiance. Data are means±s.e.m. of N=3–6 measurements. For each parameter, numbers with different letters (a to d) are significantly different (P<0.05); n.s., not significant.

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

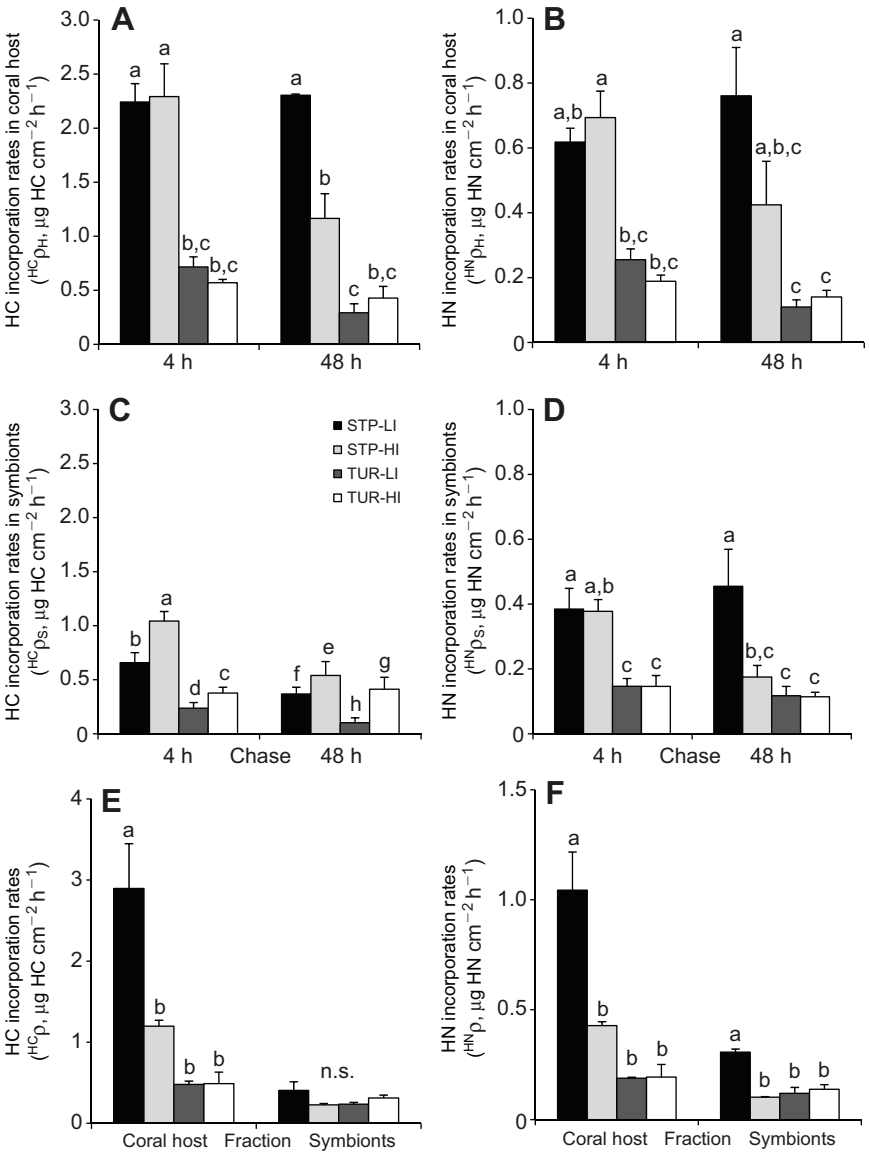

Fig. 2. Effect of irradiance on the heterotrophic nutrient incorporation rates of two species of scleractinian coral. Heterotrophic (A) carbon ($^{HC}\rho_H$) and (B) nitrogen ($^{HN}\rho_H$) in coral host; and (C) carbon ($^{HC}\rho_S$) and (D) nitrogen ($^{HN}\rho_S$) in symbionts during short-term experiment. (E) Carbon ($^{HC}\rho$), and (F) nitrogen ($^{HN}\rho$) in coral host and symbionts during long-term experiment for *Stylophora pistillata* (STP) and *Turbinaria reniformis* (TUR) maintained at low (LI) and high (HI) irradiance. Data are means±s.e.m. of N=3 measurements. For each fraction, bars with different letters (a to h) are significantly different (P<0.05); n.s., not significant.

of autotrophic carbon (AC) in the symbionts (Fig. 3C,D) in both the short- and long-term experiments.

In *S. pistillata*, HC and HN incorporation rates were higher in the host tissue than in the symbionts, in both the short- and long-term experiments (Fig. 2). Nutrients were, however, rapidly translocated and/or available to the symbionts, which were labeled 4 h after feeding. Irradiance significantly affected incorporation rates (Fig. 2; Table 2). Under low irradiance, the amount of HN and HC incorporated in each fraction remained constant between 4 and 48 h (Fig. 2A,B,D; Table 2), whereas the incorporated HC decreased by half with time under high irradiance (Fig. 2A,C; Table 2). After 48 h, 2.7 µg HC cm^{-2} h^{-1} and 1.2 µg HN cm^{-2} h^{-1} were retained within the symbiotic association under low irradiance, compared with only about half of these amounts under high irradiance (1.7 µg HC cm^{-2} h^{-1} and 0.6 µg HN cm^{-2} h^{-1}; Fig. 2A–D). HC and HN incorporation rates calculated after 1 week at both irradiance levels were proportional to the incubation length and in the same range than the rates obtained after 1 day (3.3 µg HC cm^{-2} h^{-1} and 1.4 µg HN cm^{-2} h^{-1} retained under low irradiance, compared with only half of these amounts under high irradiance, Fig. 2E,F). These results suggest that nutrients were not lost from the symbiotic association after 1 week and that there was a constant pattern of nutrient allocation between the partners. Although the total amount of HC and HN incorporated within the symbiotic association changed with time and irradiance, the nutrient allocation between the host and symbionts remained constant (62% to 88% retained in the host and 12% to 38% retained in the symbionts; supplementary material Table S3). The highest retention of HC per symbiont cell occurred under high irradiance (Table 1; supplementary material Table S2).

Turbinaria reniformis presented rates of HC and HN acquisition and retention rates in the symbiotic association two to six times lower than those measured for *S. pistillata*. Indeed, after 48 h or 7 days, 0.4–0.8 µg HC cm^{-2} h^{-1} and 0.2–0.4 µg HN cm^{-2} h^{-1} were retained depending on the irradiance level considered (Fig. 2; Table 2). Irradiance had no effect on the total amount of HN and HC incorporated in the host. However, the amount of HC incorporated in symbionts per surface area (Fig. 2C; Table 2) and per symbiont cell (Table 1; supplementary material Table S2) was higher under high irradiance. Concerning the allocation of nutrients within the symbiotic association, 49–77% was retained in the coral host versus 23–51% in the symbionts (supplementary material Table S3).

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

Table 2. Results of the repeated-measure or factorial analysis of variance (ANOVA) testing the effect of species, irradiance and time on heterotrophic carbon and nitrogen incorporation rates in symbionts and coral host

	Symbionts			Coral host		
Factor	d.f.	*P*	*F*	d.f.	*P*	*F*
Heterotrophic carbon short-term incorporation rates ($^{HC}\rho$)						
Between subjects						
Species	1	**0.0011**	34.44	1	**<0.0001**	80.19
Irradiance	1	**0.0068**	16.28	1	0.1463	2.67
Species×irradiance	1	0.8351	0.47	1	0.1529	2.57
Error	6	–	–	7	–	–
Within subjects						
Time	1	**0.0254**	8.74	1	**0.0005**	36.03
Time×species	1	0.0877	4.15	1	0.1117	3.31
Time×irradiance	1	0.9888	<0.01	1	**0.0118**	11.42
Time×species×irradiance	1	0.2258	1.82	1	**0.0009**	29.82
Error	6	–	–	7	–	–
Heterotrophic nitrogen short-term incorporation rates ($^{HN}\rho$)						
Between subjects						
Species	1	**0.0006**	29.61	1	**0.0001**	51.08
Irradiance	1	0.1726	2.24	1	0.2735	1.38
Species×irradiance	1	0.1839	2.12	1	0.3982	0.80
Error	8	–	–	8	–	–
Within subjects						
Time	1	**0.0090**	11.73	1	0.1427	2.64
Time×species	1	0.1041	3.36	1	0.7405	0.12
Time×irradiance	1	**0.0284**	7.12	1	0.1502	2.53
Time×species×irradiance	1	**0.0321**	6.71	1	**0.0327**	6.65
Error	8	–	–	8	–	–
Heterotrophic carbon long-term incorporation rates ($^{HC}\rho$)						
Species	1	0.5357	0.42	1	**0.0020**	22.95
Irradiance	1	0.4603	0.61	1	**0.0360**	6.70
Species×irradiance	1	0.0919	3.81	1	**0.0345**	6.85
Error	7	–	–	7	–	–
Heterotrophic nitrogen long-term incorporation rates ($^{HN}\rho$)						
Species	1	**0.0093**	12.63	1	**0.0012**	27.30
Irradiance	1	**0.0032**	19.24	1	**0.0220**	8.58
Species×irradiance	1	**0.0012**	27.62	1	**0.0206**	8.87
Error	7	–	–	7	–	–
Ratio of HC:HN incorporation rates						
Species	1	0.7063	0.14	1	**0.0066**	8.51
Irradiance	1	**0.0002**	17.93	1	0.7615	0.09
Species×irradiance	1	0.8418	0.04	1	0.0967	2.94
Error	29	–	–	30	–	–

N=3 replicates; significant *P*-values are bold.

Allocation of autotrophic carbon

The autotrophic carbon (AC) budget, as calculated with the equations presented in Tremblay et al. (2012, 2014) for the two coral species and the two irradiance levels are presented in Fig. 4. Only the final model is presented in this paper, to supplement the heterotrophic allocation of nutrients. The detailed results are presented in supplementary material Figs S1 and S2. There was a significant interaction between growth irradiance and species on the fate of the autotrophically acquired carbon (Table 3). In *S. pistillata*, high irradiance significantly increased the amount of carbon fixed by the symbionts and translocated to the host (T_S) as well as the amount of AC incorporated by host tissue ($^{AC}\rho_H$) and lost by symbiotic association (C_L) after 48 h (Fig. 4A,B; Table 3). Conversely, irradiance had no effect on most autotrophic parameters in *T. reniformis* (Fig. 4C,D).

At both irradiances, after 48 h, the amount of AC incorporated in the symbionts ($^{AC}\rho_S$) was higher in *S. pistillata* (from 1.7 to 2.3 µg AC cm^{-2} h^{-1}) than in *T. reniformis* (from 0.9 to 1.4 µg AC cm^{-2} h^{-1}; Fig. 4; Table 3; Tukey's test $P<0.05$). The same pattern was observed for the host tissue ($^{AC}\rho_H$, Fig. 4; Table 3), because the total amount of carbon translocated after 48 h was also higher in *S. pistillata* (Fig. 4; Table 3; Tukey's test $P<0.0001$). It indeed supplied, depending on the irradiance level, between 6.7 and 16.9 µg AC cm^{-2} h^{-1}, against only 3.7 and 5.9 µg AC cm^{-2} h^{-1} in *T. reniformis* (Fig. 4). The percentage of carbon translocation was, however, equivalent for both coral species under low irradiance (51–52%, Fig. 4A,C; Table 3; Tukey's test $P=0.9442$), but remained higher in *S. pistillata* than *T. reniformis* under high irradiance (79% vs 60%, Fig. 4B,D; Table 3; Tukey's test $P<0.001$). The amount of AC lost (C_L) in the form of symbiont and host respiration was generally twice as high in *S. pistillata* than *T. reniformis* (Fig. 4; Table 3; Tukey's test $P<0.01$), although the percentage was equivalent between the two coral species (Fig. 4; Table 3; Tukey's test $P>0.05$). Finally, the amount of AC lost in the form of particulate (POC) and dissolved (DOC) organic carbon ($^{AC}\rho_{POC}$ and $^{AC}\rho_{DOC}$) was negligible, compared with the amount respired, for both coral species under low irradiance (*t*-test, d.f.=7, $P>0.05$). It was much higher (*t*-test, d.f.=7, $P<0.01$), both in terms of percentage or total amount of AC lost for *S. pistillata* than *T. reniformis* under high irradiance (Fig. 4B,D; Table 3).

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

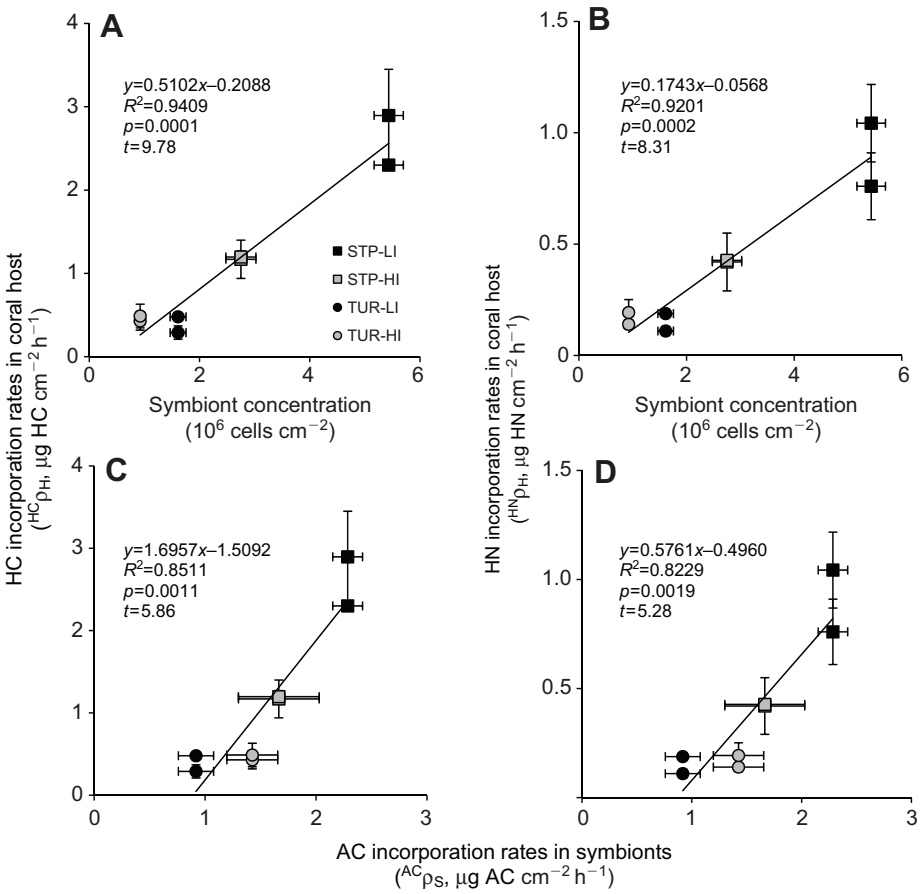

Fig. 3. Relationship between symbiont population and heterotrophic nutrient incorporation rates in coral host. Relationship between symbiont concentrations and incorporation rates in coral host of heterotrophic (A) carbon ($^{HC}\rho_H$) or (B) nitrogen ($^{HN}\rho_H$); and between autotrophic carbon incorporation rates in symbionts ($^{AC}\rho_S$) and incorporation rates in coral host of heterotrophic (C) carbon ($^{HC}\rho_H$) or (D) nitrogen ($^{HN}\rho_H$), after 48 h during short-term experiment and during long-term experiment for *Stylophora pistillata* (STP) and *Turbinaria reniformis* (TUR) maintained at low (LI) and high (HI) irradiance. Data are means±s.e.m. of N=3 measurements for incorporation rates of nutrients, or N=6 measurements for symbiont concentration.

DISCUSSION

The dual $^{13}C/^{15}N$-labeling of the coral food clearly demonstrated that there was a rapid acquisition, exchange and long-term retention of heterotrophic nutrients (both carbon and nitrogen) by host and symbionts of *S. pistillata* and *T. reniformis*, while autotrophic products were mainly respired and released as mucus in the surrounding water. Heterotrophic nitrogen (HN) was also preferentially retained over heterotrophic carbon (HC). Comparison of the nutrient acquisition in *S. pistillata* and *T. reniformis* showed that the amount of heterotrophic nutrients retained in the host tissue was holobiont dependent and also positively correlated to the symbiont concentration or the amount of autotrophic carbon (AC) retained in symbionts. Finally, there was a coupling between auto- and heterotrophy in the species *S. pistillata*. Indeed, heterotrophic nutrient acquisition and retention were higher under low irradiance, to compensate for a reduced autotrophic input and to maintain maximal growth rates.

Although the two coral species were maintained under the same conditions of irradiance and heterotrophic feeding prior to and during the experiment, and presented equivalent feeding rates (Tremblay et al., 2011, 2014), symbiont concentration was twice as high in *S. pistillata* than *T. reniformis*, leading the former species to achieve higher rates of calcification and photosynthesis per skeletal surface area. *Stylophora pistillata* also presented higher rates of auto- and heterotrophic nutrient acquisition and/or assimilation in the host tissue, in agreement with its higher metabolism. The host, the symbiont, or most probably the synergistic effect of both partners (Goulet et al., 2005) might have dictated the metabolic differences observed between the two species. For example, *T. reniformis* presented lower growth rates as well as higher protein content and metabolic rates compared with *S. pistillata*, and might have exhibited higher protein turnover rates (Gates and Edmunds, 1999). A different light environment inside the host tissue (Wangpraseurt et al., 2012, 2014), clade-specific metabolic demands (Leal et al., 2015) or different growth rates might have led to differences in symbiont concentration. Significant genotypic differences were observed in the rates of nutrient acquisition, retention and translocation per cell (Table 1) between *Symbiodinium* of each coral species (Cantin et al., 2009; Baker et al., 2013). Overall, compared with *S. pistillata*, symbionts of *T. reniformis* had a lower concentration and also presented, for each irradiance, higher gross photosynthesis and respiration rates per cell, as well as higher incorporation rates of AC and HC. These measurements are in agreement with the observation that a high metabolic demand per cell usually impairs high symbiont concentration (Wooldridge, 2013). In turn, a smaller concentration in *T. reniformis* avoided self-shading and increased the amount of light reaching each symbiont, promoting photosynthesis (Wangpraseurt et al., 2014). Overall, host–symbiont differences can have induced large intrinsic metabolic differences between the two coral species (Gates and Edmunds, 1999), which might have affected energy fluxes within the symbiosis.

Despite these differences, heterotrophic nutrients (HC and HN) were rapidly shared between the host and the symbionts in both coral species, with large bidirectional flows between the symbiotic partners. HC and HN were first obtained by the animal host through prey capture, and acquired within 4 h by the symbionts, from the coelenteric digestion or direct translocation, as already observed in *O. arbuscula*, *O. diffusa, Montipora capitata* and *Porites compressa* (Piniak et al., 2003; Piniak and Lipschultz, 2004; Hughes et al., 2010). The re-translocation of heterotrophic nutrients

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

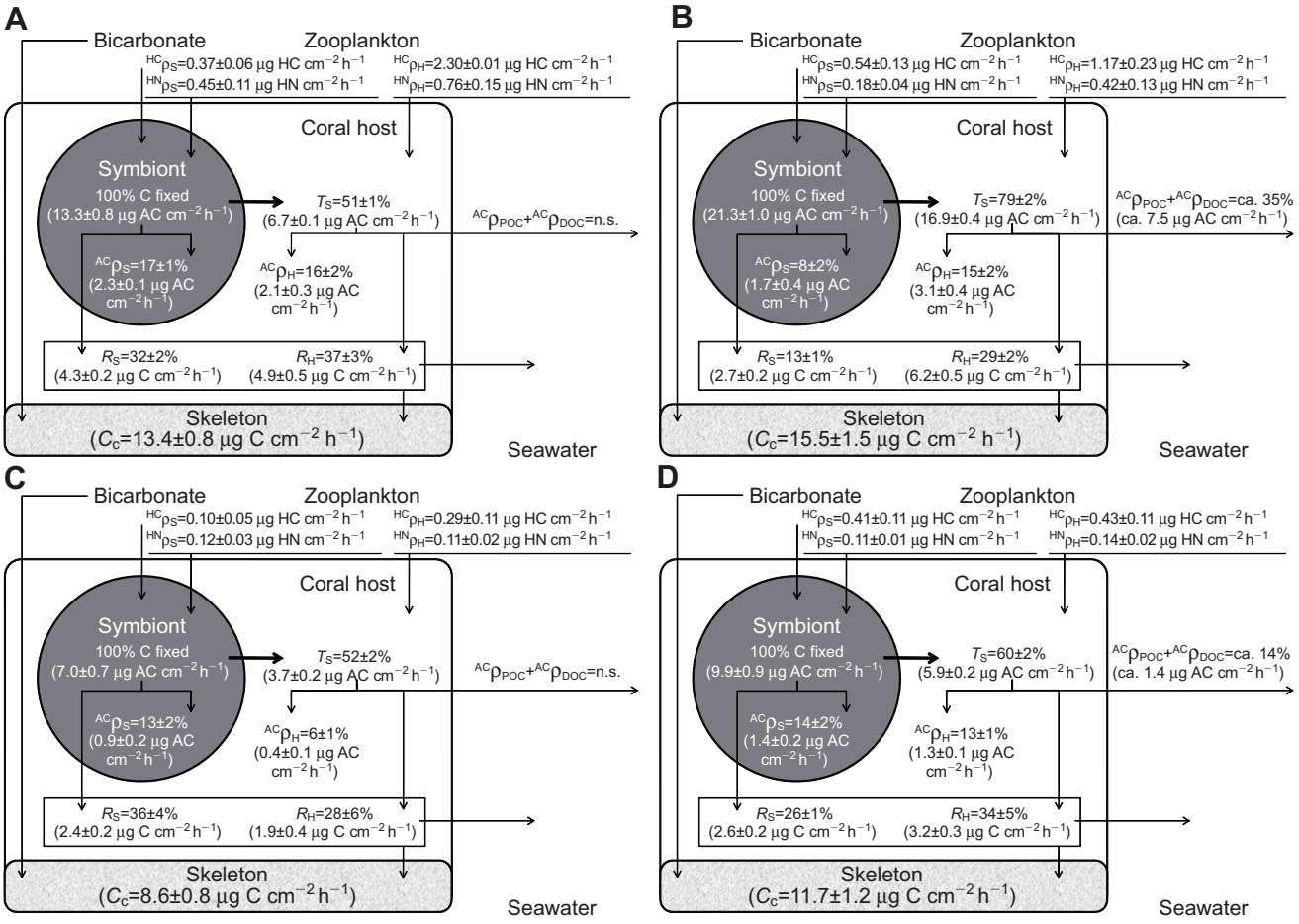

Fig. 4. Mass-balanced results of photosynthate translocation and carbon budget. Results for *Stylophora pistillata* maintained under (A) low and (B) high irradiance or *Turbinaria reniformis* maintained under (C) low and (D) high irradiance, based on $^{13}C/^{15}N$ experiments after 48 h of incubation. Symbols are defined in list of symbols and abbreviations and in the text. Data represent means±s.e.m. of N=3 measurements for $H^{13}CO_3^-$-based rates ($^{AC}\rho_S$, $^{AC}\rho_H$, $^{AC}\rho_{POC}$+ $^{AC}\rho_{DOC}$ and T_S) and $^{13}C/^{15}N$-labeled prey-based rates ($^{HC}\rho_S$ and $^{HC}\rho_H$) or N=6 measurements for rates of photosynthesis (P_C), respiration (R_S and R_H) and calcification (C_C).

from the symbionts to the host has been observed only once in *O. diffusa* (Piniak and Lipschultz, 2004), but is confirmed in this study, under low irradiance (Fig. 2; supplementary material Table S2). Indeed, while HC incorporation in *S. pistillata* host increased from 77% to 86% (+9%) between 4 and 48 h, and to 88% (+2%) after 7 days, a parallel and corresponding decrease was observed in the symbionts, from 23% to 14% (−9%) between 4 h and 48 h and to 12% (−2%) after 7 days. The same pattern was observed for incorporation of HN in *S. pistillata*. In *T. reniformis*, HN was first translocated from the host to the symbionts between 4 and 48 h, and re-translocated to the host after 7 days. HN incorporation rates thus decreased in the host tissue from 64% to 49% (−15%) between 4 h and 48 h and then increased to 62% (+13%) after 7 days, while the converse was observed in the symbionts. Overall, these observations suggest that in both species, nutrients were recycled between the partners, so that they could be incorporated into molecules of interest. These observations confirm *in situ* measurements of stable isotope signature and lipid composition of the tissue of red sea coral species, which have suggested a high efficiency of carbon recycling between the symbiotic partners (Alamaru et al., 2009). Over the long term, symbionts retained between 12% and 44% of the total HC and HN. These values are comparable to those previously estimated with ^{14}C for symbionts of *Astrangia danae* (Szmant-Froelich, 1981), and hydra (Cook, 1972) and with ^{15}N for symbionts of *O. arbuscula* (Piniak et al., 2003). However, the amount of HN acquired per symbiont cell was equivalent for both coral species and irradiance levels, and remained relatively low (0.06×10^{-6} to 0.12×10^{-6} µg HN cell^{-1} h^{-1}) compared with HC incorporation rates, which might explain the discrepancy observed in the literature concerning the symbiont capacity to acquire heterotrophic nutrients (Piniak et al., 2003; Mills et al., 2004). In addition, the fate of HN within the symbiotic association, which is shared both by the host and symbionts, is different from the fate of the dissolved inorganic nitrogen (DIN) which is retained by the symbionts for their own use and development (Grover et al., 2002, 2003). This might explain why DIN tends to disrupt the symbiosis at high concentrations (Wiedenmann et al., 2013), whereas HN tends to improve the corals' health and metabolism (Grottoli et al., 2006; Ferrier-Pagès et al., 2010). Also, compared with the uptake rates of natural concentrations of DIN by the algae, 2 h of feeding per day brings, in *S. pistillata*, 10 times more nitrogen to the symbiotic association.

There was a long-term retention of heterotrophic nutrients (HC and HN) within the symbiosis, conversely to photosynthates (AC), which were used to meet immediate metabolic demands. The amounts of HN, but also HC, remaining in the symbiotic associations after 1 week of feeding were indeed equivalent to those

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

Table 3. Repeated-measured analysis of variance (ANOVA) testing the effect of species, irradiance and time on autotrophic carbon incorporation rates and percentage of fixed carbon in symbionts and coral host as well as amount and percentage of carbon lost and translocated

		Amount (μg carbon cm^{-2} h^{-1})		Percentage (%)	
Factor	d.f.	*P*	*F*	*P*	*F*
Autotrophic carbon incorporation rate ($^{AC}\rho_S$) and fixed carbon remaining (C_R) in symbionts					
Between subjects					
Species	1	**<0.0001**	73.40	0.6200	0.27
Irradiance	1	0.5799	0.33	**0.0022**	19.48
Species×irradiance	1	**0.0012**	24.13	**0.0007**	28.14
Error	8	–	–	–	–
Within subjects					
Time	2	**<0.0001**	22.79	**<0.0001**	23.05
Time×species	2	0.1059	2.59	0.7812	0.25
Time×irradiance	2	0.9608	0.04	0.4719	0.79
Time×species×irradiance	2	0.6763	0.40	0.5048	0.71
Error	16	–	–	–	–
Autotrophic carbon incorporation rate ($^{AC}\rho_H$) and fixed carbon remaining (C_R) in coral host					
Between subjects					
Species	1	**<0.0001**	78.16	**0.0258**	7.46
Irradiance	1	**0.0128**	10.19	0.5823	0.33
Species×irradiance	1	0.4248	0.71	0.1955	2.00
Error	8	–	–	–	–
Within subjects					
Time	2	**0.0435**	3.84	**0.0424**	3.87
Time×species	2	**0.0226**	4.85	**0.0247**	4.71
Time×irradiance	2	0.5018	0.72	0.2175	1.68
Time×species×irradiance	2	0.9141	0.09	0.4987	0.73
Error	16	–	–	–	–
Autotrophic carbon translocation (T_S)					
Between subjects					
Species	1	**<0.0001**	371.75	**0.0004**	33.94
Irradiance	1	**<0.0001**	349.33	**<0.0001**	192.47
Species×irradiance	1	**<0.0001**	157.62	**<0.0001**	77.11
Error	8	–	–	–	–
Within subjects					
Time	2	**<0.0001**	22.79	**<0.0001**	23.05
Time×species	2	0.1059	2.59	0.7812	0.25
Time×irradiance	2	0.9608	0.04	0.4719	0.79
Time×species×irradiance	2	0.6763	0.40	0.5048	0.71
Error	16	–	–	–	–
Autotrophic carbon lost (C_L)					
Between subjects					
Species	1	**<0.0001**	269.75	0.2126	1.83
Irradiance	1	**<0.0001**	208.94	0.0304	6.89
Species×irradiance	1	**<0.0001**	90.52	**0.0071**	12.85
Error	8	–	–	–	–
Within subjects					
Time	2	**0.0002**	15.95	**0.0001**	16.78
Time×species	2	**0.0375**	4.06	0.0901	2.81
Time×irradiance	2	0.6384	0.46	0.1892	1.85
Time×species×irradiance	2	0.9690	0.03	0.7502	0.29
Error	16	-	-	-	-

N=3 replicates; significant *P*-values are bold.

measured after 48 h of a single day of feeding, whereas more than 65% of photosynthates were lost within 2 days as respiration, POC and DOC (Fig. 4). Such food partitioning has only been reported once in corals (Hughes et al., 2010) and in a sea anemone (Bachar et al., 2007). However, together, these studies highlight a different cycling of auto- and heterotrophic carbon between the components of marine anthozoan symbioses and the importance of heterotrophic nutrient as building blocks or long-term source of energy for these symbioses. The ratios of HC to HN incorporated were equivalent in both species, and lower than the one of the *A. salina* prey, suggesting that HN was preferentially retained in coral tissue compared to HC. This is in agreement with the idea that tropical corals should favor the acquisition and conservation of nitrogen because of the N-poor diet of the translocated algal photosynthates. Although HN was preferred over HC, the rates of HC retention in the host tissue and/or symbionts ($^{HC}\rho_H$ and $^{HC}\rho_s$, Fig. 2) were in the same range, or higher than those measured for AC ($^{AC}\rho_H$ and $^{AC}\rho_s$, Fig. 4), confirming that the two carbon sources have different nutritional values.

A coupling between auto- and heterotrophy was also observed in the species *S. pistillata*, which presented a significant difference in symbiont and chlorophyll concentrations as well as rates of photosynthesis and carbon acquisition between high and low irradiance. Heterotrophic nutrient acquisition and retention were significantly higher under low irradiance (Fig. 2), to compensate for a 50% reduction in autotrophic nutrient acquisition and

The Journal of Experimental Biology (2015) 218, 1223-1234 doi:10.1242/jeb.115303

translocation and to keep maximal rates of calcification as well as the same carbon budget in all conditions (Fig. 4): the sum of the HC incorporated in host tissue at each irradiance level (2.3 and 1.2 µg HC cm^{-2} h^{-1} at low and high irradiance, respectively), with the AC respired by the host (4.9 and 6.2 µg carbon cm^{-2} h^{-1}) and incorporated in the host tissue (2.1 and 3.1 µg AC cm^{-2} h^{-1}) gave an equivalent carbon budget at both irradiance levels after 48 h. A shift from auto- to heterotrophy was also noticed in *S. pistillata* colonies sampled along a depth gradient in the Red Sea (Alamaru et al., 2009), suggesting that this species indeed shows a great plasticity in the acquisition of nutrients. A very recent paper (Leal et al., 2015) performed with sea anemones associated with different *Symbiodinium* clades found the contrary, that higher autotrophy (in terms of total carbon translocation) was strongly associated with increased heterotrophy. This observation is not supported by our results, although we observed a significant relationship between symbiont concentration and the amount of heterotrophic nutrients remaining in host tissue (Fig. 3A,B). This positive association may be due to several non-mutually exclusive reasons: (1) food supply during the five weeks of acclimation to the experimental conditions has enhanced symbiont concentration in host tissue differently according to the coral and symbiont species and/or environmental conditions (clade A in symbiosis with *S. pistillata* might for example, have had a higher growth compared with clades C and D in symbiosis with *T. reniformis*); (2) a high symbiont concentration in coral tissue has a higher metabolic demand (Wooldridge, 2009), and has driven a higher heterotrophy; (3) a high symbiont concentration, and thus an increased autotrophic nutrient availability to the host might help to support the metabolically demanding heterotrophic processes; (4) increased heterotrophic contribution is critical to the assimilation of photosynthetic carbon into host biomass.

In conclusion, this study provides a comprehensive picture of the trophic ecology of the coral-dinoflagellate symbiosis, and particularly of the nutrient exchanges between the two partners. It provides evidence of the complexity of the host-symbiont relationship, and shows that the acquisition and allocation of nutrients depends on the host and symbiont combination. The association between *S. pistillata* and *Symbiodinium* clade A, rapidly modulated its mixotrophic nutrition, and shifted from autotrophy to heterotrophy to conserve an equivalent nutritional state and maintain high rates of calcification. The association between *T. reniformis* and *Symbiodinium* clades C and D had a different trophic ecology, since it conserved the same auto- and heterotrophic nutrient acquisition at the two irradiances investigated. Understanding the coral trophic ecology is especially important in the context of bleaching events, as not all stable host-dinoflagellate symbioses have the same trophic functioning and are thus not equally nutritionally advantageous. For species like *S. pistillata*, their resistance to a stress impairing symbiont photosynthesis will be strongly dependent on the abundance and health of the zooplankton community in the water column. This study has also shown that the cycling of auto- and heterotrophic carbon between the components of the holobiont is very different, and that the nature of this difference can vary between holobionts and environments. Nonetheless, heterotrophy is an important process for the acquisition of nutrients, especially nitrogen, and the build up of the coral biomass, and may explain the particular resistance and/or resilience of some coral species to stress.

MATERIALS AND METHODS

Experimental design

Three large colonies of two species of scleractinian corals, originating from the Red Sea and maintained in the aquaria of the Centre Scientifique de Monaco, were used: *S. pistillata* (suborder *Astrocoeniina*) containing *Symbiodinium* clade A, and *T. reniformis* (suborder *Dendrophylliina*) containing clades C and D on the same colony. *Symbiodinium* clades were identified using partial chloroplast large subunit (23S) rDNA sequences (Santos et al., 2002). A total of 80 nubbins per species (26–27 nubbins per colony) was prepared by cutting the apical branches of *S. pistillata* or portions of *T. reniformis*. They were then equally divided into eight tanks of 20 l volume (three to four nubbins per colony, species and tank) and cultured for 4 weeks until tissue entirely covered the skeleton. Tanks were maintained in an open flow system (renewal rate of 10 l h^{-1}), with seawater containing low levels of inorganic and organic nutrients (Ferrier-Pagès et al., 1998), under an irradiance level of 120 µmol photons m^{-2} s^{-1} (12 h:12 h light:dark photoperiod), and at a constant temperature of 25.0± 0.5°C. After these first 4 weeks, irradiance was increased in four of the eight tanks to 250 µmol photons m^{-2} s^{-1} (hereafter called HI tanks) while the remaining tanks were kept at 120 µmol photons m^{-2} s^{-1} (hereafter called LI tanks). Corals were fed twice a week for 5 weeks with *Artemia salina* nauplii, after which a series of physiological and tracer measurements were made (24 h at least after the last feeding).

Physiological measurements

Calcification rates were determined for six nubbins per species per irradiance (two nubbins per colony), using the buoyant weight technique (Jokiel et al., 1978; Davies, 1989). Carbon allocation to calcification (C_C) was calculated according to the formula: $C_C=M_{Sk}\times12/100$, where M_{Sk} is the amount of $CaCO_3$ produced in µg and 12/100 is the ratio of molecular masses of carbon (12) and $CaCO_3$ (100). Protein content of the coral tissue was measured on the same nubbins according to Smith et al. (1985) using the BC Assay Kit (Uptima, Interchim, France) after extraction in 1 mol l^{-1} NaOH at 90°C for 30 min. The standard curve was established using bovine serum albumin, and the absorbance was read on a spectrofluorometer Xenius (Safas, Monaco).

Rates of respiration (R) and net photosynthesis (P_n) were assessed on six nubbins per species per irradiance (two per colony) using oxygen respirometry according to Hoogenboom et al. (2010). Temperature was kept constant at 25.0±0.5°C and irradiances were equal to 0, 120 and 250 µmol photons m^{-2} s^{-1}. Rates of gross photosynthesis (P_g) were calculated by adding R to P_n. The gross autotrophic carbon (AC) acquisition (P_C) during the 12 h light period, and the daily carbon respired (R_C) were calculated for each treatment. For this purpose, oxygen fluxes were converted to carbon equivalents based on molar weights, as $P_C=P_g\times12/PQ$ and $R_C=R\times12\times RQ$ (Anthony and Fabricius, 2000), where PQ and RQ are photosynthetic and respiratory quotients equal to 1.1 mol O_2:mol carbon and 0.8 mol C:mol O_2, respectively (Muscatine et al., 1981; Gattuso and Jaubert, 1990). After these measurements, samples were frozen for the determination of symbiont and chlorophyll (chl) concentrations, according to Rodolfo-Metalpa et al. (2006) for symbionts, and Jeffrey and Humphrey (1975) for chl *a* and c_2. Chl pigments were extracted in 99% acetone at 4°C over 24 h. All data were normalized to the skeletal surface area, which was measured using the wax-dipping technique (Stimson and Kinzie, 1991) or per symbiont cell.

In addition to the above measurements, respiration rates of freshly isolated symbionts (R_S) were determined for six nubbins per species per irradiance (two per colony) according to Tremblay et al. (2012, 2014). Host respiration (R_H) was obtained by subtracting R_S from R_C.

Allocation of heterotrophic nitrogen and carbon

The heterotrophic carbon (HC) and nitrogen (HN) incorporation in the symbionts and coral host was obtained using $^{13}C/^{15}N$-labeled *Artemia salina* prey. For this purpose, a large batch of the microalgae *Dunaliella* sp. was grown in a Conway medium enriched with 2 mmol l^{-1} of $NaH^{13}CO_3$ (98 atom %^{13}C, cat. no. 372382, Sigma-Aldrich, St Louis, MO, USA) and 1 mmol l^{-1} of $^{15}NH_4Cl$ (98 atom %^{15}N, cat. no. 299251, Sigma-Aldrich). After being hatched, a large batch of *A. salina* nauplii was grown for 2 days in the $^{13}C/^{15}N$-labeled culture of *Dunaliella* sp. *Artemia salina* were then isolated by filtration on a 20 µm mesh, divided into equal portions and frozen at −20°C. A portion of $^{13}C/^{15}N$-labeled *A. salina* corresponded to 53.3±0.8 mg dry weight (or ∼300 prey l^{-1}) with 290±9 µg HC mg^{-1} and 70±2 µg HN mg^{-1} (C:N ratio=4.2). The ^{13}C% and ^{15}N% of the $^{13}C/^{15}N$-

labeled *A. salina* were equal to 2.293±0.001% and 14.4±0.2%, respectively, and were measured with a mass spectrometer as described below. Un-enriched *Dunaliella* sp. and *A. salina* were also grown in parallel (^{13}C%=1.148±0.001% and ^{15}N%=0.468±0.001%).

At the end of the 5 weeks incubation at the two irradiance levels, short and long-term feeding experiments were performed to assess the short- and long-term allocation of nutrients as well as the short- and long term losses of heterotrophic nutrients from the symbiotic association. For the short-term experiment, nine nubbins per species per irradiance (three per colony) were fed once, during 5 h, with two portions of ^{13}C/^{15}N-enriched *A. salina*. Prey digestion has been assessed using visual observations of polyp dissections, as previously performed by several investigators (e.g. Sebens, 1981; Sebens and Johnson, 1991; Houlbrèque et al., 2004; Palardy et al., 2005; Grottoli et al., 2006). These observations showed that no more *A. salina* or pieces of *A. salina* could be observed in the coelenteron of *S. pistillata* or *T. reniformis* 4 h after the feeding trial stopped. Three nubbins per species and irradiance (one per colony) were thus sampled 4 and 48 h after the end of the feeding day and immediately frozen at −20°C. The three remaining nubbins per species and per irradiance were kept being fed during six days for 2 h daily (or a total of 17 h) with one portion of ^{13}C/^{15}N-enriched *A. salina* and sampled 24 h after the last feeding.

Allocation of autotrophic carbon

Allocation of AC was performed according to the protocol fully detailed in (Tremblay et al., 2012, 2014). Briefly, nine nubbins per species per irradiance (three nubbins per colony) were incubated in seawater enriched with a final concentration of 0.6 mmol l^{-1} $NaH^{13}CO_3$ (98 atom %^{13}C, cat. no. 372382, Sigma-Aldrich), giving a final 23% ^{13}C enrichment of the incubation medium. After 5 h incubation (pulse), corals were transferred in non-enriched seawater (chase). Three nubbins per species and irradiance (one nubbin per colony) were removed from the incubation medium after the chase intervals of 0, 4 and 48 h and immediately frozen at −20°C. Four control nubbins per species and irradiance (one per tank) were sampled without being labeled with ^{15}N/^{13}C-*Artemia* or $H^{13}CO_3^-$. The samples were treated as described below.

Sample treatment

For ^{13}C and ^{15}N samples, tissue was detached from the skeleton using an air-brush in 0.45 µm-filtered seawater. The slurry was homogenized using a potter tissue grinder, and the host and symbiont fractions were then separated by centrifugation according to Tremblay et al. (2012, 2014). Cross-contaminations were checked in these previous experiments. Samples were flash-frozen in liquid nitrogen and freeze-dried until analysis. The %^{13}C, and %^{15}N, as well as the carbon and nitrogen content of the animal tissue and symbionts were determined with a mass spectrometer (Delta Plus, Thermofisher Scientific, Bremen, Germany) coupled via a type III interface with a C/N analyzer (Flash EA, Thermofisher Scientific).

The HC or HN incorporation rates in symbionts ($^{HC}\rho_S$ and $^{HN}\rho_S$, respectively) and coral host ($^{HC}\rho_H$ and $^{HN}\rho_H$, respectively) were calculated using a modification of the equation of Grover et al. (2002), adapted to corals from the equation of Dugdale and Wilkerson (1986). These equations are similar to those used in the AC model (Tremblay et al., 2012, 2014):

$$^{HC}\rho = \frac{(C_{meas} - C_{nat}) \times M_{sample} \times M_C}{(C_{prey} - C_{meas}) \times t_{inc} \times S}, \quad (1)$$

or

$$^{HN}\rho = \frac{(N_{meas} - N_{nat}) \times M_{sample} \times M_N}{(N_{prey} - N_{meas}) \times t_{inc} \times S}, \quad (2)$$

where C_{meas} and C_{nat} or N_{meas} and N_{nat} are the percentages of ^{13}C or ^{15}N measured in enriched and control samples, respectively; C_{prey} and N_{prey} are the percentages of ^{13}C and ^{15}N in the prey (2.293% and 14.4%, respectively); M_{sample} is the mass of the sample (mg); M_C and M_N are the carbon and nitrogen content per symbiont or host tissue biomass (µg mg^{-1}); S is the surface area (cm^2); t_{inc} is the feeding length (5 h for the short-term experiment and 17 h for the long-term experiment). The heterotrophic incorporation rates ($^{HC}\rho$ and $^{HN}\rho$) are expressed in µg cm^{-2} h^{-1} or per cell^{-1} h^{-1}. The percentage of HC or HN in coral host and symbionts were obtained by dividing the incorporation rates in coral host or symbionts by the total incorporation rates within the symbiotic association.

The AC budget was calculated exactly as described in Tremblay et al. (2012, 2014). These equations allow the calculations of the AC incorporation rates in symbionts ($^{AC}\rho_S$) and coral host ($^{AC}\rho_H$), the percentage of AC remaining (C_R) in the symbiotic association, the AC lost (C_L=R_C+$^{AC}\rho_{POC}$+$^{AC}\rho_{DOC}$) by this association (as respiration, POC and DOC) and the rates of AC translocation from the symbionts to the host (T_S=P_C−$^{AC}\rho_S$−R_S).

Statistical analysis

All parameters were expressed as means±s.e.m. Data were checked for normality using a Kolmogorov–Smirnov's test with Lilliefors correction, and for variance homoscedasticity using a Levene's test. When the normality condition was not fulfilled, data were transformed (natural logarithms). The effect of species and irradiance on the physiological parameters was tested using factorial analyses of variance (ANOVA) with two factors (species and irradiance). Significant differences for AC, HC and HN incorporation rates, percentage of AC remaining in the symbiotic association, AC lost and translocation were tested using a repeated measures ANOVA. Colonies were considered as 'subjects', while species and irradiance were the between subject factors. The third factor was time over the course of the chase interval, and had two (4 and 48 h) or three (0, 4 and 48 h) levels. When there were significant differences, the analyses were followed by *a posteriori* testing (Tukey's test). Preliminary partly nested ANOVA tests were used to test the tank effect. This effect was not significant for any of the parameters so this factor was excluded from analyses (Underwood, 1997). Linear regressions, using standard least-squares techniques, were used to estimate the relationship between mean symbiont concentration or mean incorporation rates of AC in symbionts and mean HC or HN incorporation rates in coral host after 48 h during short-term experiment and during long-term experiment. Differences in the amount of AC lost as respiration, POC and DOC (C_L), after 48 h and total respiration (R_C) were tested using a *t*-test (d.f.=NC_L+NR_C−2=3+6−2=7). Differences between factors were considered significant for *P*-values <0.05. Statistics were performed using Systat 13 (Systat Software, Chicago, IL, USA).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

P.T., J.F.M., R.G. and C.F.P. conceived and designed the experiments; P.T. and J.F.M. performed the experiments; P.T. analyzed the data; P.T., J.F.M., R.G. and C.F.P. wrote the paper.

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Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.115303/-/DC1

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