

## Effect of light and feeding on the fatty acid and sterol composition of zooxanthellae and host tissue isolated from the scleractinian coral *Turbinaria reniformis*

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### Abstract

The fatty acid and sterol compositions of zooxanthellae and animal fractions of the scleractinian coral *Turbinaria reniformis* were investigated under different light and feeding conditions, to study the symbiont-host exchanges. Nubbins were maintained during 6 weeks under two light levels (100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and 300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and two feeding levels (starved and fed with zooplankton) in a factorial experiment. There were greater proportions of some polyunsaturated fatty acids (PUFA; e.g., C18:4 n-3, C20:5 n-3, C22:6 n-3) in the zooxanthellae than in the host, suggesting that these PUFA were synthesized by the algae and transferred to the animal. Conversely, C20:4 n-6 exhibited a greater proportion in the host and might have been synthesized by the animal. Light affected the chlorophyll content, the rates of photosynthesis, and the lipid production of all coral samples. Corals maintained in high-light conditions had lower relative phytol content but higher concentrations of fatty acids (FA) and sterols than the shaded corals. Feeding also affected coral metabolism, but differently according to the light level and despite the fact that the host did not directly incorporate the zooplankton lipids (PUFA and cholesterol). In low light, feeding resulted in an increase of growth rates and storage lipid concentrations, mainly saturated fatty acids (SAFA) and membrane constituents (PUFA and sterols). In high light, the lipid energy from the food was directed toward an increase in calcification, as well as in chlorophyll content and protein content. This study highlights the importance of feeding in sustaining coral metabolism, especially when light, or stress events, is limiting photosynthesis.

Corals can meet their energetic requirements either via autotrophy through their symbiotic association with dinoflagellates called zooxanthellae (Muscatine et al. 1981) or via heterotrophy, i.e., capture of zooplankton and particulate organic matter (Goreau et al. 1971; Sebens et al. 1996). Zooxanthellae are known to transfer more than 90% of their photosynthates to their host (Muscatine et al. 1981), explaining the exceptional development of corals in oligotrophic environments. Corals are also heterotrophs that are able to catch large amounts of zooplankton (Goreau et al. 1971; Sebens et al. 1996; Yahel et al. 2005) as well as dissolved and particulate organic matter (Anthony and Fabricius 2000). Heterotrophy was proved to significantly enhance the zooxanthellae density, chlorophyll content, as well as the rates of growth and photosynthesis (Anthony and Fabricius 2000; Ferrier-Pagès et al. 2003; Houlbrèque et al. 2003). Both autotrophy and heterotrophy supply corals with major compounds, such as glycerol and other lipids (Crossland et al. 1980; Grottoli et al. 2006), which play an essential

role in coral metabolism at all levels. Lipids are important energy reserves, mainly stored in the animal tissue as wax esters and triglycerides (Muscatine and Cernichiaro 1969; Oku et al. 2002; Grottoli et al. 2004), or in the membranes as sterols and polyunsaturated fatty acids (PUFA; Tchernov et al. 2004). These reserves are, for example, used in the reproduction process (Ward 1995) or are oxidized to generate energy for survival during bleaching events (Yamashiro et al. 2005; Grottoli et al. 2006; Rodrigues and Grottoli 2007). Lipids are also respired to support metabolic needs or excreted as mucus (Crossland et al. 1980).

Since lipid composition is often specific to particular groups of organisms (Volkman et al. 1989), the analysis of lipids, such as fatty acids (FA) and sterols, gives useful information about their autotrophic or heterotrophic origin (Napolitano et al. 1997). In the case of FA, animals cannot insert double bonds beyond the  $\Delta^9$  position, and they cannot synthesize 18:2 n-6 and 18:3 n-3 (Bachok et al. 2006). These FA are essential for animals to further synthesize the n-6 and n-3 PUFA (Lehninger 1985; Papina et al. 2003). As a consequence, corals acquire PUFA through external diet (Meyers 1979; Al-Moghrabi et al. 1995) or through zooxanthellae photosynthates (Papina et al. 2003; Zhukova and Titlyanov 2003). Similarly, analysis of sterols may allow different food regimes to be distinguished because zooplankton contains large quantity of cholesterol, while algae mainly contain phytosterols (Volkman 1986).

Many studies have revealed information on autotrophic FA production, by analyzing lipids in cultured and freshly isolated zooxanthellae (Al-Moghrabi et al. 1995; Zhukova and Titlyanov 2006) or in bulk symbiont samples (Yama-

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### Acknowledgments

The International Atomic Energy Agency (IAEA) is grateful for the support provided to its Marine Environment Laboratories by the Government of the Principality of Monaco. The suggestions of three anonymous reviewers greatly improved this paper.

shiro et al. 1999, 2005). But, only Papina et al. (2003) determined the FA composition for each fraction (algae, animal) of the same coral colony. Also the work by Yamashiro et al. (1999) is the only report on sterol composition. The first aim of this study was therefore to acquire a better knowledge on the production of autotrophic lipids (FA, sterols, and alcohols) and their transfer to the host in the scleractinian coral *Turbinaria reniformis*. For this purpose, coral colonies were experimentally grown under two controlled light levels in order to obtain limited and saturated rates of photosynthesis. The produced lipids were analyzed in parallel in the algal and animal fractions of the same coral colony.

As far as heterotrophy is concerned, few data exist on the effect of feeding on the growth of coral species (Wellington 1982; Anthony and Fabricius 2000; Houlbrèque et al. 2003) or on the cross-effect of light and feeding on coral physiology (Grottoli and Wellington 1999; Grottoli 2002; Houlbrèque et al. 2003). Studies on the role of feeding in lipid production also remain scarce and controversial (Al-Moghrabi et al. 1995; Anthony and Fabricius 2000). Whereas Anthony and Fabricius (2000) found no significant change in lipid content of *Goniastrea retiformis* and *Porites cylindrical* with sediment feeding, Al-Moghrabi et al. (1995) measured a higher PUFA content in *Galaxea fascicularis* fed with *Artemia nauplii*. The second aim of this study was therefore to experimentally investigate the effect of feeding, under two different light levels, both on tissue and skeletal growth of *T. reniformis*, and on its lipid composition.

## Methods

**Experiments**—Experiments were carried out with nubbins of the scleractinian symbiotic coral *T. reniformis*. Colonies have been collected in the Red Sea and maintained in aquaria for several months, at a light level of  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Members of *Turbinaria* species usually live in turbid coastal areas (Veron 2000), and are supposed to rely mainly on heterotrophy. Forty-eight nubbins were obtained by cutting six parent colonies into eight pieces that were  $\sim 2$  cm in diameter, which were distributed into eight 10-L tanks so that each tank contained one nubbin from each parent colony. Nubbins were allowed to heal for 3 weeks before starting the experiments. Tanks were continuously supplied with seawater pumped from a 50-m depth, filtered, and heated to  $26^\circ\text{C}$ . Parameters such as temperature, salinity, pH, and  $\text{pCO}_2$  were constant throughout the experiment.

The experimental design, which ran for 6 weeks, included two different light levels ( $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively;  $12 \text{ h d}^{-1}$ ), crossed with two feeding regimes (starved [S] and fed [F] twice per week). Two tanks were assigned for each condition. The following treatments were therefore achieved: starved at  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (100 S and 300 S, respectively), and fed at  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (100 F and 300 F, respectively). Temperature was maintained at a constant using temper-

ature controllers and heaters. Light was provided by Hydrargyrum quartz iodide (HQI) lamps (Philips), without ultraviolet radiation (UVR) and the lowest light level was obtained using filters between the lamp and the aquaria.

Natural zooplankton, with a rich energetic value compared to commercially available *Artemia* prey, was supplied to the groups of fed corals as described in Houlbrèque et al. (2003). During the whole experiment (6 weeks), corals were fed twice per week for 3 h with a 250-mL solution of zooplankton ( $800 \text{ prey L}^{-1}$ ). Feeding of corals upon zooplankton was checked under a binocular. At the end of the feeding period, water was entirely renewed.

During the 6-week incubation, coral growth was monitored every week as described below. At completion of the experiment, rates of photosynthesis were also measured. All nubbins were then sampled for the determination of lipid biomarkers, as well as chlorophyll and protein concentrations. Samples were kept frozen ( $-80^\circ\text{C}$ ) until analyses.

**Physiological parameters**—Growth was monitored once a week using the buoyant weight technique (Jokiel et al. 1978; UNESCO 1981) on six nubbins in each condition. Chlorophyll and protein contents were measured on six nubbins in each condition according to Houlbrèque et al. (2003), and normalized to the surface area as described below.

Net photosynthesis and respiration were determined on five nubbins in each condition. Each nubbin was placed in a glass chamber filled with filtered seawater (FSW) at a constant  $26^\circ\text{C}$  and was continuously stirred with a magnetic bar. Measurements were performed at  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and in the dark. The chamber contained a Strathkelvin 928<sup>®</sup> oxygen ( $\text{O}_2$ ) electrode, which was calibrated into air-saturated water (100%  $\text{O}_2$ ) and sodium sulfite saturated solution (absence of  $\text{O}_2$ ), prior to the measurement. The  $\text{O}_2$  measurement was recorded with a computer every 10 s. Surface area was determined wrapping the skeleton with aluminium foil (Marsh 1970) and results were expressed as  $\mu\text{mol O}_2 \text{ h}^{-1} \text{ cm}^{-2}$ .

**Isolation of the zooxanthellae and host tissue**—For lipid compound specific analyses, the tissue of three nubbins (from three different mother colonies) was detached from the skeleton using a water pick and was homogenized with a Potter tissue grinder. The slurry of the three microcolonies was pooled because large quantities are required. Furthermore, this enabled the formation of an integrated signal because no replicate was done, due to the time-consuming protocol.

The homogenate was then centrifuged at  $3000 \times g$  for 5 min. The supernatant, which contained the animal tissue, was centrifuged three times ( $3000 \times g$ ; 5 min) to remove the remaining zooxanthellae and was frozen until further analyses. The pellet containing the zooxanthellae was rinsed three times with 20 mL of FSW, and was further purified from the remaining host debris. Four procedures were tested to obtain pure zooxanthellae: centrifugation,

filtration on 50- $\mu\text{m}$  and 10- $\mu\text{m}$  mesh nets, sucrose and Percoll density gradients. Microscopic observations showed that a large contamination of the zooxanthellae by host tissue remained after the completion of the first three protocols. We therefore used the Percoll density gradient (Tytler and Davies 1983) to isolate clean and viable zooxanthellae. Following this procedure, the pellet was ultracentrifuged in 14 mL of 75% Percoll solution (70,000  $\times$  g; 30 min; 10°C), creating a density gradient: Pure zooxanthellae accumulated in a layer at the bottom of the centrifugation tube while lighter fragments (animal debris with a few zooxanthellae) remained on top of the Percoll solution. Pure zooxanthellae were washed with FSW, centrifuged (3000  $\times$  g; 5 min; three times) and the purification was checked microscopically.

*Compound-specific lipid analyses*—Compound-specific lipid analyses were carried out on the whole coral samples to measure their concentrations and, on the isolated zooxanthellae and host tissue, to identify lipid compounds specific to each compartment. Each sample was a composite pool of three nubbins from three different colonies.

*Lipid extraction*: Samples were thawed and spiked with two internal standards (cholic acid and 5 $\alpha$ -androstane-3 $\beta$ -ol). The whole coral and the isolated zooxanthellae composite samples were plunged into 12 mL of dichloromethane ( $\text{CH}_2\text{Cl}_2$ ):methanol (MeOH; 2:1), stirred, and sonicated for 10 min each. Host-tissue composite samples were extracted with 7 mL of  $\text{CH}_2\text{Cl}_2$ , and stirred for 10 min. The lipid extracts were collected after centrifugation (3000 g; 5 min). The extraction was repeated twice more and the three aliquot extracts from the same composite sample were pooled. Four milliliters of MeOH were added to the total extracts before they were concentrated on a rotary evaporator. The extracts were saponified using 1 mL of potassium hydroxide (KOH) 6% + 1 mL of Milli-Q water (80°C; 1 h). Isolations of the neutral and acidic fractions were done according to the protocol developed by Tolosa and de Mora (2004). Control FSW analysis was carried out to determine blank levels.

*Compound identification and quantification*: The neutral and acidic fractions were derivatized, respectively, with bis-trimethylsilyl-trifluoroacetamide and boron trifluoride methanol  $\text{BF}_3$ :MeOH, prior to gas chromatographic (GC) analyses (Tolosa and De Mora 2004).

Confirmation of peak identity was obtained using a GC coupled to a mass spectrometer (GC-MS; Hewlett-Packard HP 5889B MS "Engine") operated in the electron impact mode at 70 eV.

Quantification of neutral compounds was performed on a Hewlett-Packard HP 5890 Series II with a flame ionization detector (FID) and an on-column injector. The column was an HP-Ultra 2 (25 m  $\times$  0.198 mm i.d.  $\times$  0.33  $\mu\text{m}$  film thickness), and the detector temperature was held at 320°C. The oven temperature was programmed at 4°C min<sup>-1</sup> from 60°C to 310°C, then held 20 min. Quantification of acidic compounds was carried out as

previously described, but with a split-splitless injector and two different columns: (a) an HP-5 (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$  film thickness) and (b) a polar BPX-70 (SGE, 60 m  $\times$  0.32 mm  $\times$  0.5  $\mu\text{m}$  film thickness). The oven temperature for the HP-5 was programmed from 60°C (0.5 min hold) to 310°C at 3°C min<sup>-1</sup> and maintained at 310°C for 30 min. The GC oven for the BPX-70 was programmed from 60°C (0.5-min hold) to 250°C, at 3°C min<sup>-1</sup>.

*Statistical analysis*—Results of the physiological parameters are reported as mean  $\pm$  SD. The interaction between light and feeding was tested on the physiological parameters using a two-way ANOVA and the StatView software. Results were considered significantly different for values of  $p < 0.05$ .

## Results

*Physiological parameters*—Effect of feeding and light: Physiological data obtained for the four coral stocks are presented in Table 1. Table 2 shows that light or feeding significantly affected the chlorophyll contents, which were higher at low light and in fed corals. Feeding, at both light levels, also significantly increased the rates of calcification. Conversely, high light significantly increased the rates of photosynthesis and respiration. Finally, protein concentration was significantly higher in fed corals maintained at high-light conditions.

*Lipid concentrations in the whole coral colony (animal + zooxanthellae)*—Figure 1a shows the lipid content per surface area ( $\mu\text{g cm}^{-2}$ ) of the corals maintained under the four different conditions. FA were the major lipid components (59–73% of total lipids), followed by sterols (23–34%), *n*-alcohols (2–6%), and phytol (<1%). Feeding had a different effect on corals depending on the light level. For corals maintained at low light, total FA and sterols were twice as high in fed as in starved corals (Fig. 1a). Conversely, feeding decreased the lipid content of corals maintained at the higher light level and in particular that of sterols. High light doubled concentrations of all lipid classes of starved corals (Fig. 1), except those of phytol.

*Lipid composition in zooxanthellae and host*—Because the quantity of analyzed material was unknown, lipid analyses carried out on isolated zooxanthellae and host are only reported as relative compositions (e.g., each FA as percentage of total FA, each sterol as percentage of total sterols, and each aliphatic alcohol as percentage of total aliphatic alcohols). Compounds measured in zooxanthellae and host tissue were the same as for the analyses of the whole coral samples.

*Fatty acids*: The zooxanthellae presented a different FA distribution pattern compared to the host (Table 3; Fig. 2). For clarity, Fig. 2 shows only the data obtained during low-light conditions since those obtained during high light were quite similar. In zooxanthellae, C16 FA was predominant, averaging 22% of the total FA. C20:5 n-3

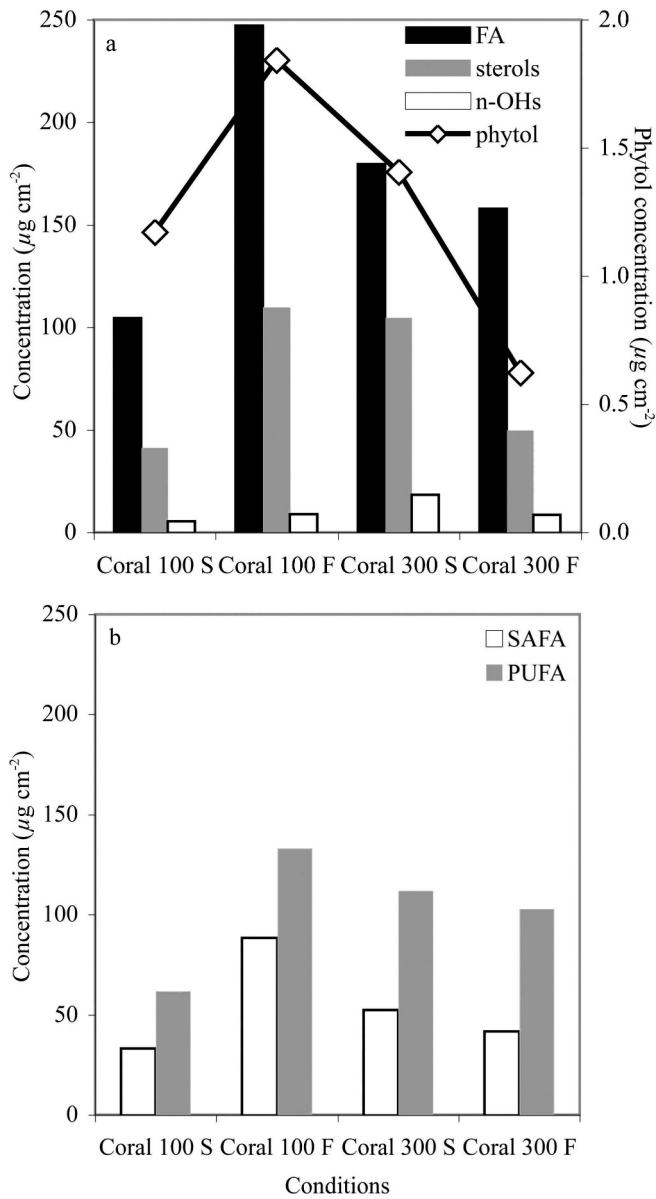


Fig. 1. Concentration ( $\mu\text{g cm}^{-2}$ ) of (a) the different lipid classes and (b) SAFA and PUFA of the whole coral colony (animal + zooxanthellae) in the different experimental conditions (corals exposed to an intensity light level of  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; starved [S] or fed [F]). No standard deviation is available since analyses were carried out on three pooled nubbins. Relative standard deviation of the analytical protocol calculated through the recovery yields of the two internal lipid standards was better than 15% ( $n = 12$ ).

and C22:6 n-3 were also important components (9–13%), followed by C18:4 n-6 (7–10%) and C18:3 n-6 (6–8%). In the animal tissue, C16, C18, and C20:4 n-6 were the dominant FA compounds, averaging 33, 16, and 11% of the total FA. Overall, the FA in the host tissue showed a smaller proportion of PUFA (34%), and especially of n-3 PUFA (7%) than the zooxanthella material (51% and 30%, respectively). Conversely, SAFA and n-6 PUFA were higher in host tissue than in zooxanthellae (53% vs. 37%, respectively, for SAFA and 26% vs. 21%, respectively, for

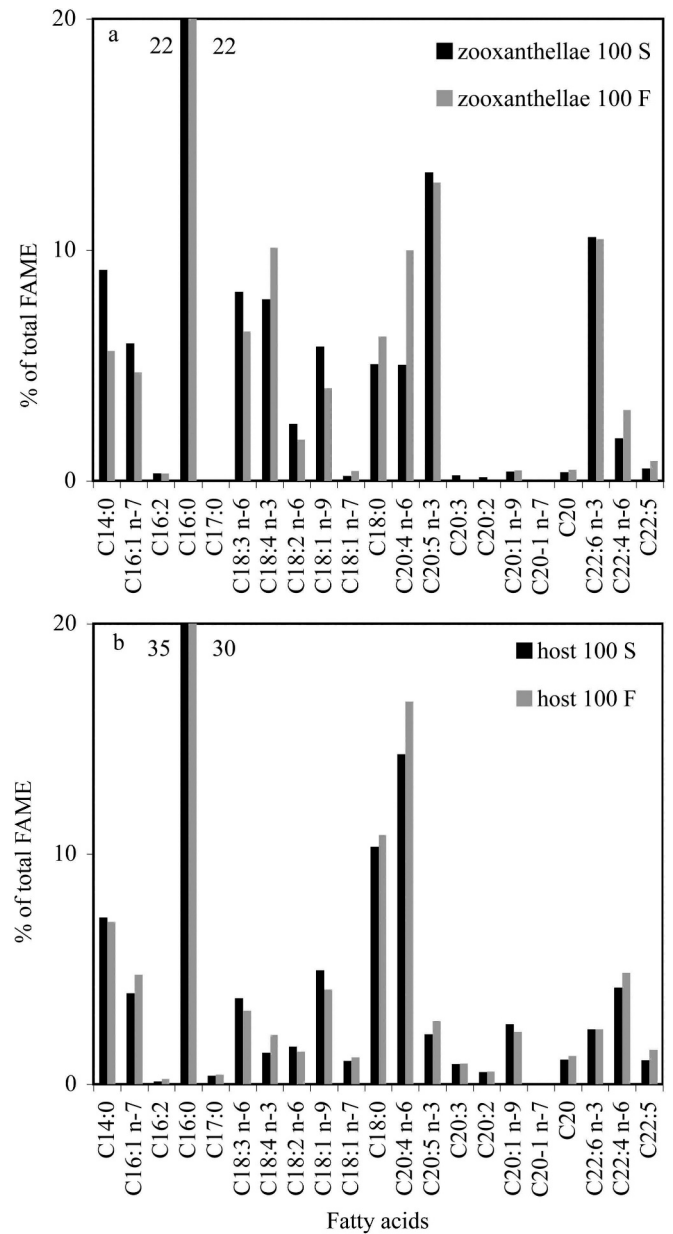


Fig. 2. Fatty acid distribution in (a) zooxanthellae and (b) host tissue from fed (F) and starved (S) corals.

n-6 PUFA). In zooxanthellae, the proportion of n-3 PUFA was inversely related to light intensity (27% vs. 32% for  $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively; Table 3).

Sterol: In both zooxanthellae and host tissue, 24 $\alpha$ -methylcholest-5-en-3 $\beta$ -ol (C<sub>28</sub> $\Delta^5$ ) was the predominant sterol (averaging 72% and 75%, respectively; Table 4). A higher proportion of the 24-propylidene cholesterol was observed in the zooxanthellae compared to the host material (15% and 10%, respectively). High light significantly increased the relative contribution of 24-propylidene cholesterol in zooxanthellae. In the host, feeding resulted in an increase of the 24-propylidene cholesterol percentage at low light and of C<sub>27</sub> $\Delta^5$  at high light.

Table 1. Physiological parameters for *Turbinaria reniformis* corals maintained during 6 weeks under four different conditions: light (100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and 300  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) and feeding (fed [F], starved [S]). Mean  $\pm$  standard deviation ( $n = 6$  for all parameters except photosynthesis and respiration for which  $n = 5$ ).

	100 S	100 F	300 S	300 F
Calcification rates ( $\text{mg g}^{-1} \text{ d}^{-1}$ )	3.38 $\pm$ 0.44	4.03 $\pm$ 0.42	3.59 $\pm$ 1.25	5.03 $\pm$ 0.36
Chlorophyll <i>a</i> ( $\mu\text{g cm}^{-2}$ )	3.76 $\pm$ 0.29	6.29 $\pm$ 1.24	1.21 $\pm$ 0.03	2.08 $\pm$ 0.46
Chlorophyll <i>c</i> <sub>2</sub> ( $\mu\text{g cm}^{-2}$ )	2.72 $\pm$ 0.58	3.01 $\pm$ 0.61	0.88 $\pm$ 0.16	1.83 $\pm$ 0.56
Proteins ( $\text{mg cm}^{-2}$ )	1.74 $\pm$ 0.12	1.47 $\pm$ 0.03	1.53 $\pm$ 0.03	1.91 $\pm$ 0.14
Net photosynthesis ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ cm}^{-2}$ )	0.28 $\pm$ 0.03	0.34 $\pm$ 0.09	0.46 $\pm$ 0.06	0.50 $\pm$ 0.03
Respiration ( $\mu\text{mol O}_2 \text{ h}^{-1} \text{ cm}^{-2}$ )	-0.27 $\pm$ 0.02	-0.28 $\pm$ 0.08	-0.44 $\pm$ 0.10	-0.37 $\pm$ 0.02

**Alcohols:** The major alcohol compounds in the zooxanthellae were phytol—a chlorophyll *a* (Chl *a*)—derived product—and *n*-C<sub>16</sub>OH, which accounted for 70–87% and 13–30% of total alcohols, respectively (Table 5). Host tissue contained *n*-C<sub>14</sub>OH to *n*-C<sub>20</sub>OH with a predominance of *n*-C<sub>16</sub>OH (78%). In contrast to algal cells, the relative phytol content in the host tissue was very low. While feeding did not seem to have any effect, here, the proportion of phytol decreased with increasing light (87% vs. 71% at 100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and 300  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , respectively).

**Lipid composition in zooplankton—**Concerning fatty acids, zooplankton was characterized by high proportions of C22:6 n-3 FA, C16 FA, and C20:5 n-3 FA, which constituted 32%, 16%, and 15%, respectively, of the total FA (Table 3). The major sterol compound was cholesterol (C<sub>27</sub> $\Delta^5$ ; 54%; Table 4), since it results from the conversion of algal sterols by zooplankton (Volkman 1986). Cholesta-5,22-dien-3 $\beta$ -ol (C<sub>27</sub> $\Delta^{5,22}$ ) as well as 24-methylcholesta-5,22(E)-dien-3 $\beta$ -ol (brassicasterol, C<sub>28</sub> $\Delta^{5,22}$ ) were also important compounds, accounting for 20% and 16%, respectively of the total sterols. For alcohols, the ubiquitous *n*-C<sub>16</sub>OH constituted 62%, the monounsaturated long-chain (C<sub>20</sub>–C<sub>22</sub>OH) 10% and phytol <1% of the total alcohols. Sterol together with FA composition suggested a preferential grazing on dinoflagellates and diatoms. Phytoplankton FA are generally assimilated and incorporated unmodified into the zooplankton storage lipids (Lee et al. 2006) whereas sterols are de-alkylated and converted into C<sub>27</sub> $\Delta^5$ . These compounds might be then transferred to the coral by zooplankton ingestion.

Table 2. Results of the two-way ANOVAs testing the effects of light and feeding on the physiological parameters of fed and starved corals maintained under 100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and 300  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Significant values ( $p < 0.05$ ) are in italics.

	Effect of light	Effect feeding	Effect of light $\times$ feeding
Chlorophyll <i>a</i>	<0.001	0.002	0.073
Chlorophyll <i>c</i> <sub>2</sub>	0.001	0.010	0.317
Proteins	0.461	0.987	0.001
Calcification rates	0.060	0.002	0.200
Net photosynthesis	0.022	0.544	0.786
Respiration	0.007	0.955	0.123

## Discussion

**Lipid content and physiological parameters—**Most of the corals are heterotrophs despite the fact that they can meet most of their energetic requirements through the transfer of the symbionts' photosynthates. Information on the relative heterotrophic dependence of corals in relation to other environmental parameters such as light is still lacking (Anthony and Fabricius 2000; Houlbrèque et al. 2003). We are thus providing new insights into the interactions between autotrophy and heterotrophy and into the role of lipids in coral metabolism.

Light affected the lipid content of *T. reniformis*, as observed in other symbiotic organisms (Stimson 1987; Harland et al. 1992). In starved corals, low light decreased lipid concentrations (Fig. 1a,b), because photosynthetically fixed C might not meet the needs for both the coral metabolism and lipid accumulation in the tissue (Anthony et al. 2002). This reduced energy investment was also shown in the corals *Goniastrea retiformis* and *Porites cylindrica* (Anthony and Fabricius 2000) and in the *Nannochloropsis* algae (Sukenic et al. 1989). At low light, however, feeding increased lipid concentrations and rates of calcification, suggesting that the energy gained by feeding goes into these two processes. The effect of heterotrophy on total lipid content was previously highlighted in healthy (Al-Moghrabi et al. 1995) and bleached corals (Grottoli et al. 2006; Rodrigues and Grottoli 2007).

In contrast to low-light conditions, a substantial decrease in sterol and alcohol concentrations was observed in the fed corals at high light, whereas FA concentrations differed less between starved and fed nubbins (Fig. 1a,b). The energy obtained from feeding was preferentially directed toward an increase in chlorophyll concentrations as well as in skeletal growth (Table 1). We can hypothesize that feeding at high light level also increased lipid concentrations, but that skeletal and tissue growth rapidly induced lipid consumption (mainly sterols and *n*-alcohols), as has already been demonstrated with growing cells (Ward 1995; Oku et al. 2002). The response of *T. reniformis* to feeding in the present study was similar to the one observed in *Stylophora pistillata* maintained under the same light level (300  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), for which feeding enhanced all physiological parameters and especially skeletal growth (Houlbrèque et al. 2003, 2004). It also confirms previous results measured in the coral *Goniastrea retiformis* (Anthony and Fabricius 2000), for which the

Table 3. Fatty acid composition (% of the total FA) of zooplankton, and of the zooxanthellae (Zoox) and host tissue (Host). Fatty acids were designated as  $C_n:p$  n- $x$  with  $n$  being the number of carbon atoms,  $p$  being the number of double bonds, the first double bond being located between  $x$  and  $x + 1$  relative to the terminal methyl group (e.g., 20:5 n-3 indicates 20 carbon, 5 double bonds, and ultimate double-bond 3 carbons from the terminal methyl group). F, fed corals; S, starved corals.

% of total FA	Zooplankton	Zoox				Host			
		100 S	100 F	300 S	300 F	100 S	100 F	300 S	300 F
C12	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1
C14:1	0.0	0.6	0.4	0.5	0.5	0.2	0.2	0.1	0.1
C14	6.6	9.1	5.6	6.4	7.7	7.3	7.0	4.2	5.3
C15	0.6	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1
C16:2	0.1	0.3	0.3	0.2	0.2	0.1	0.2	0.1	0.1
C16:1 n-7	2.7	5.9	4.7	3.2	4.0	4.0	4.8	2.3	2.9
C16	16.0	21.6	21.6	22.2	23.9	34.8	30.4	35.9	34.7
C17	0.1	0.0	0.0	0.0	0.0	0.4	0.4	0.3	0.3
C18:3 n-6	3.0	8.2	6.5	7.6	7.5	3.8	3.2	3.6	3.4
C18:4 n-3	2.7	7.9	10.1	7.0	7.0	1.4	2.1	0.8	1.0
C18:2 n-6	6.6	2.5	1.8	2.5	2.4	1.6	1.4	1.8	1.7
C18:1 n-9	1.6	5.8	4.0	6.6	6.2	4.9	4.1	6.7	5.3
C18:1 n-7	0.0	0.2	0.4	0.5	0.4	1.0	1.2	1.6	1.2
C18	6.3	5.0	6.2	8.3	6.9	10.3	10.8	11.4	11.7
C20:4 n-6	0.0	5.0	10.0	9.1	7.7	14.3	16.6	13.5	14.8
C20:5 n-3	15.2	13.4	12.9	8.7	9.5	2.2	2.8	1.6	1.8
C20:4 n-3	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	0.0	0.2	0.0	0.3	0.3	0.9	0.9	1.3	1.1
C20:2 n-6	1.1	0.2	0.0	0.4	0.3	0.5	0.6	0.9	0.8
C20:1 n-9	1.4	0.4	0.5	0.8	0.6	2.6	2.3	3.2	2.7
C20:1 n-7	0.6	0.0	0.0	0.0	0.0	0.2	0.3	0.3	0.2
C20	0.2	0.4	0.5	0.7	0.7	1.1	1.2	1.3	1.2
C22:6 n-3	31.9	10.6	10.5	10.8	10.3	2.4	2.4	2.6	2.3
C22:4 n-6	0.0	1.9	3.1	3.4	2.9	4.2	4.9	4.3	4.6
C22:5 n-3	0.8	0.5	0.9	0.7	0.7	1.0	1.5	1.2	1.4
C22:3 n-6	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.2
C22:1 n-11	0.3	0.0	0.0	0.0	0.0	0.3	0.3	0.4	0.5
C22	0.1	0.2	0.0	0.3	0.2	0.1	0.0	0.1	0.1
Σ SAFA	30.0	36.4	33.9	37.9	39.5	54.1	50.0	53.5	53.6
Σ MUFA	6.6	13.0	10.0	11.5	11.7	13.2	13.2	14.6	12.9
Σ PUFA	62.6	50.6	56.1	50.6	48.8	32.7	36.8	31.9	33.5
Σ n-3 PUFA	51.7	32.3	34.4	27.1	27.5	7.0	8.8	6.2	6.6
Σ n-6 PUFA	10.7	18.0	21.4	23.3	21.1	25.5	27.8	25.7	26.8
Σ n-3 PUFA : Σ n-6 PUFA	4.8	1.8	1.6	1.2	1.3	0.3	0.3	0.2	0.2
(Σ SAFA+Σ MUFA) : Σ PUFA	0.58	0.98	0.78	0.97	1.05	2.06	1.72	2.14	1.99

Table 4. Sterol composition (% of total sterols) of the zooplankton and of the separated zooxanthellae (Zoox) and host tissue (Host). <sup>1</sup>C<sub>26</sub>Δ<sup>5,22</sup>: 24-Norcholesta-5,22(E)-dien-3β-ol; C<sub>27</sub>Δ<sup>5,22</sup>: Cholesta-5,22(E)-dien-3β-ol; C<sub>27</sub>Δ<sup>5</sup>: Cholest-5-en-3 β-ol (cholesterol); C<sub>28</sub>Δ<sup>5,22</sup>: 24-Methylcholesta-5,22(E)-dien-3β-ol; C<sub>28</sub>Δ<sup>5</sup>: 24-Methylcholesta-5,-en-3β-ol; C<sub>28</sub>Δ<sup>5,24(28)</sup>: 24-Methylcholesta-5,24(28)-dien-3β-ol; C<sub>29</sub>Δ<sup>5,22</sup>: 24-Ethylcholesta-5,22(E)-dien-3β-ol; C<sub>29</sub>Δ<sup>5</sup>: 24-Ethylcholest-5-en-3β-ol; C<sub>30</sub>Δ<sup>5,24(28)</sup>: 24-Propylcholesta-5,24(28)-dien-3β-ol (24-propylidene cholesterol). F, fed corals; S, starved corals.

% of total sterols	Zooplankton	Zoox				Host			
		100 S	100 F	300 S	300 F	100 S	100 F	300 S	300 F
C <sub>26</sub> Δ <sup>5,22</sup>	3.0	–	–	–	–	–	–	–	–
C <sub>27</sub> Δ <sup>5,22</sup>	20.3	–	–	–	–	–	–	–	–
C <sub>27</sub> Δ <sup>5</sup>	54.1	6.7	6.3	6.2	6.3	8.0	8.0	7.8	8.7
C <sub>28</sub> Δ <sup>5,22</sup>	16.0	6.2	5.8	7.1	6.8	7.4	6.4	7.1	6.8
C <sub>28</sub> Δ <sup>5</sup>	–	73.2	72.5	70.4	70.0	76.0	75.1	74.7	74.1
C <sub>28</sub> Δ <sup>5,24(28)</sup>	2.7	–	–	–	–	–	–	–	–
C <sub>29</sub> Δ <sup>5,22</sup>	0.5	–	–	–	–	–	–	–	–
C <sub>29</sub> Δ <sup>5</sup>	1.4	–	–	–	–	–	–	–	–
C <sub>30</sub> Δ <sup>5,24(28)</sup>	0.8	13.9	15.4	16.3	16.8	8.6	10.4	10.4	10.4

Table 5. Alcohol composition (% of total alcohols) of the zooplankton and of the separated zooxanthellae (Zoox) and host tissue (Host). F, fed corals; S, starved corals.

% of total alcohols	Zooplankton	Zoox				Host			
		100 S	100 F	300 S	300 F	100 S	100 F	300 S	300 F
C14-OH	15	—	—	—	—	1.8	1.9	1.4	1.4
C15-OH	1	—	—	—	—	—	—	—	—
C16:2-OH	—	—	—	—	—	0.4	0.6	0.4	0.4
C16:1-OH	2.1	—	—	—	—	2.5	3.4	3.4	2.8
C16-OH	62	13.3	13.1	30.8	22.1	82.8	80.0	73.1	77.7
C17-OH	—	—	—	—	4.7	0.6	1.2	1.0	1.3
C18:2-OH	—	—	—	—	—	1.4	1.1	2.2	2.1
C18:1-OH	2.5	—	—	—	—	3.1	2.3	5.5	4.3
C18-OH	4.2	—	—	—	—	4.4	3.2	4.7	4.7
C20-OH	0.9	—	—	—	—	2.0	4.5	7.8	4.6
C20:1-OH	4.6	—	—	—	—	—	—	—	—
C22-OH	0.9	—	—	—	—	—	—	—	—
C22:1-OH	3.8	—	—	—	—	—	—	—	—
C24-OH	0.9	—	—	—	—	—	—	—	—
C24:1-OH	1.7	—	—	—	—	—	—	—	—
Phytol	0.7	86.7	86.9	69.2	73.2	0.9	1.8	0.5	0.7

highest growth rate was obtained at high-light and feeding conditions.

*Lipid composition in zooxanthellae*—Following the work of Papina et al. (2003), this study is the second one that investigates the lipid composition of zooxanthellae and tissue extracted from the same coral colony. Lipid composition was previously assessed in the entire animal (Latyshev et al. 1991; Harland et al. 1993; Yamashiro et al. 1999) or in zooxanthellae in culture (CZ; Al-Moghrabi et al. 1995), these latter having a different metabolism than the in hospite zooxanthellae especially concerning their photosynthetic processes (Goiran et al. 1996; Allemand et al. 1998). Finally, studies performed on freshly isolated zooxanthellae (FIZ) were not coupled with measures on the animal host (Zhukova and Titlyanov 2003, 2006). This coupling is, however, important in order to assess the transfer of “autotrophic” lipids.

The FA distribution in the zooxanthellae (Fig. 2a) is rather similar to previous reports, with a majority of C16, and PUFA (C20:5 n-3 and C22:6 n-3, C18:3 n-6, and C18:4 n-3; Latyshev et al. 1991; Al-Moghrabi et al. 1995; Papina et al. 2003). C22:6 n-3 and C18:4 n-3 are considered to be specific Dinophyceae markers, since C22:6 n-3 is rare in other microalgae (Viso and Marty 1993; Papina et al. 2003; Zhukova and Titlyanov 2003). As expected, this composition was slightly different from the CZ, in which C18:4 n-3 was missing (Al-Moghrabi et al. 1995). The dinoflagellate markers C18:5 n-3 and C18:3 n-3 were also missing, unlike other studies (Al-Moghrabi et al. 1995; Papina et al. 2003), perhaps due to the fact that they can be easily metabolized or due to interspecific variations.

Light level influenced the zooxanthellae production of PUFA, since high light induced a decrease in the n-3 PUFA (33% vs. 27%, respectively; Table 3), which are thylakoid membrane constituents. This decrease might be linked to the parallel decrease in chlorophyll and phytol concentrations. Similar observations were made on microalgae

(Sukenic et al. 1989), dinoflagellates in culture (Parrish et al. 1994), and zooxanthellae (Zhukova and Titlyanov 2006).

Conversely to light, the effect of host feeding on the zooxanthellae has never been investigated. The present study shows that “fed” zooxanthellae maintained at low light increased the percentage of PUFA compounds (Table 3), and in particular the C20:4 n-6, which is the major PUFA compound of the host tissue. These results suggest that the animal might synthesize de novo and partially transfer this compound to the zooxanthellae or that zooxanthellae preferentially accumulate this compound when the animal is fed with zooplankton. This trend is different at high light and may be due to the consumption of lipids used for the coral metabolism.

Concerning the other classes of lipids, no previous study has investigated the effect of light and feeding on their composition. This study demonstrated that zooxanthellae were mostly enriched in C<sub>28</sub>Δ<sup>5</sup> and 24-propylidene cholesterol (Table 4). The C<sub>28</sub>Δ<sup>5</sup> proportion seemed to slightly increase under low-light conditions, suggesting that it might have a function in thylakoid membranes. No substantial effect of feeding was observed on the sterol composition of zooxanthellae. C<sub>16</sub>OH and phytol were the unique alcohols detected in the zooxanthellae material. A decrease in phytol production was observed at high light (87% vs. 72%, respectively; Table 5), and can be linked to the decrease in Chl *a*, from which it is derived.

*Lipid composition of the animal*—Transfer of lipids between the zooxanthellae and the animal: PUFA were important components of the host tissue (34% of total FA in average; Table 3). Their proportions were similar to those found in other studies (Harland et al. 1993; Al-Moghrabi et al. 1995; Bachok et al. 2006). This dominance of PUFA is a major characteristic of marine lipids, even if the proportions vary between species (Meyers 1979;

Yamashiro et al. 1999) or with temperature (Meyers 1979). The proportion of PUFA, especially n-3 series (mostly C20:5 n-3 and C22:6 n-3) were larger in the zooxanthellae than in the host (Table 3; Fig. 2), suggesting that these compounds are first produced in the algae and then transferred to the animal (Papina et al. 2003). These suggestions were confirmed by the two following observations: (1) animals have a limited ability to synthesize long-chain PUFA with more than four double bonds, which are mostly obtained through the diet (Volkman et al. 1989; Viso and Marty 1993); (2) C18:4 n-3 and C20:5 n-3, phytoplankton markers, decreased with light simultaneously in the animal and zooxanthellae fractions, which confirms their algal origin (Papina et al. 2003).

C16 FA was the major product of the animal fraction (Fig. 2), where it was found in relatively larger proportions than in the zooxanthellae. Consequently, the ratio ( $\Sigma$  SAFA +  $\Sigma$  MUFA): $\Sigma$  PUFA was also slightly higher in the host, confirming that the host tissue contains larger lipid reserves. C20:4 n-6 is the second major product, being either selectively concentrated from zooxanthellae products or, more likely, synthesized de novo by the host by  $\Delta 5$  and  $\Delta 6$  desaturation of the algal C18:2 n-6.

Although feeding influenced most of the physiological parameters of the corals and their total lipid concentrations, it affected only slightly the FA distribution in the coral tissue. Indeed, very little increase of the C20:5 n-3 and C22:6 n-3 FA was observed in fed host-tissue, despite the fact that these FA were abundant in zooplankton (Table 3). The lack of increase in zooplankton FA in our study might be due to the fact that most of the components derived from zooplankton were either respired or/and transferred from the animal to the algae, which transformed them into essential FA, and relocated to the animal. Patton et al. (1977) indeed suggested that there is a translocation of acetyl units from the coral to the zooxanthellae, which then synthesize the majority of coral lipids. They also suggested that diet PUFA might not be found in host tissue due to its oxidation to acetate, in which form it would be absorbed by the zooxanthellae and reconverted into fatty acids of a higher degree of unsaturation.

Yamashiro et al. (1999) carried out the only other study concerning sterols and determined the existence of C27 to C30 sterols. Among the four sterols identified, only C<sub>27</sub> $\Delta^5$  and C<sub>28</sub> $\Delta^5$  were in common with those of the present study. So far 24-propylidene cholesterol has not been reported in corals, and it was preferentially concentrated in the zooxanthellae. In contrast, C<sub>27</sub> $\Delta^5$  as well as C<sub>28</sub> $\Delta^5$  were preferentially concentrated in the host tissue. Similarly to the FA, no increase in the predominant zooplankton sterol C<sub>27</sub> $\Delta^5$  was observed under feeding (Table 4).

In conclusion, this experimental study has shown that feeding sustains lipid production in *T. reniformis*, particularly when light, i.e., zooxanthellae photosynthesis, is limited. Part of the food input was directly transformed into FA by the zooxanthellae and shared by the two symbionts. An effect of feeding on the maintenance of lipid stocks under reduced photosynthesis was observed in situ for other coral species during a bleaching event (Grottoli et al. 2006; Rodrigues and Grottoli 2007). These observations

suggest that feeding might be an important parameter for corals in deep waters or whenever stress reduces photosynthesis. When photosynthesis is maximal, heterotrophic assimilation of nutrients leads to a general increase in tissue and skeletal growth rather than lipid reserves. This study is one of the few investigating the lipid composition of each coral partner and showed that FA were the main lipid compounds found both in the animal and algal fractions. PUFA, especially n-3 series (mostly C20:5 n-3 and C22:6 n-3) showed larger proportions in the zooxanthellae than in the host (Table 3; Fig. 2), suggesting that these compounds are first produced in the algae and then transferred to the animal. Additional investigations taking into account both algal and animal fractions are necessary to further study the symbiosis.

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Received: 6 September 2007

Accepted: 29 April 2008

Amended: 4 June 2008