The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T. reniformis* under a short-term temperature increase

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Abstract

Worldwide increase in seawater temperature represents one of the major threats affecting corals, which experience bleaching, and thereafter a significant decrease in photosynthesis and calcification. The impact of bleaching on coral physiology may be exacerbated when coupled with eutrophication, i.e., increasing plankton, inorganic nutrient concentrations, sedimentation and turbidity due to coastal urbanization. Whereas zooplankton provision (heterotrophy) may alleviate the negative consequences of thermal stress, inorganic nutrient supply may exacerbate them, which creates a paradox. Our experimental study aims to disentangle the effects of these two components of eutrophication on the physiological response of *Turbinaria reniformis* subject to normal and to a short-term temperature increase. Additionally, three different inorganic nutrient ratios were tested to assess the influence of nutrient stoichiometry on coral physiology: control (ambient SW 0.5 μM N and 0.1 μM P), N only (ambient + 2 μM N) and N + P (ambient + 2 μM N and + 0.5 μM P). Our results show a deleterious effect of a 2 μM nitrate enrichment alone (N) on coral photosynthetic processes under thermal stress as well as on calcification rates when associated with heterotrophy. On the contrary, a coupled nitrate and phosphorus enrichment (N + P) maintained coral metabolism and calcification during thermal stress and enhanced them when combined with heterotrophy. Broadly, our results shed light on the tight relationship existing between inorganic nutrient availability and heterotrophy. Moreover, it assesses the relevance of N: P stoichiometry as a determining factor for the health of the holobiont that may be adapted to specific nutrient ratios in its surrounding environment.

Rising seawater temperature and eutrophication are currently two of the most serious factors threatening coral reefs throughout the world (Fabricius 2011; Fabricius et al. 2011; Fabricius et al. 2013). Over the last 30 yrs, global climate change has indeed increased average oceanic temperature by approximately 0.2°C per decade (Hansen et al. 2006). Therefore, the world's oceans are warmer now than at any point in the last 50 yrs and seawater temperature is expected to continue increasing. Thermal stress has detrimental effects on many marine organisms, some of which have already started to reach the limits of their capacity to persist (Visser 2008). In symbiotic corals, high temperatures induce bleach-ing (i.e., the loss of the dinoflagellate symbionts of the genus *Symbiodinium* sp.), thus weakening the symbiotic association (Brown 1997). *Symbiodinium*, through inorganic nutrient acquisition and photosynthesis, provide 75–100% of the daily metabolic requirements of their host (Grottoli et al. 2006; Tremblay et al. 2012). Therefore, bleaching induces the loss of the coral's major source of nutrition and can cause widespread mortality (Glynn 2012). In addition, human activities such as increased coastal fertilizer use, land clearing, and urbanization contribute to eutrophication (enhanced nutrients, particles, and sedimentation levels) of coastal sites and have modified the nutrient stoichiometry in these ecosystems (Sterner and Elser 2002). Eutrophication alters coral health because it enhances algal growth and induces competition for space and light (Bruno et al. 2009; Norström et al. 2009; De'ath and Fabricius 2010; Graham

et al. 2015). It has also been suggested that elevated inorganic nutrient levels directly impact corals by decreasing their thermal thresholds for bleaching. Nutrient addition indeed tends to enhance symbiont growth inside the coral host tissue, increasing oxidative stress during warm seasons (Nordemar et al. 2003; Wooldridge 2009; Wooldridge and Done 2009; Wiedenmann et al. 2013; Vega Thurber et al. 2014). Therefore, there is evidence to suggest that corals living in nutrient-rich environments that are also experiencing thermal stress may be particularly endangered.

However, several laboratory experiments have also shown that nutrient supplementation may increase the ability of corals to withstand climate change stress because it can offset the negative effect of a temperature increase by favoring symbiont growth and photosynthesis. Nutrients can be acquired either via heterotrophy, the capture of zooplankton prey and or particulate organic matter (POM) by the coral host, or via autotrophy, via the uptake of inorganic nutrients (e.g., nitrate, ammonium, phosphate) by the symbionts. Aside from improving coral metabolism under nonstressful conditions (Houlbrèque and Ferrier-Pagès 2009), several studies have indeed identified coral heterotrophy as an indicator of resilience to bleaching stress (Grottoli et al. 2006; Rodrigues and Grottoli 2007; Palardy et al. 2008; Connolly et al. 2012). In their model, Anthony et al. (2009) stated that increased heterotrophy was a good predictor of survivorship and reduced mortality risk during a bleaching event, and Tolosa et al. (2011) discovered that zooplankton feeding played an essential role in sustaining *Turbinaria reniformis* metabolism under thermal stress. Furthermore, moderate inorganic nitrogen supply has been shown to promote coral growth and metabolism (Tanaka et al. 2007; Sawall et al. 2011), especially under elevated pCO_2 (Langdon and Atkinson 2005; Holcomb et al. 2010; Chauvin et al. 2011), or thermal stress (Béraud et al. 2013). Based on these studies, there are lines of evidence to suggest that corals thriving in nutrient-rich environments may be more resilient to stress than others, if not in competition with algae.

In view of this conflicting evidence, more research is needed to understand the effects of heterotrophy and/or inorganic nutrient supplementation on coral metabolism during environmental stresses. Several hypotheses for the differing results have been put forward. In their meta-analysis of the effects of eutrophication on corals, Shantz and Burkepile (2014) assert that the physiological response of corals to eutrophication is context-dependent. Thus, when comparing studies, several parameters such as coral taxa, coral morphology, nutrient concentration and nitrogen source, and type (i.e., ammonium vs. nitrate, organic vs. inorganic) have to be taken into account. More importantly, the stoichiometry of the N: P ratio needs to be close to the N: P ratio of the coral tissue, i.e., a complete lack of phosphate with nitrate enrichment may be unfavorable for the symbiosis (Wiedenmann et al. 2013).

In coastal ecosystems, eutrophication enhances both POM and inorganic nutrient concentrations. Nutrient-rich reefs tend to have higher gross production as well as higher heterotrophic consumption than oligotrophic reefs (Mioche and Cuet 1999; Szmant 2002). To date, only two studies have attempted to decipher the effect of the different components of eutrophication (POM, inorganic nutrient, sedimentation and turbidity) on coral growth and survival (Fabricius 2005; Fabricius et al. 2013). The first study by Fabricius (2005) highlighted sedimentation and turbidity as the two major factors of stress for coral growth. Conversely, their model suggested an increase in growth with POM, and a moderate reduction in growth with dissolved inorganic nutrient enrichment. Fabricius et al. (2013), however, later observed low coral growth under a coupled enrichment in dissolved inorganic nutrients (nitrate) and POM. In view of these contrasting observations on coral growth, more work is needed to improve our knowledge of the effect of eutrophication on the coral physiological response in terms of photosynthesis, calcification, and tissue growth. In this experimental study, we sought to disentangle the combined effect of POM (heterotrophy) and inorganic nutrient enrichment from their individual effects on the physiological response of the scleractinian coral *T. reniformis* maintained under ambient temperature conditions as well as during short-term thermal stress. In addition, we implemented different N : P ratios (4 : 1, 5 : 1, and 20 : 1) of inorganic nutrients in seawater to assess the effect of nutrient stoichiometry on coral physiology and symbiotic interactions. We hypothesize that (1) heterotrophy has a different effect than inorganic nutrient supplementation on coral metabolism; (2) the inorganic N : P ratio shapes the metabolic response of *T. reniformis* under both normal and stressful conditions; nitrate enrichment being more detrimental to coral physiology than a combined enrichment in nitrate and phosphate (Wiedenmann et al. 2013); (3) the combined effect of heterotrophy and inorganic nutrient supplementation differs from their individual effects on coral physiology.

Materials and procedures

Experimental set-up

The widely distributed scleractinian coral *T. reniformis* (Dendrophylliidae) was used in this study due to its ability to thrive in turbid and organically rich environments (Veron 2000; Anthony et al. 2006). Five large colonies were sampled between 5 m and 10 m depth in different locations of the Northern Red Sea, brought back to the laboratory, and tagged with an electronic microchip implant to account for parent colony before being used to produce a total of 456 nubbins of about the same size (4 cm diameter). Nubbins were then evenly distributed into 12 independent 20 L tanks (38 nubbins per tank), maintained under the following controlled conditions: aquaria were continuously supplied with

Table 1. Experimental conditions for the six different treatments: Control condition (C), Control condition + zooplankton provision (Heterotrophy) (CH), Nitrate enrichment (N), Nitrate enrichment + Heterotrophy (NH), Nitrate and phosphorus enrichment (NP), Nitrate and phosphorus enrichment + Heterotrophy (NPH).

	Treatments					
Characteristics	C	CH	N	NH	NP	NPH
Natural seawater*	x	x	x	x	x	x
Supply of 2000 artemia d^{-1}		x		x		x
2 μM nitrate addition			x	x	x	x
0.5 μM phosphate addition					x	x

*(< 0.05 μM N and < 0.1 μM P).
The "x" indicates presence of each of the characteristics for each treatment.

oligotrophic seawater containing a low amount of nutrients (~ 0.5 μM nitrate (NO_3) and ~ 0.1 μM phosphate, PO_4) at a flow rate of 20 L h^{-1}. A constant seawater temperature of 25°C $\pm$ 0.5°C was regulated using submersible resistance heaters (Visi-ThermH Deluxe, Aquarium Systems, France) and metal halide lamps (Philips, HPIT 400W, Distrilamp, Bossee, France) provided a constant irradiance of 200 μmol photons m^{-2} s^{-1} inside the tanks (12: 12 light: dark). Submersible pumps ensured water mixing in the tanks. Nubbins were kept unfed for four weeks to prevent any impact of heterotrophy on the physiological responses of corals before performing the experiments described below. After the acclimation period, six different nutritional conditions were established (in duplicated tanks) as described in Table 1 and below. Tanks were cleaned weekly to avoid any algal proliferation.

Four control tanks were maintained in duplicate under low inorganic nutrient concentrations (as described above), with or without zooplankton (heterotrophy). In four other tanks, supplied with or without zooplankton, nitrate was added to reach a N: P ratio of 20: 1. Finally, in the last four tanks, maintained with or without zooplankton, nitrate and phosphate were added in parallel to reach a N: P ratio of 4 : 1. Zooplankton was provided in the "heterotrophic" tanks every evening for 5 d per week, by means of ca. 2000 *Artemia salina* (in total) that were left in the tanks until they were eaten or diluted with the seawater renewal. For the nitrate and nitrate-phosphate enriched conditions, tanks were, respectively, supplied with a solution of sodium nitrate ($NaNO_3$) alone or in combination with sodium dihydrogenophosphate (NaH_2PO_4). Stock solutions were continuously pumped from individual batch tanks via a peristaltic pump and delivered to the experimental tanks at a final concentration of 2.0 μM for $NaNO_3$ and 0.5 μM for NaH_2PO_4. The stock solutions were renewed every 3 d and added to the tanks at a constant flow rate of 0.3 L h^{-1}.

Inorganic nutrient concentrations in the water were monitored in each tank and in triplicate twice a week using an Autoanalyzer (Alliance Instrument, AMS, France) according to Tréguer and Le Corre (1975). After 3 weeks under these experimental conditions (T_0), temperature was increased from 25°C $\pm$ 0.5°C to reach 30°C $\pm$ 0.5°C over a 10 day-period and was kept constant for 7 d (T_1). This thermal stress was chosen according to the highest temperature observed in the northern Red Sea (Sawall et al. 2014). After 17 d under thermal stress, all tanks were brought back to 25°C $\pm$ 0.5°C over 10 d for two additional weeks to assess recovery (T_2). Several measurements (described below) were performed to assess physiology and photophysiology of *T. reniformis* at the end of each temperature step (T_0, T_1, and T_2).

Measurements

Photosynthesis and respiration rates, total chlorophyll, symbiont density, and protein determination

Rates of net photosynthesis (Pn) and respiration (R) were assessed at 200 and 0 μmol photons m^{-2} s^{-1} on six randomly selected nubbins per treatment in 50 mL glass chambers supplied with filtered seawater (FSW). Chambers were equipped with a Unisense optode (oxygen-sensitive minisensor) connected to the Oxy-4 software (Chanel fiber-optic oxygen meter, Presens, Regensburg, Germany). The optodes were calibrated before each experiment against air-saturated and nitrogen-saturated seawater (for the 100% and 0% oxygen, respectively). Stir bars were used to continuously mix the water. Light was provided by a metal halide lamp (Philips, HPIT 400W, Distrilamp, Bossee, France). Pn and R were calculated by regressing oxygen production or consumption against time. Gross photosynthesis was estimated by adding the absolute value of R to Pn. At the end of each incubation, nubbins were frozen overnight (-20°C) before the subsequent determination on the same nubbins of symbiont density, total chlorophyll, and protein concentration according to Hoogenboom et al. (2010). Briefly, tissue was removed from the skeleton of each nubbin with an air-pick, and collected in 10 mL of 0.45 μM FSW. Tissue slurry was then homogenized with a potter grinder and divided into 5 mL subsamples. The first subsample was used for the determination of zooxanthellae density and protein concentration. Density of symbionts was quantified (in 500 μL) using an inverted microscope (Leica, Wetzlar, Germany) and the Histolab 5.2.3 image analysis software (Microvision, Every, France). Protein concentration was assessed according to Smith et al. (1985) using a BCAssay Protein Quantification Kit (Uptima, Interchim) and a Xenius® spectrofluorometer (SAFAS, Monaco). The second 5 mL subsample was centrifuged at 8000 $\times$ g for 10 min. Supernatant was removed and the symbionts resuspended in 5 mL acetone for chlorophyll a (Chl a) and c_2 extractions. Chlorophyll concentration was then determined according to the method of Jeffrey and

Humphrey (1975) with a spectrophotometer. Data were normalized to the upper side surface area (cm^2) of each nubbin, using the wax-dipping method from Veal et al. (2010).

Relative electron transport rate

Measurements were performed on six nubbins per treatment as for photosynthesis. A pulse amplitude modulation (PAM) fluorometer [DIVING-PAM, Walz, Germany (Schreiber et al. 1986)] was used to measure the minimal (F_0) and maximal (F_m) Chl *a* fluorescence after 5 min of dark adaptation by applying a weak pulse red light (max. intensity < 1 mol photon m^{-2} s^{-1}, width 3μs, frequency 0.6kHz) and a saturating pulse of actinic light (max. intensity 5000 μmol photon m^{-2} s^{-1}, pulse width 800 ms) on coral nubbins placed under a constant distance from the optical fiber. The rapid light curve function of the PAM assessed the relative electron transport rate (rETR) after exposing nubbins to eight light intensities (from 69 μmol photons s^{-1} m^{-2} to 1500 μmol photons s^{-1} m^{-2}) over 10 s.

Feeding rates

Feeding rate experiments were conducted as described in Houlbrèque et al. (2004). Each coral nubbin was incubated in individual Plexiglas flumes of 1 L, specially designed for this kind of measurement according to Levy et al. (2001), based on the procedures described by Vogel and LaBarbera (1978). Flow speed was approximately 4 cm s^{-1} as this was found to allow for maximum feeding in *T. reniformis* (Ferrier-Pagès et al. 2010), and chambers were placed in a water bath maintaining constant temperature. Measurements were run at T_0, T_1, and T_2 on fed nubbins only (Table 1, $n = 6$ per treatment). When polyps were fully expanded, the experimental run was started by delivering 1000 *A. salina* nauplii L^{-1} according to Gori et al. (2015), previously quantified using a Bogorov counting chamber. Six replicated 10 mL seawater samples were taken from the flow chamber just after the start of the experiment (0 min), then at 15 min, 30 min, 45 min, and 60 min. *A. salina* nauplii in each sample were counted and immediately replaced in the chambers to avoid any concentration decrease. Three control experiments were also conducted at each temperature step (prey only, without corals) to account for possible concentration decrease due to nauplii becoming trapped in the flume. Feeding rates were calculated as the decrease in prey concentration against time and normalized to number of polyps.

Calcification rates

Growth rates ($n = 10$ per treatment) were monitored weekly using the buoyant weight technique (Davies 1989). Growth data are presented as percent weight increase per day, using the equation: [(*F-I*)/(*I* × number of days)] × 100, where *F* is the weight of each nubbin at the end of a given time step (T_0, T_1, T_2) and *I* is the weight at the beginning of a given time step.

Statistical analyses

Statistical analyses were processed using the program JMP Version 11.0.0. Data were checked for normality using the Kolmogorov–Smirnov test with Lilliefors correction and for homoscedasticity using Levene's test. When conditions were not fulfilled, a data transformation (natural logarithms) was used. Preliminary partly nested ANOVA tests were used to test for any possible tank effect. This effect was not significant for any of the parameters so this factor was excluded from analyses (Underwood 1997). A general linear model for parametric repeated measure analysis of variance (ANOVA) was performed on the feeding and growth rate response variables using "Temperature" as dependent variable and "Inorganic" enrichment, "Organic" feeding (zooplankton provision) and the corresponding sample as categorical predictors. The assumption of sphericity (independency of the repeated measures) was tested. When not fulfilled, the hypotheses were tested using the multivariate approach (Wilks test) for repeated measurements. A Three-Way ANOVA was then performed on all other studied variables with "Inorganic" enrichment, "Organic" feeding, and "Temperature" as factors. When significant differences appeared, analyses were followed by a posteriori testing (Tukey's HSD test). *p*-values were considered significant for $p < 0.05$.

Assessment

Evolution of the treatments during the experiment

During heat-stress, unfed control corals (C) significantly decreased their symbiont density (Table 4, Tukey HSD, $p = 0.007$) as well as their protein content (Table 4, Tukey HSD, $p = 0.035$). In addition, the rETR of all control corals (C and CH) was lower during thermal stress (Fig. 2, Tukey HSD, $p < 0.0055$), while respiration rates and Pg were increased (Fig. 1, Tukey HSD, $p < 0.0445$ and $p < 0.0013$, respectively). During the recovery period, Pg of fed corals and R of unfed corals decreased and returned to their basal level measured at 25°C (Tukey HSD, $p = 0.028$ and $p = 0.0002$, respectively, compared to 30°C).

During thermal stress in the nitrate-enriched treatments, rETRmax of unfed colonies (Fig. 2, Tukey HSD, $p < 0.0001$ and Tukey HSD, $p = 0.047$, respectively) and calcification rates of fed colonies (Fig. 2, Table 5, Tukey HSD, $p = 0.02$, respectively) significantly decreased. Pg increased slightly for NH corals (Tukey HSD, $p = 0.0115$), while no difference was observed for N nubbins compared to control conditions (Tukey HSD, $p > 0.05$). During the recovery period, calcification rates of fed corals remained very low, while protein content largely increased compared to 25°C and 30°C (Table 5, Tukey HSD, $p < 0.012$). Symbiont density of unfed corals significantly increased compared to 30°C (Tukey HSD, $p = 0.0046$).

NP-enriched corals (NP and NPH) significantly changed their physiology throughout the entire study. At 30°C, Pn of
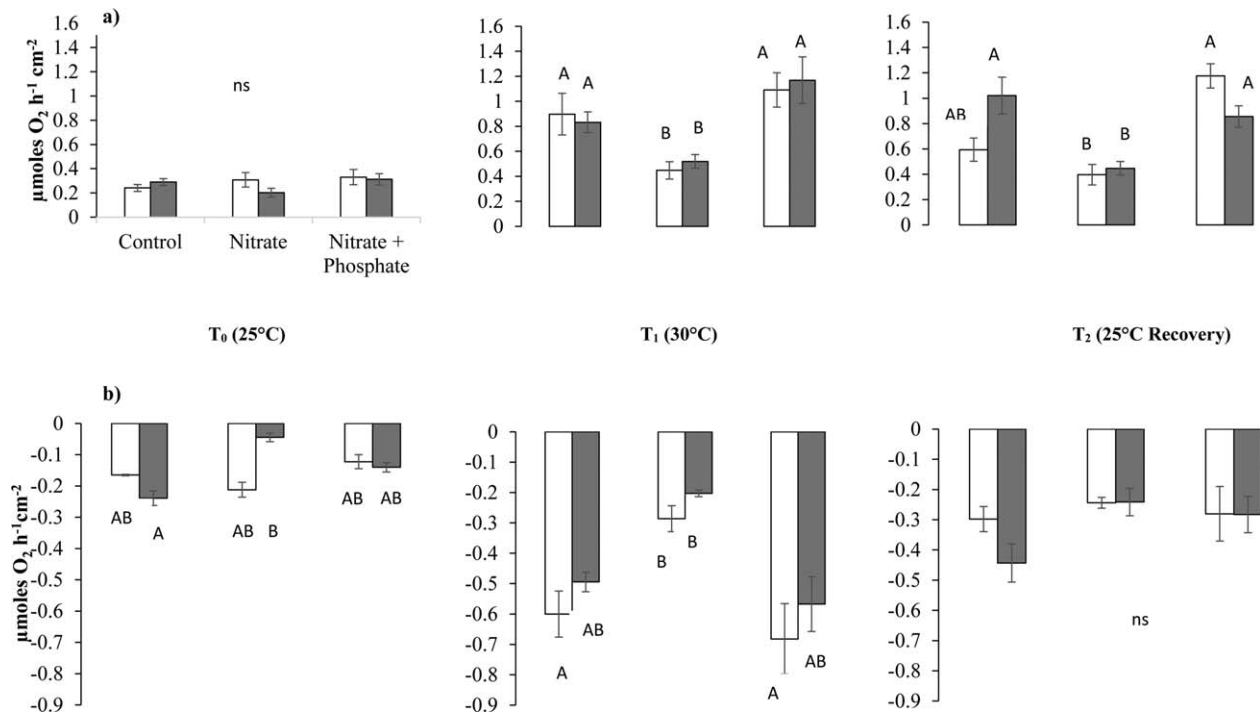
Fig. 1. Rates of gross photosynthesis and respiration (μmol O_2 h^{-1} cm^{-2}) for the different nutritional treatments according to the temperature steps from left to right (T_0, T_1, T_2). Data are presented as mean $\pm$ 1 SE. Letters represent statistical differences based on post hoc Tukey's HSD test ($p < 0.05$). Dark bars represent zooplankton (heterotrophic) supply.

all colonies, R of unfed colonies, and calcification of fed colonies significantly increased compared to respective rates at 25°C (Figs. 1, 2, Tables 2, 3 and 5 Tukey HSD, $p < 0.01$, $p < 0.0001$, and $p = 0.0061$). During recovery, respiration rates in unfed corals returned to their basal values (Tukey HSD, $p < 0.0001$) and rETR max values decreased compared to 25°C and 30°C (Tukey HSD, $p < 0.0065$).

Comparison between treatments at each temperature step

At 25°C, NPH colonies presented a higher chlorophyll content per surface area compared to the other corals, except NP (Table 4, Tukey HSD, $p < 0.0112$), as well as higher calcification rates compared to NH nubbins (Fig. 2, Table 5, Tukey HSD, $p < 0.05$) and higher feeding rates (Fig. 2, Table 5, Tukey HSD, $p < 0.03$). In addition, the rate of Pn was higher for NP corals than for C and CH (Fig. 1, Tukey HSD, $p < 0.0414$). Respiration rates were lower in NH and NP corals compared to CH corals (Tukey HSD, $p < 0.0083$).

At 30°C, NP and NPH treatments maintained higher chlorophyll content than C and CH regimes (Fig. 3, Tukey HSD, $p < 0.0408$). NPH corals also showed the highest zooxanthellae density compared to NH, C, and CH (Tukey HSD, $p < 0.035$). During thermal stress, nitrate enriched corals (N and NH) presented the largest amount of differences compared to the other treatments. NH nubbins had significantly

less protein than NPH and CH corals (Tukey HSD, $p < 0.0422$). N nubbins showed lower rates of Pn (Tukey HSD, $p < 0.0185$) than NP and NPH corals. N and NH nubbins showed lower rates of R than C and NP corals (Tukey HSD, $p < 0.029$) and the lowest Pg compared to all other treatments (Tukey HSD, $p < 0.012$). In addition, NH corals showed the lowest calcification rate compared to all other treatments (Table 5, Tukey HSD, $p < 0.0446$) except N nubbins, while NPH showed the highest calcification rates (Tukey HSD, $p < 0.006$). Feeding rates were higher in NPH and NH nubbins compared to CH corals (Table 5, Tukey HSD, $p < 0.0288$).

After 2 weeks recovery, Pn remained higher in NP-enriched corals (NP and NPH) than in N-enriched (N and NH) and C corals (Tukey HSD, $p < 0.0001$). Pg was higher in NP and CH treatment nubbins than in C, N, and NH regimes (Tukey HSD, $p < 0.036$). N and NH corals showed higher symbiont densities compared to control corals (C and CH) (Tukey HSD, $p < 0.002$).

Discussion

This study investigated the effect of two components of eutrophication, namely inorganic nutrient enrichment and zooplankton supplementation (heterotrophy), in combination or alone, on the physiology of the scleractinian coral *T.*
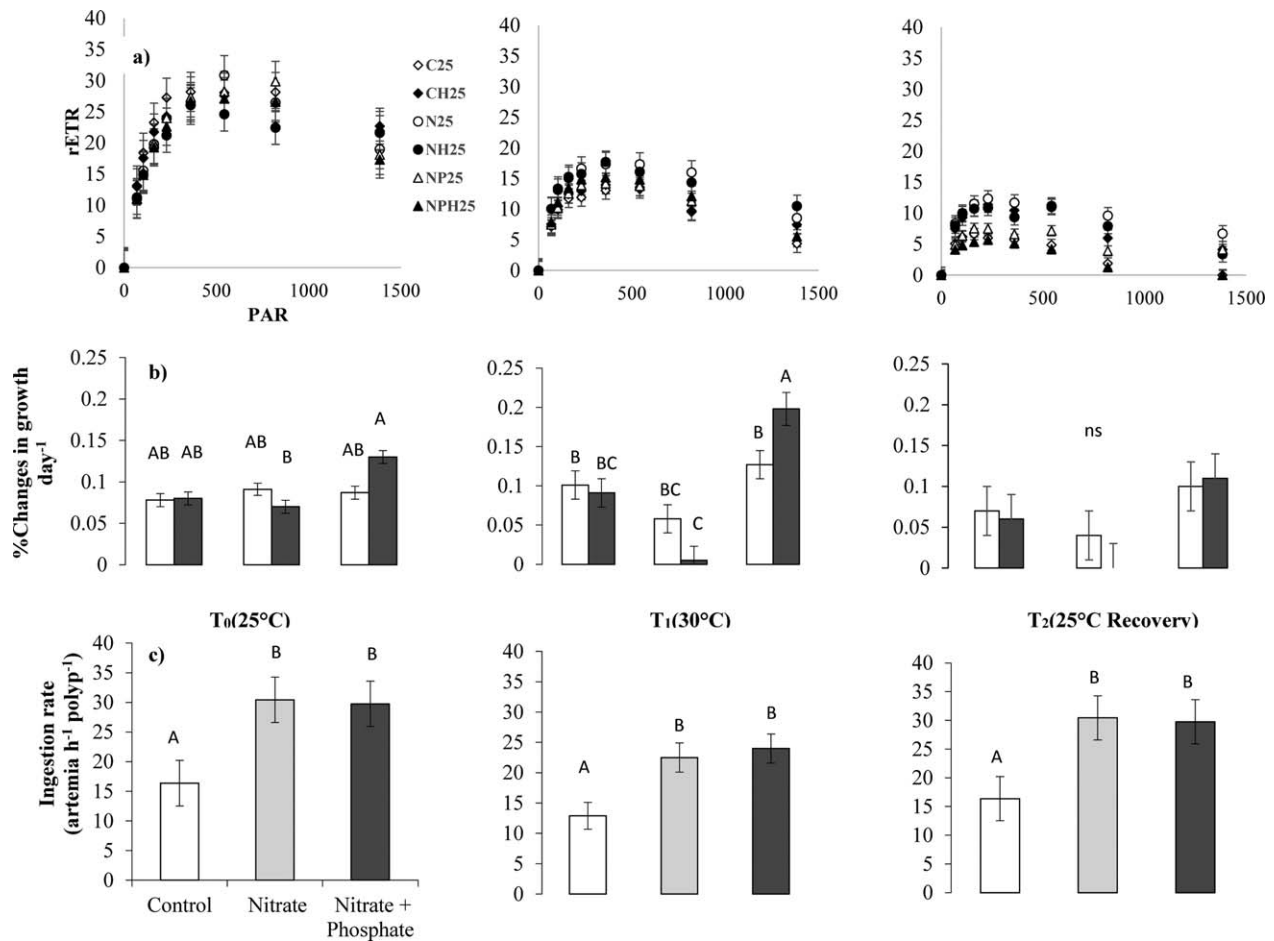
Fig. 2. (**a**) rETR max values, (**b**) Rates of calcification (% change in growth per day) and (**c**) feeding (Nb artemia h^{-1} polyp^{-1}) for the different nutritional treatments according to the temperature steps from left to right (T_0, T_1, T_2). Data are presented as mean $\pm$ 1 SE. Letters represent statistical differences based on post hoc Tukey's HSD test ($p < 0.05$). Dark bars represent zooplankton (heterotrophic) supply.

reniformis exposed to normal or stressful temperatures. In addition, three different inorganic N: P ratios were also tested to evaluate the effect of nutrient stoichiometry on coral physiology. It is still unclear whether corals exhibit synergistic or antagonistic responses when simultaneously exposed to heat stress and eutrophication because heterotrophy may help sustain coral metabolism during stress events, whereas inorganic nutrient supply can have deleterious effects. The results observed allow identification of some of the mechanisms involved in the coral response to heat stress and nutrient availability. We showed that inorganic nutrient enrichment did not exacerbate coral bleaching susceptibility in *T. reniformis*, which regulated its symbiont population even in nutrient-enriched environments. However, inorganic nutrient stoichiometry was a key factor in explaining coral fitness during thermal stress, providing support for the hypothesis that corals and their symbionts may be adapted to specific nutrient ratios. In addition, heterotrophy and inorganic nutrient enrichment were related in *T. reniformis*,

whereby zooplankton feeding rates increased under thermal stress only when inorganic nutrients were also available. Moreover, heterotrophy in the presence of a balanced inorganic nutrient stoichiometry enhanced coral growth during the thermal stress period. On the contrary, heterotrophy with an imbalanced inorganic nutrient stoichiometry exacerbated the negative effect of heat stress on coral growth.

Under nonstressful conditions, the usual consequence of inorganic nutrient supplementation on symbiotic corals is a significant increase in symbiont growth and density inside the coral tissues (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989; Dubinsky et al. 1990; Stambler et al. 1991; Muller-Parker et al. 1994) which can lead to a decrease in calcification rate, especially when nitrate is used as the nitrogen source (Marubini and Davies 1996; Fabricius 2005; Silverman et al. 2007). In our study, however, *T. reniformis* did not change its symbiont concentration with nutrient enrichment, nor decrease its rate of calcification at 25°C. This observation suggests either: (1) a carefully regulated control
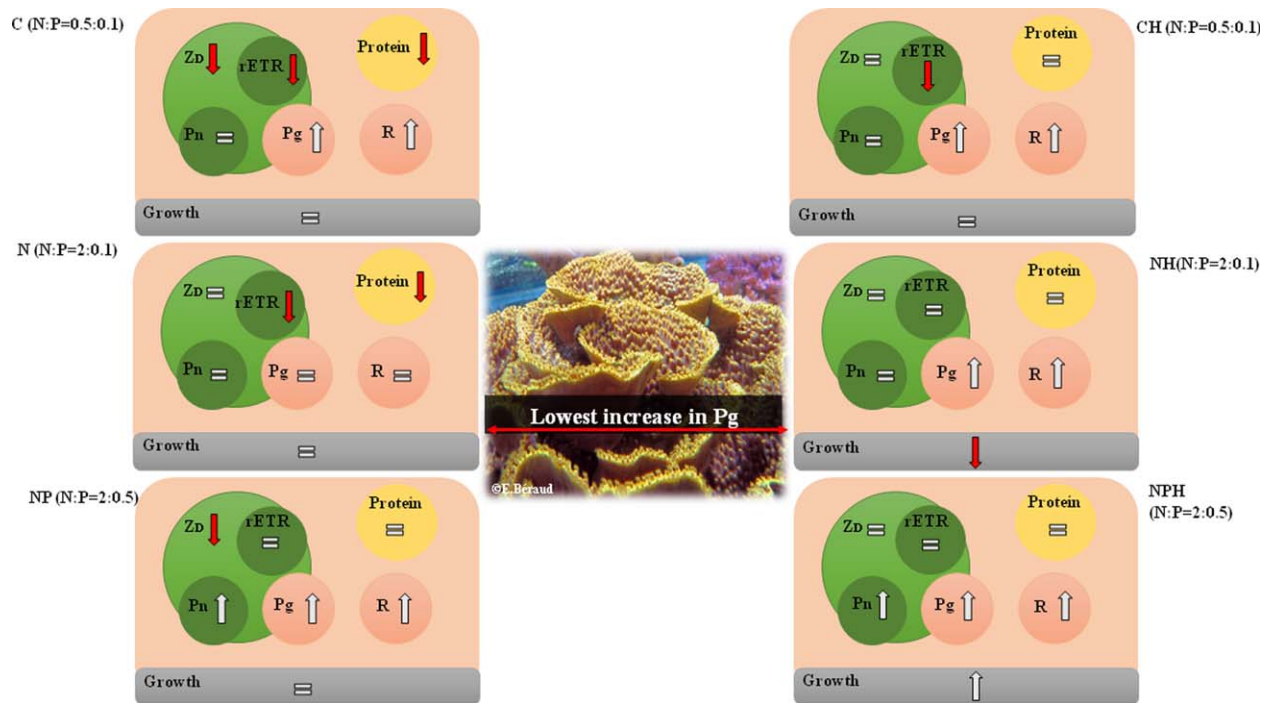
Fig. 3. Budgets highlighting the major changes in the physiological parameters between 25°C and 30°C. The arrows indicate a significant increase or decrease (up or down arrows, respectively), whereas the equal sign indicates no significant changes.

Table 2. Changes observed (as %) in physiological parameters at and between the different time steps (25°C, 30°C, and 25°C Recovery).

Comparisons	Parameter	C	CH	N	NH	NP	NPH
		% change between enriched treatments and control conditions					
At 25°C	Chlorophyll content	—	—	—	—	**+350%**	**+450%**
		(C)	(C)	(BC)	(BC)	(AB)	(A)
At 30°C	Chlorophyll content	—	—	—	—	**+280%**	**+311%**
		(B)	(B)	(AB)	(AB)	(A)	(A)
	Symbiont density	—	—	—	—	—	**+236%**
		(B)	(B)	(B)	(B)	(B)	(A)
	Protein content	—	—	—	**−48%**	—	—
		(AB)	(A)	(AB)	(B)	(AB)	(A)
At 25°C recovery	Chlorophyll content	—	**−371%**	—	—	—	**+514%**
		(AB)	(C)	(BC)	(BC)	(B)	(A)
	Symbiont density	—	—	**+285%**	—	—	—
		(B)	(B)	(A)	(AB)	(AB)	(AB)
		% change within each treatment					
Between 25°C and 30°C	Symbiont density	**−52%**	—	—	—	**−47%**	—
	Protein content	**−37%**	—	**−38%**	—	—	—
Between 30°C and −25°C recovery	Symbiont density	—	—	**+266%**	—	—	—
	Protein content	—	—	—	**+482%**	—	—

Letters indicate significant differences after performing Tukey's HSD post hoc tests.

of symbionts by the animal host, (2) a limitation of algal density by light availability which has restricted symbionts from occupying deeper tissues in corals (Wangpraseurt et al. 2012), as observed in other corals with highly perforate skeletons (Jones and Yellowlees 1997), or (3) a reallocation of energy to other processes, such as chlorophyll production,

Table 3. Summary of ANOVAs testing the effect of the different time steps (T) (25°, 30°, and 25R °C), Inorganic nutrient enrichment (I), and Organic feeding (zooplankton provision) (O) on *T. reniformis* main physiological parameters. Bold face numbers indicates $p < 0.05$.

Source of variation	df	Sum of squares	F	p
Zoox. density (Nb Zoox cm^{-2})				
T	2	6044006007	11.1932	**<0.0001**
I	2	2624173771	4.8598	**0.0103**
T × I	4	5169596097	4.7869	**0.0017**
O	1	170779219	0.6325	0.4288
T × O	2	454644507	0.8420	0.4347
I × O	2	1220422046	2.2602	0.1111
T × I × O	4	1115478181	1.0329	0.3957
Chlorophyll (μg Chl (a + c$_2$)) cm^{-2}				
T	2	0.0416158	0.4891	0.6150
I	2	1.5730277	18.4890	**<0.0001**
T × I	4	0.0015235	0.0358	0.8504
O	1	0.1646167	0.9674	0.4302
T × O	2	0.0070177	0.0825	0.9209
I × O	2	0.0610869	0.7180	0.4909
T × I × O	4	0.1435669	0.8437	0.5017
Protein (μg cm^{-2})				
T	2	283468.76	20.2754	**<0.0001**
I	2	621.99	0.0445	0.9565
T × I	4	35659.37	1.2753	0.2869
O	1	152.34	0.0218	0.8830
T × O	2	8686.62	0.6213	0.5399
I × O	2	2773.42	0.1984	0.8205
T × I × O	4	72202.5	2.5822	**0.0435**

Table 4. Summary of ANOVAs testing the effect of the different time steps (T) (25°, 30°, and 25R °C), Inorganic nutrient enrichment (I), and Organic feeding (zooplankton provision) (O) on *T. reniformis* main physiological photosynthetic parameters. Bold face numbers indicates $p < 0.05$.

Source of variation	df	Sum of squares	F	p
ETR				
T	2	5097.8614	215.5329	**<0.0001**
I	2	73.4705	3.1063	0.0518
T × I	4	152.2962	3.2195	**0.0182**
O	1	13.0689	1.1051	0.2972
T × O	2	54.2981	2.2957	0.1092
I × O	2	78.6251	3.3242	**0.0425**
T × I × O	4	74.5125	1.5752	0.1921
Net photosynthesis (μmol O$_2$ h^{-1} cm^{-2})				
T	2	1.6928	23.6108	**<0.0001**
I	2	1.5857	22.1175	**<0.0001**
T × I	4	0.4132	2.8818	**0.0270**
O	1	0.0405	1.1296	0.2907
T × O	2	0.0637	0.8883	0.4150
I × O	2	0.0203	0.2830	0.7542
T × I × O	4	0.0385	0.2687	0.8974
Respiration (μmol O$_2$ h^{-1} cm^{-2})				
T	2	3.2974	46.3595	**<0.0001**
I	2	0.2742	3.8546	**0.0248**
T × I	4	0.5638	3.9634	**0.0052**
O	1	0.0097	0.2736	0.6022
T × O	2	0.0595	0.8365	0.4366
I × O	2	0.0578	0.8126	0.4469
T × I × O	4	0.1905	1.3390	0.2617
Gross photosynthesis (μmol O$_2$ h^{-1} cm^{-2})				
T	2	12.4146	53.0252	**<0.0001**
I	2	6.0940	26.0290	**<0.0001**
T × I	4	1.1381	2.4306	0.0586
O	1	0.0194	0.1658	0.6854
T × O	2	0.1367	0.5841	0.5611
I × O	2	0.5286	2.2581	0.1143
T × I × O	4	1.2939	2.7634	**0.0366**

which was indeed significantly enhanced by inorganic nutrient enrichment. Our inorganic nutrient enrichment was also much lower than those commonly applied in previous experiments (Nordemar et al. 2003; Voss and Richardson 2006; Tanaka et al. 2007; Holcomb et al. 2010; Wiedenmann et al. 2013; Tanaka et al. 2014b) and might not have been sufficient to significantly change symbiont growth.

Under thermal stress, heterotrophy and/or inorganic nutrient supplementation affected the corals' physiological response differently depending on whether they were provided alone or in combination compared to control corals that did not receive any nutrients. Control corals significantly increased their respiration rates at 30°C, suggesting that the holobiont invested more heavily in maintenance and repair processes associated with metabolic costs incurred during heat stress (Warner et al. 1996; Takahashi et al. 2004; Abrego et al. 2008). This additional cost was afforded by increasing photosynthetic rate compared to rates at 25°C, using energetic reserves such as proteins, which significantly decreased, and by reducing growth rate

and efficiency (rETR$_{max}$) of the symbionts. Conversely to the response of control nubbins, corals that were supplemented only with zooplankton food maintained symbiont density and protein content at the same level at 30°C that they did at 25°C because they had access to a supplemental source of energy. These results confirm the positive effect of heterotrophy regarding resistance to thermal stress (Borell and Bischof 2008; Ferrier-Pagès et al. 2010; Connolly et al. 2012). The response of *T. reniformis* to an inorganic nutrient enrichment during thermal stress was different from the two other feeding conditions and complex,

Table 5. Summary of repeated-measured ANOVA testing the effect of Inorganic nutrient enrichment (I) and Organic feeding (zooplankton provision) (O) according to the different time steps (T) on Growth and Feeding rates*. Bold face numbers indicates $p < 0.05$.

Source of variation	df	F	p
Growth (% change d^{-1})			
T	2	6.1830	**0.0039**
I	2	15.5391	**<0.0001**
T × I	4	9.1835	**<0.0001**
O	1	0.0182	0.8933
T × O	2	1.3379	0.2713
I × O	2	3.6217	**0.0336**
T × I × O	4	3.4939	0.0102
Feeding (Nb Artemia h^{-1} $polyp^{-1}$)			
T	2	8.0095	**0.0071**
I	2	7.6711	**0.0071**
T × I	4	1.2618	0.3148

*The feeding rate parameter was tested with Inorganic nutrient enrichment (I) through the different timesteps (T).

depending on the nutrient stoichiometry and presence or absence of zooplankton food.

It has been suggested and recently shown that corals living in inorganic nutrient enriched seawater have exacerbated bleaching responses to thermal stress (Cunning and Baker 2013; Tanaka et al. 2014a) because nutrients enhance symbiont density, which in turn generate an excessive production of harmful oxygen radicals (Cunning and Baker 2013; Wooldridge 2013) in addition to decreasing photosynthetic rates per symbiont cell (Stambler et al. 1991). As a direct consequence of the unchanged symbiont density in *T. reniformis*, inorganic nutrient addition and thermal stress did not increase the percentage of symbionts lost, suggesting that bleaching in these conditions is species- and context-dependent. Increased heterotrophic feeding under the combination of thermal stress and inorganic nutrient supply might have also avoided excessive bleaching. Our results are consistent with previously published ideas regarding greater food intake by corals in more productive waters (Wang et al. 2015) and with literature reporting that elevated temperature can increase coral feeding rates (Grottoli et al. 2006; Ferrier-Pagès et al. 2010). The mechanisms involved in the coupling between inorganic nutrient uptake of the symbionts and heterotrophy of the coral host are still unclear; however, in agreement with our observations, symbiont carbon translocation rates and host prey ingestion were positively correlated in sea anemones (Leal et al. 2015). The explanation given was that autotrophic energy is necessary to the host to afford the metabolic demand of feeding and digestion (Leal et al. 2015). In our study, inorganic nutrient supplementation might have increased the quality and quantity of sym-

biont photosynthates, thereby increasing the host prey ingestion.

Under inorganic nutrient enrichment and thermal stress, a significant decrease in gross photosynthesis and calcification of *T. reniformis* was observed when nitrate addition was not combined with phosphorus. The decrease was exacerbated when nitrate enrichment was combined with heterotrophy. It is well known in food web studies that organism performance depends not only on the concentrations but also on the proportions of C, N, and P in seawater (Van de Waal et al. 2009). In corals, N : P ratios of host tissue and symbionts is ca. 50: 1 (Godinot et al. 2011b) and is much higher than the Redfield ratio of 16: 1, suggesting a phosphorus limitation with nutrient concentrations measured on the reefs and in the control condition of this study (0.5 μM N and 0.1 μM P). It is, therefore, predictable that seawater enrichment in nitrogen alone will increase this phosphorus limitation. It was observed in two previous studies that an imbalanced nitrate: phosphorus ratio exacerbated phosphorus-starvation and bleaching in several coral species (Wiedenmann et al. 2013), or decreased the amount of photosynthates translocated from the symbionts to the host in *Stylophora pistillata* (Ezzat et al. 2015). This effect is due to the fact that the reduction of nitrate into ammonium is a costly energetic process (Patterson et al. 2010; Dagenais-Bellefeuille and Morse 2013), diverting electrons from other metabolic pathways such as photosynthesis. Our results are another piece of evidence supporting the two previous studies that there is a detrimental effect of an imbalanced nitrate: phosphorus ratio on the metabolism of *T. reniformis*, which can affect symbiont density, photosynthesis or calcification depending on the species and stress amplitude.

Heterotrophy exacerbated the impact of nitrate enrichment on calcification rates as there was a 94% reduction in growth compared to the control. A similar effect on calcification by a simultaneous exposure to organic-enriched sediments and nitrate was observed in the corals *Montipora tuberculosa* and *Acropora millepora* (Fabricius et al. 2013). The explanation given was that organic matter might have stimulated bacterial growth, thereby increasing disease susceptibility and reducing growth rates. As our laboratory treatments likely did not stimulate the growth of seawater bacteria, we suggest that calcification could have been impacted by the production of deleterious reactive nitrogen species (RNS) following accumulation of nitrate into the animal tissues (Lesser 2006; Roth 2014). RNS act together with reactive oxygen species to damage cells, and this process can be exacerbated during thermal stress (Lesser 2006). Nitrate may accumulate in coral tissue when corals are fed because food is already rich in nitrogen, specifically ammonium, produced by host catabolism (Wang et al. 2015). As ammonium is preferred to nitrate (Raven et al. 1992), it is used by symbionts instead of nitrate, which may then accumulate

inside the host cells. Furthermore, heterotrophy might have increased the N: P imbalance, inducing a higher phosphate limitation. If the Growth Rate Hypothesis (Elser et al. 2000) [which proposed that P concentrations in organisms vary predictably with their investment in RNA, which in turn gives rise to variation in growth rates and other physiological processes] is applicable to corals, this might be an explanation for the decreased growth. However, more integrated work is needed to decipher which of the above hypotheses apply to corals. It is important to note that during the recovery period, corals in the N and NH treatments were still exhibiting low calcification and Pg. These data indicate that a 2 week recovery period from a short-term thermal stress is not long enough for corals in nitrate-enriched treatments to recover, if they can recover at all.

In contrast to nitrate-enrichment alone, a double enrichment in nitrate and phosphate concentration in the water (balanced N: P ratio), with or without heterotrophic feeding, greatly enhanced coral metabolism at 25°C and was mostly beneficial for corals at elevated temperature. We indeed observed a stimulation of symbiont growth and chlorophyll synthesis, enhancing the net O_2 production and maintaining rETR max values at 30°C. Additionally, increased calcification rate in the NPH treatment certainly appears to have been enhanced by heterotrophic feeding, as NPH nubbins consistently fed more than control corals. This double enrichment undoubtedly demonstrates that many corals are greatly limited in phosphorus under normal conditions, and this limitation can become problematic under thermal stress and/or under nitrogen enrichment alone. Because phosphorus is a key nutrient for marine organisms (Benitez-Nelson 2000; Elser et al. 2007) it has been shown that phosphorus supplementation increased the metabolism of some corals such as *Stylophora pistillata* (Godinot et al. 2011b), which showed an increased alkaline phosphatase activity when bleached to use the dissolved organic phosphorus present in seawater (Godinot et al. 2013). Although phosphorus was also suggested to decrease calcification rates in corals when present in very high concentrations in seawater ($\geq 2 \mu M$) (Kinsey and Davies 1979; Ferrier-Pages et al. 2000; Koop et al. 2001), a minimum amount may be essential to coral metabolism. Clearly, more studies are needed to assess the minimal requirements for phosphorus in coral species, depending on their living conditions.

In conclusion, although zooplankton feeding in seawater generally enhances coral metabolism, especially under thermal stress (Houlbrèque and Ferrier-Pagès 2009), it is deleterious for corals in the presence of elevated inorganic nutrient concentrations with an unbalanced N: P ratio. This scenario is unfortunately the case in many eutrophicated areas where loading of inorganic nitrogen is prevalent, such as in southwest Japan or in the Caribbean (Umezawa et al. 2002; Lapointe et al. 2005; Pastore 2014). The mechanisms involved in this combined negative effect still remain unde-

termined, but may be linked to a P-limitation in N-enriched corals, which prevent the construction of phospholipidic membranes (Tchernov et al. 2004; Wiedenmann et al. 2013) or the buildup of adenosine triphosphate (ATP) reserves. On the contrary, when inorganic nutrients have a balanced N: P ratio and are maintained in relatively low concentrations (that remain undetermined) they may not affect coral metabolism under thermal stress and they may even enhance coral metabolism when combined with heterotrophy. Clearly, the different effect is due to the supply of phosphorus for coral metabolism. This deleterious effect of phosphorus limitation coupled to other stressors may particularly affect branching coral species, which often have higher metabolism and thus higher phosphorus needs (Godinot et al. 2011a), and may change the diversity and species composition of coral reefs in the future.

References

Abrego, D., K. E. Ulstrup, B. L. Willis, and M. J. van Oppen. 2008. Species–specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. Proc. R. Soc. B Biol. Sci. **275**: 2273–2282. doi:10.1098/rspb.2008.0180

Anthony, K. R. N. 2006. Enhanced energy status of corals on coastal, high-turbidity reefs. Mar. Ecol. Prog. Ser. **319**: 111–116. doi:10.3354/meps319111

Anthony, K., M. O. Hoogenboom, J. A. Maynard, A. G. Grottoli, and R. Middlebrook. 2009. Energetics approach to predicting mortality risk from environmental stress: A case study of coral bleaching. Funct. Ecol. **23**: 539–550. doi:10.1111/j.1365-2435.2008.01531.x

Benitez-Nelson, C. R. 2000. The biogeochemical cycling of phosphorus in marine systems. Earth Sci. Rev. **51**: 109–135. doi:10.1016/S0012-8252(00)00018-0

Béraud, E., F. Gevaert, C. Rottier, and C. Ferrier-Pagès. 2013. The response of the scleractinian coral Turbinaria reniformis to thermal stress depends on the nitrogen status of the coral holobiont. J Exp. Biol. **216**: 2665–2674. doi:10.1242/jeb.085183

Borell, E. M., and K. Bischof. 2008. Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress. Oecologia **157**: 593–601. doi:10.1007/s00442-008-1102-2

Brown, B. E. 1997. Coral bleaching: Causes and consequences. Coral Reefs **16**: S129–S138. doi:10.1007/s003380050249

Bruno, J. F., H. Sweatman, W. F. Precht, E. R. Selig, and V. G. Schutte. 2009. Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs. Ecology **90**: 1478–1484. doi:10.1890/08-1781.1

Chauvin, A., V. Denis, and P. Cuet. 2011. Is the response of coral calcification to seawater acidification related to nutrient loading? Coral Reefs **30**: 911–923. doi:10.1007/s00338-011-0786-7

Connolly, S., M. Lopez-Yglesias, and K. Anthony. 2012. Food availability promotes rapid recovery from thermal stress in a scleractinian coral. Coral Reefs **31**: 951–960. doi:10.1007/s00338-012-0925-9

Cunning, R., and A. C. Baker. 2013. Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. **3**: 259–262. doi:10.1038/nclimate1711

Dagenais-Bellefeuille, S., and D. Morse. 2013. Putting the N in dinoflagellates. Front Microbiol **4**. doi:10.3389/fmicb.2013.00369

Davies, P. S. 1989. Short-term growth measurements of corals using an accurate buoyant weighing technique. Mar. Biol. **101**: 389–395. doi:10.1007/BF00428135

De'ath, G., and K. Fabricius. 2010. Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecol. Appl. **20**: 840–850. doi:10.1890/08-2023.1

Dubinsky, Z., N. Stambler, M. Ben-Zion, L. McCloskey, L. Muscatine, and P. Falkowski. 1990. The effect of external nutrient resources on the optical properties and photosynthetic efficiency of Stylophora pistillata. Proc. R Soc. Lond. B Biol. Sci. **239**: 231–246. doi:10.1098/rspb.1990.0015

Elser, J. J., and others. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature **408**: 578–580. doi:10.1038/35046058

Elser, J. J., and others. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10**: 1135–1142. doi:10.1111/j.1461-0248.2007.01113.x

Ezzat, L., J.-F. Maguer, R. Grover, and C. Ferrier-Pagès. 2015. New insights into carbon acquisition and exchanges within the coral–dinoflagellate symbiosis under NH4+ and NO3− supply. Proc. R Soc. B **282**: 20150610. doi:10.1098/rspb.2015.0610

Fabricius, K. E. 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. Mar. Pollut. Bull. **50**: 125–146. doi:10.1016/j.marpolbul.2004.11.028

Fabricius, K. E. 2011. Factors determining the resilience of coral reefs to eutrophication: A review and conceptual model, p. 493–505. Coral Reefs: An ecosystem in transition. Springer. doi:10.1007/978-94-007-0114-4_28

Fabricius, K. E., and others. 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat. Clim. Chang. **1**: 165–169.

Fabricius, K. E., S. Cséke, C. Humphrey, and G. De'ath. 2013. Does trophic status enhance or reduce the thermal tolerance of scleractinian corals? A review, experiment and conceptual framework. PloS One **8**: e54399. doi:10.1371/journal.pone.0054399

Ferrier-Pages, C., J.-P. Gattuso, S. Dallot, and J. Jaubert. 2000. Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral Stylophora pistillata. Coral Reefs **19**: 103–113. doi:10.1007/s003380000078

Ferrier-Pagès, C., C. Rottier, E. Beraud, and O. Levy. 2010. Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. J. Exp. Mar. Biol. Ecol. **390**: 118–124. doi:10.1016/j.jembe.2010.05.007

Glynn, P. W. 2012. Global warming and widespread coral mortality: Evidence of first coral reef extinctions, p. 103–119. *In* Lee Hannah [ed.], Saving a million species. Springer.

Godinot, C., R. Grover, D. Allemand, and C. Ferrier-Pagès. 2011a. High phosphate uptake requirements of the scleractinian coral Stylophora pistillata. J. Exp. Biol. **214**: 2749–2754. doi:10.1242/jeb.054239

Godinot, C., C. Ferrier-Pages, P. Montagna, and R. Grover. 2011b. Tissue and skeletal changes in the scleractinian coral Stylophora pistillata Esper 1797 under phosphate enrichment. J. Exp. Mar. Biol. Ecol. **409**: 200–207. doi:10.1016/j.jembe.2011.08.022

Godinot, C., C. Ferrier-Pagès, S. Sikorski, and R. Grover. 2013. Alkaline phosphatase activity of reef-building corals. Limnol. Oceanogr. **58**: 227–234. doi:10.4319/lo.2013.58.1.0227

Gori, A., S. Reynaud, C. Orejas, and C. Ferrier-Pagès. 2015. The influence of flow velocity and temperature on zooplankton capture rates by the cold-water coral Dendrophyllia cornigera. J. Exp. Mar. Biol. Ecol. **466**: 92–97. doi:10.1016/j.jembe.2015.02.004

Graham, N. A., S. Jennings, M. A. MacNeil, D. Mouillot, and S. K. Wilson. 2015. Predicting climate-driven regime shifts versus rebound potential in coral reefs. Nature. **518**: 94–97. doi:10.1038/nature14140

Grottoli, A. G., L. J. Rodrigues, and J. E. Palardy. 2006. Heterotrophic plasticity and resilience in bleached corals. Nature **440**: 1186–1189. doi:10.1038/nature04565

Hansen, J., M. Sato, R. Ruedy, K. Lo, D. W. Lea, and M. Medina-Elizade. 2006. Global temperature change. Proc. Natl. Acad. Sci. USA **103**: 14288–14293. doi:10.1073/pnas.0606291103

Hoegh-Guldberg, O., and G. J. Smith. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals Stylophora pistillata Esper and Seriatopora hystrix Dana. J. Exp. Mar. Biol. Ecol. **129**: 279–303. doi:10.1016/0022-0981(89)90109-3

Holcomb, M., D. C. McCorkle, and A. L. Cohen. 2010. Long-term effects of nutrient and CO2 enrichment on the temperate coral Astrangia poculata (Ellis and Solander, 1786). J. Exp. Mar. Biol. Ecol. **386**: 27–33. doi:10.1016/j.jembe.2010.02.007

Hoogenboom, M., E. Beraud, and C. Ferrier-Pagès. 2010. Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral Cladocora caespitosa. Coral Reefs **29**: 21–29. doi:10.1007/s00338-009-0558-9

Houlbrèque, F., E. Tambutté, D. Allemand, and C. Ferrier-Pagès. 2004. Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian

coral Stylophora pistillata. J. Exp. Biol. **207**: 1461–1469. doi:10.1242/jeb.00911

Houlbrèque, F., and C. Ferrier-Pagès. 2009. Heterotrophy in tropical scleractinian corals. Biol. Rev. **84**: 1–17. doi:10.1111/j.1469-185X.2008.00058.x

Jeffrey, S. T., and G. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. **167**: 191–194.

Jones, R. J., and D. Yellowlees. 1997. Regulation and control of intracellular algae (= zooxanthellae) in hard corals. Philos. Trans. R Soc. Lond. Ser. B Biol. Sci. **352**: 457–468. doi:10.1098/rstb.1997.0033

Kinsey, D. W., and P. J. Davies. 1979. Effects of elevated nitrogen and phosphorus on coral reef growth1. Limnol. Oceanogr. **24**: 935–940. doi:10.4319/lo.1979.24.5.0935

Koop, K., and others. 2001. ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Mar. Pollut. Bull. **42**: 91–120. doi:10.1016/S0025-326X(00)00181-8

Langdon, C., and M. Atkinson. 2005. Effect of elevated pCO2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res. Oceans (1978–2012) **110**: C09S07. doi:10.1029/2004JC002576

Lapointe, B. E., P. J. Barile, M. M. Littler, and D. S. Littler. 2005. Macroalgal blooms on southeast Florida coral reefs: II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen. Harmful Algae **4**: 1106–1122. doi:10.1016/j.hal.2005.06.002

Leal, M. C., K. Hoadley, D. T. Pettay, A. Grajales, R. Calado, and M. E. Warner. 2015. Symbiont type influences trophic plasticity of a model cnidarian-dinoflagellate symbiosis. J. Exp. Biol. **218**: 858–863. doi:10.1242/jeb.115519

Lesser, M. P. 2006. Oxidative stress in marine environments: Biochemistry and physiological ecology. Annu. Rev. Physiol. **68**: 253–278. doi:10.1146/annurev.physiol.68.040104.110001

Levy, O., L. Mizrahi, N. Chadwick-Furman, and Y. Achituv. 2001. Factors controlling the expansion behavior of Favia favus (Cnidaria: Scleractinia): Effects of light, flow, and planktonic prey. Biol. Bull. **200**: 118–126. doi:10.2307/1543305

Marubini, F., and P. Davies. 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar. Biol. **127**: 319–328. doi:10.1007/BF00942117

Mioche, D., and P. Cuet. 1999. Métabolisme du carbone, des carbonates et des sels nutritifs en saison chaude, sur un récif frangeant soumis à une pression anthropique (île de la Réunion, océan Indien). Comptes Rendus de l'Acad. des Sci. Ser. IIA Earth Planet. Sci. **329**: 53–59. doi:10.1016/S1251-8050(99)80227-4

Muller-Parker, et al. 1994. This article was published in Pacific Science journal **48**: 273–283. doi:10.1007/BF00346316

Muller-Parker, G., C. Cook, and C. D'elia. 1994. Elemental composition of the coral Pocillopora damicornis exposed to elevated seawater ammonium. doi:10.1007/BF00346316

Muscatine, L., P. G. Falkowski, Z. Dubinsky, P. A. Cook, and L. R. McCloskey. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. R Soc. Lond. B Biol. Sci. **236**: 311–324. doi:10.1098/rspb.1989.0025

Nordemar, I., M. Nyström, and R. Dizon. 2003. Effects of elevated seawater temperature and nitrate enrichment on the branching coral Porites cylindrica in the absence of particulate food. Mar. Biol. **142**: 669–677. doi:10.1007/s00227-002-0989-0

Norström, A. V., M. Nyström, J. Lokrantz, and C. Folke. 2009. Alternative states on coral reefs: Beyond coral-macroalgal phase shifts. Mar. Ecol. Prog. Ser. **376**: 295–306. doi:10.3354/meps07815

Palardy, J. E., L. J. Rodrigues, and A. G. Grottoli. 2008. The importance of zooplankton to the daily metabolic carbon requirements of healthy and bleached corals at two depths. J. Exp. Mar. Biol. Ecol. **367**: 180–188. doi:10.1016/j.jembe.2008.09.015

Pastore, M. 2014. The impact of nitrogen eutrophication on caribbean coral reefs: A review*. Concept 37.

Patterson, K., T. Cakmak, A. Cooper, I. Lager, A. G. Rasmusson, and M. A. Escobar. 2010. Distinct signalling pathways and transcriptome response signatures differentiate ammonium-and nitrate-supplied plants. Plant Cell Environ. **33**: 1486–1501.

Raven, J. A., B. Wollenweber, and L. L. Handley. 1992. A comparison of ammonium and nitrate as nitrogen sources for photolithotrophs. New Phytol. **121**: 19–32. doi:10.1111/j.1469-8137.1992.tb01088.x

Rodrigues, L. J., and A. G. Grottoli. 2007. Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol. Oceanogr. **52**: 1874–1882. doi:10.4319/lo.2007.52.5.1874

Roth, M. S. 2014. The engine of the reef: photobiology of the coral–algal symbiosis. Front. Microbiol. **5**. doi:10.3389/fmicb.2014.00422

Sawall, Y., M. C. Teichberg, J. Seemann, M. Litaay, J. Jompa, and C. Richter. 2011. Nutritional status and metabolism of the coral Stylophora subseriata along a eutrophication gradient in Spermonde Archipelago (Indonesia). Coral Reefs **30**: 841–853. doi:10.1007/s00338-011-0764-0

Sawall, Y., A. Al-Sofyani, E. Banguera-Hinestroza, and C. R. Voolstra. 2014. Spatio-temporal analyses of Symbiodinium physiology of the coral Pocillopora verrucosa along large-scale nutrient and temperature gradients in the Red Sea. PLos One **9**: e103179. doi:10.1371/journal.pone.0103179

Schreiber, U., U. Schliwa, and W. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of

modulation fluorometer. Photosynth. Res. **10**: 51–62. doi: 10.1007/BF00024185

Shantz, A. A., and D. E. Burkepile. 2014. Context-dependent effects of nutrient loading on the coral-algal mutualism. Ecology **95**: 1995–2005. doi:10.1890/13-1407.1

Silverman, J., B. Lazar, and J. Erez. 2007. Effect of aragonite saturation, temperature, and nutrients on the community calcification rate of a coral reef. J. Geophys. Res. Oceans (1978–2012) **112**: C05004. doi:10.1029/2006JC003770

Smith, P., and others. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**: 76–85. doi: 10.1016/0003-2697(85)90442-7

Stambler, N., N. Popper, Z. Dubinsky, and J. Stimson. 1991. Effects of nutrient enrichment and water motion on the coral Pocillopora damicornis. Pac. Sci **45**: 299–307. doi: 10.1016/S0022-0981(05)80006-1

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton Univ Press.

Szmant, A. M. 2002. Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? Estuaries **25**: 743–766. doi:10.1007/BF02804903

Takahashi, S., T. Nakamura, M. Sakamizu, R. van Woesik, and H. Yamasaki. 2004. Repair machinery of symbiotic photosynthesis as the primary target of heat stress for reef-building corals. Plant Cell Physiol. **45**: 251–255. doi: 10.1093/pcp/pch028

Tanaka, Y., T. Miyajima, I. Koike, T. Hayashibara, and H. Ogawa. 2007. Imbalanced coral growth between organic tissue and carbonate skeleton caused by nutrient enrichment. Limnol. Oceanogr. **52**: 1139–1146. doi:10.4319/lo.2007.52.3.1139

Tanaka, Y., M. Inoue, T. Nakamura, A. Suzuki, and K. Sakai. 2014a. Loss of zooxanthellae in a coral under high seawater temperature and nutrient enrichment. J. Exp. Mar. Biol. Ecol. **457**: 220–225. doi:10.1016/j.jembe.2014.04.019

Tanaka, Y., A. Iguchi, K. Nishida, M. Inoue, T. Nakamura, A. Suzuki, and K. Sakai. 2014b. Nutrient availability affects the response of juvenile corals and the endosymbionts to ocean acidification. Limnol. Oceanogr. **59**: 1468–1476. doi:10.4319/lo.2014.59.5.1468

Tchernov, D., M. Y. Gorbunov, C. de Vargas, S. N. Yadav, A. J. Milligan, M. Häggblom, and P. G. Falkowski. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc. Natl. Acad. Sci. USA **101**: 13531–13535. doi:10.1073/pnas.0402907101][15340154

Tolosa, I., C. Treignier, R. Grover, and C. Ferrier-Pagès. 2011. Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral Turbinaria reniformis. Coral Reefs **30**: 763–774. doi:10.1007/s00338-011-0753-3

Tréguer, P., and P. Le Corre. 1975. Manuel d'analyse des sels nutrifs dans l'eau de mer: utilisation de l'Autoanalyzer II Technicon (R). Univ. de Bretagne occidentale.

Tremblay, P., R. Grover, J. F. Maguer, L. Legendre, and C. Ferrier-Pagès. 2012. Autotrophic carbon budget in coral tissue: A new ^{13}C-based model of photosynthate translocation. J. Exp. Biol. **215**: 1384–1393. doi:10.1242/jeb.065201

Umezawa, Y., T. Miyajima, H. Kayanne, and I. Koike. 2002. Significance of groundwater nitrogen discharge into coral reefs at Ishigaki Island, southwest of Japan. Coral Reefs **21**: 346–356. doi:10.4319/lo.2002.47.5.1405

Underwood, A. 1997. Environmental decision-making and the precautionary principle: What does this principle mean in environmental sampling practice? Landsc. Urban Plan. **37**: 137–146. doi:10.1016/S0169-2046(97)80000-X

Van de Waal, D. B., A. M. Verschoor, J. M. Verspagen, E. van Donk, and J. Huisman. 2009. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Front. Ecol. Environ. **8**: 145–152. doi:10.1890/080178

Veal, C., M. Carmi, M. Fine, and O. Hoegh-Guldberg. 2010. Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. Coral Reefs **29**: 893–897. doi:10.1007/s00338-010-0647-9

Vega Thurber, R. L., D. E. Burkepile, C. Fuchs, A. A. Shantz, R. McMinds, and J. R. Zaneveld. 2014. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob. Chang. Biol. **20**: 544–554. doi: 10.1111/gcb.12450

Veron, J. 2000. Corals of the world, v. 1–3. Australian Institute of Marine Science, Townsville.

Visser, M. E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. Proc. R Soc. Lond. B Biol. Sci. **275**: 649–659. doi:10.1098/rspb.2007.0997

Vogel, S., and M. LaBarbera. 1978. Simple flow tanks for research and teaching. Bioscience **28**: 638–643. doi: 10.2307/1307394

Voss, J. D., and L. L. Richardson. 2006. Nutrient enrichment enhances black band disease progression in corals. Coral Reefs **25**: 569–576. doi:10.1007/s00338-006-0131-8

Wang, X., D. Sigman, A. Cohen, D. Sinclair, R. Sherrell, M. Weigand, D. Erler, and H. Ren. 2015. Isotopic composition of skeleton-bound organic nitrogen in reef-building symbiotic corals: A new method and proxy evaluation at Bermuda. Geochim. Cosmochim. Acta **148**: 179–190. doi: 10.1016/j.gca.2014.09.017

Wangpraseurt, D., A. W. Larkum, P. J. Ralph, and M. Kühl. 2012. Light gradients and optical microniches in coral tissues. Front. Microbiol. **3**. doi:10.3389/fmicb.2012.00316]

Warner, M., W. Fitt, and G. Schmidt. 1996. The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: A novel approach. Plant Cell Environ. **19**: 291–299. doi:10.1111/j.1365-3040.1996.tb00251.x

Wiedenmann, J., C. D'Angelo, E. G. Smith, A. N. Hunt, F.-E. Legiret, A. D. Postle, and E. P. Achterberg. 2013. Nutrient

enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. **3**: 160–164. doi:10.1038/nclimate1661

Wooldridge, S. A. 2009. Water quality and coral bleaching thresholds: Formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. Mar. Pollut. Bull. **58**: 745–751. doi:10.1016/j.marpolbul.2008.12.013

Wooldridge, S. 2013. Breakdown of the coral-algae symbiosis: Towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences **10**: 1647–1658. doi:10.5194/bg-10-1647-2013

Wooldridge, S. A., and T. J. Done. 2009. Improved water quality can ameliorate effects of climate change on corals. Ecol. Appl. **19**: 1492–1499. doi:10.1890/08-0963.1