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Photoacclimatization in the zooxanthellae of *Pocillopora verrucosa* and comparison with a pelagic algal community

Photoacclimatation comparée des zooxanthelles de *Pocillopora verrucosa* et d'une communauté algale pélagique

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Abstract

Photoacclimatization of zooxanthellae extracted from the coral *Pocillopora verrucosa* was studied through the determination of pigments, light absorption and photosynthetic parameters, for samples collected in summer and winter between 1 and 40 m on a northwestern reef of Tahiti (French Polynesia). The same measurements were also performed on phytoplanktonic samples collected at a stable oceanic site north of the island. For the zooxanthellae, the variations with depth of all the parameters were generally of small amplitude. Seasonal differences were also observed. The photosynthetic to non-photosynthetic pigments ratio was higher at depth in both seasons and was higher in winter. The intracellular concentration of chlorophyll *a* and photosynthetic pigments was higher in winter, as was the photosynthetic pigments/chlorophyll *a* ratio, whereas the non-photosynthetic pigments/chlorophyll *a* ratio was higher in summer. Variations in the light absorption properties were also small. The photosynthetic parameters showed limited changes with depth with the largest variations (a factor of ~2) observed for P^{B}_{max}. The trends observed for the phytoplankton assemblage were generally of much higher amplitudes than for the zooxanthellae (e.g. for photosynthetic to non-photosynthetic pigments ratio or the saturation parameter, E_k). These results suggest that, in the very clear Polynesian waters, the amount of energy that reaches the zooxanthellae of *P. verrucosa* is not variable enough in the 1–40 m depth range to result in a drastic modification of the photosynthetic apparatus of the algae.

Résumé

La photoacclimatation de zooxanthelles extraites de colonies de *P. verrucosa* est examinée au travers de leurs caractéristiques pigmentaires, d'absorption de la lumière et de leurs paramètres photosynthétiques, pour des échantillons prélevés en hiver et en été australs entre 1 et 40 m sur un récif du nord ouest de Tahiti (Polynésie française). Les mêmes mesures ont été réalisées sur des échantillons de phytoplancton prélevés sur un site océanique au nord de l'île. Pour tous les paramètres étudiés, les zooxanthelles présentent de faibles variations avec la profondeur et des différences saisonnières sont observées. Le rapport des pigments photosynthétiques sur les pigments non-photosynthétiques augmente avec la profondeur en été et en hiver durant lequel ce rapport est plus élevé. La concentration intracellulaire en chlorophylle *a* et en pigments photosynthétiques est plus élevée en hiver et en profondeur tandis que le rapport pigments non-photosynthétiques/chlorophylle *a* est plus élevé en été. Les propriétes d'absorption de la lumière sont très stables avec la profondeur ou la saison. Les paramètres photosynthétiques varient peu, la plus forte variation étant observée pour P^{B}_{max} (~ facteur 2). Les variations observées pour le phytoplancton sont, en général, de plus fortes amplitudes que celles des zooxanthelles. Ces résultats indiquent que, dans les eaux claires de la Polynésie française, la quantité d'énergie qui atteint les zooxanthelles de *P. verrucosa* ne change pas suffisamment

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entre 1 et 40 m pour induire de modifications majeures de l'appareil photosynthétique de ces algues.

Keywords: Zooxanthellae; Photoacclimatization; *Pocillopora verrucosa*; Phytoplankton; Coral reefs

Mots clés: Zooxanthelles; Photoacclimatation; *Pocillopora verrucosa*; Phytoplancton; Récifs coralliens

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1. Introduction

Photosynthesis and calcification are light-dependent processes through which hermatypic, zooxanthellate corals play a major role in the geochemical cycles of coral reef ecosystems. The high gross primary production of coral reef environments, relative to that of the surrounding open ocean, results, to a large extent, from the photosynthetic activity of zooxanthellate corals (Muscatine, 1990; Crossland et al., 1991; Gattuso et al., 1999). Depending on species, corals live and grow at various depths, and some species are commonly found from the surface down to 40–50 m. Along such a depth range, the underwater radiative field changes in terms of available flux and spectral composition, so that shallow water corals and deeper corals experience differing light regimes. These differences and the wide bathymetric distribution of many hermatypic coral species suggest that the photosynthetic apparatus of the zooxanthellae has acclimatized to the photic environment they live in.

As pointed out by Falkowski et al. (1990), changes associated with the characteristics of the light field involve various aspects of the biological organization of corals, from molecular to colony level. Numerous studies have been devoted to these adaptive processes in corals, either with the whole organism (animal + algae) or with isolated or cultured zooxanthellae (see review in Falkowski et al., 1990, and more recently, e.g. Iglesias-Prieto and Trench, 1994, 1997; Lesser et al., 2000).

Like for other algae, the characteristics of the photosynthetic apparatus depend, to a large extent, on the interactions between the zooxanthellae and the light that reaches them. Pigment content, light absorption efficiency and photosynthetic parameters are descriptors of the photosynthetic machinery and can thus be used to assess differences that develop under distinct light regimes. A commonly observed trend in earlier light-related studies is a darkening of the coral colonies with increasing depth or lower light levels, which results mainly from an increase in zooxanthella pigment content (Wyman et al., 1987; Falkowski et al., 1993). Changes in the light absorption capability of zooxanthellae as a function of light have also been evidenced in several works. On a per cell basis, light absorption efficiency was shown to increase with decreasing growth irradiance (Dustan, 1982), whereas it generally decreased with decreasing growth irradiance when normalized to chlorophyll *a* (chl *a*) concentration as a result of cellular pigment packaging (Wyman et al., 1987; Lesser et al., 2000), a typical phenomenon in phytoplankton (Kirk, 1975).

Several studies examined the response of photosynthesis to growth irradiance for either the whole association or isolated zooxanthellae and were based on the determination of the so-called "*P* vs. *E* curve" (following the SI nomenclature for irradiance, *E*). Both oxygen evolution and carbon fixation have been documented. When expressed on a cell basis, the initial slope of the *P* vs. *E* curve was generally observed to increase with depth, whereas the opposite trend was evidenced for the maximum rate of photosynthesis (reviewed by Falkowski et al., 1990). These changes were generally associated with variations in the size (i.e. number of pigment molecules) of photosynthetic units (Titlyanov et al., 1980; Falkowski and Dubinsky, 1981; Dustan, 1982), but Iglesias-Prieto and Trench (1994, 1997) demonstrated that a simultaneous increase in the number of photosynthetic units could also occur. When expressed on a chlorophyll *a* basis, the photosynthetic parameters of zooxanthellae have shown typical photoacclimatory response patterns in some cases, whereas in some other cases, no marked trends were observed. On some occasions, trends inverse to what may be expected during photoacclimatory processes were also reported (Dubinsky et al., 1984; Wyman et al., 1987).

These sometimes contradictory observations actually reflect the complexity of these organisms, which are influenced by various factors related to the plant–animal association and environmental parameters. This complexity (and the variability that inherently results from it) is even more evidenced by recent studies showing different physiological responses in different species of zooxanthellae (Iglesias-Prieto and Trench, 1994, 1997) and the presence of different strains of zooxanthellae within the same host (Rowan and Knowlton, 1995). Moreover, seasonal variations in various physiological parameters of corals have also been demonstrated recently (Fagoonee et al., 1999; Brown et al., 1999). Finally, beside the characteristics of the in-water light field, it has also been shown that the light environment of the zooxanthellae is influenced by the presence of the animal host (Kühl et al., 1995).

In this paper, we present a detailed study of the photoacclimatization strategy of a population of zooxanthellae from *Pocillopora verrucosa* by examining some characteristics of their photosynthetic apparatus along the natural depth gradient of their habitat. We here refer to photoacclimatization, as the present study deals with properties regulated by the light regime the algal cells experienced in their natural environment (as determined by the depth where their animal host lived). With this aim, pigment content, light absorption capacity and photosynthetic parameters were simultaneously measured on zooxanthellae isolated from samples of *P. verrucosa* collected at various depths in the extremely clear waters of the outer reef slope of Tahiti Island (French Polynesia) in July and December 1994. As sampling took place during two contrasted seasons (winter and summer), seasonal differences were also examined. For comparison with the zooxanthellae, a phytoplankton assemblage sampled at an oceanic site with similarly clear oligotrophic waters and a very stable vertical distribution was also investigated as part of the Olipac cruise (JGOFS-France, November 1994) in the vicinity of the island. Phytoplankton samples were collected throughout the water column, and the same parameters as above were determined.

2. Materials and methods

2.1. Sampling

Sun-exposed colonies of *P. verrucosa* (Ellis and Solander, 1786) were sampled between 1 and 40 m on the outer slope of a northwestern reef of Tahiti (French Polynesia) during the austral winter (July, six depths) and summer (December, seven depths) of 1994. At each depth, three to six colonies were sampled (around 10:00 AM, local time), by collecting three to four tips from the top of each colony. The total number of colonies sampled is 28 in winter and 24 in summer. The samples were kept in the dark and cooled at 4 °C to avoid pigment degradation. For the photosynthesis measurements, the samples were kept in the dark at in situ temperature for less than 2 h prior to the analyses. All analyses were performed on zooxanthellae extracted from the tips using jets of GF/F filtered seawater. The extracted surface area of each tip was assessed by the aluminum foil technique, and cell numerical densities were obtained from hemocytometer or microscope counts. The pigment and light absorption measurements were carried out on aliquots of the algal suspension from each tip of a colony and were subsequently averaged to represent that colony. For the photosynthesis experiments, aliquots of the zooxanthella suspensions from the same colony were pooled together (see details below). The oceanic site (16°S–150°W) was sampled for 5 days (November 25–29, 1994). The mixed layer was less than 20 m thick, and the density gradient (>0.01 kg m^{-4}) below it ensured a very stable vertical distribution of algal populations (Claustre et al., 1999) which was thus adapted to the prevailing light regime, allowing a meaningful comparison with the zooxanthellae.

2.2. Pigment analyses

Pigments were analyzed by HPLC following the procedure described in Vidussi et al. (1996). For the zooxanthellae, non-photosynthetic pigments (NPP) are defined as the sum of diadinoxanthin, diatoxanthin and β-carotene, while the photosynthetic pigments (PP, excluding chlorophyll *a*) comprise chlorophyll c_2 and peridinin-equivalent. For the phytoplankton, only the samples collected near local noon were considered here ($N = 5$ for the phytoplankton pigment data at each depth). The comparison between phytoplankton and zooxanthellae is restricted to pigments present in eukaryotes, because it can only be made on the basis of pigments that can be univocally attributed to well identified and comparable taxa. For the pelagic samples, PP is thus defined as the sum of chlorophyll *c*, peridinin, fucoxanthin, 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin, whereas NPP is defined as the sum of diadinoxanthin and diatoxanthin.

2.3. Specific absorption coefficient and in situ light measurements

The determination of chl *a*-specific absorption coefficient spectra of the zooxanthellae and phytoplankton followed protocols commonly used for free living algal cells (Bricaud and Stramski, 1990; Allali et al., 1997) and were determined with an integrating sphere (Li-Cor 1800-12s) interfaced with a spectroradiometer (Li-Cor UW-1800). In-water downward irradiance was also measured between 400 and 700 nm using the same spectroradiometer. To compare zooxanthella and phytoplankton data under similar radiative regimes (i.e. same relative photosynthetic available radiation, PAR), the geometrical depth, z (in meters), is replaced by the dimensionless optical depth, $\zeta = K_d(\mathrm{PAR}) \cdot z$, where $K_d(\mathrm{PAR})$ is the diffuse attenuation coefficient for PAR.

2.4. Photosynthetic parameters

During the summer experiment, photosynthetic parameters of the zooxanthellae were estimated from P vs. E curves determined using the "radial photosynthetron" (Babin et al., 1994). For these measurements, the 3–4 aliquots of suspension corresponding to a given depth and a given colony were pooled together. The rate of carbon fixation (P) was determined as described in Parsons et al. (1984). After normalization to the chl *a* concentration, the initial slope of each P^B vs. E curve (α^B) was estimated by linear regression. The maximum rate of carbon fixation ($P^B{}_{\max}$) was set equal to the maximum value of the curve, as this approach was found more robust than curve fitting to the experimental data. The maximum quantum yield of carbon fixation ($\phi_{C\ \max}$) is given by the ratio of α^B to the mean chl *a*-specific absorption coefficient of algae weighted by the irradiance spectrum measured within the incubation chambers. The saturation parameter, E_k, is defined as the

P^{B}_{max}/α^{B} ratio. The photosynthetic parameters of the phytoplankton community were determined only once at the oceanic site, using the same photosynthetron and the protocol described in Babin et al. (1996).

2.5. *Statistical analyses*

For both the zooxanthella and phytoplankton samples, Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks was performed to compare the different depths. When an ANOVA revealed significant differences, a Noether multiple comparison test was applied at the 0.05 significance level to identify the differences among the depths. In addition, two-way analyses of variance were performed to compare similar depths between the two sampling periods (i.e. 5, 10, 15 and 25 m). An additional comparison at 35 m was also included in the seasonal analysis after pooling together the 30 and 40 m data from the winter experiment.

3. Results

3.1. *Hydrological and optical conditions*

In French Polynesia, waters from the outer reef slope are homogeneous, warm with little variation (25–28 °C) and very clear, with a diffuse attenuation coefficient for PAR, K_d(PAR), typically around 0.05 m^{-1} (Maritorena and Guillocheau, 1996). The lower limit of the euphotic depth (where PAR is reduced to 1% of its surface value) is around 90–100 m. Optical properties are very stable throughout the year, and only temporarily affected by land drainage during heavy rains or tropical depression events. The incident PAR irradiance around noon for sunny days was typically around 500 W m^{-2} (about 2350 µmol quanta m^{-2} s^{-1}) in summer, and 340 W m^{-2} (about 1600 µmol quanta m^{-2} s^{-1}) in winter. Seasonal variations in daily integrated irradiation are larger, as day length varies from 11 to 13 h. As a result, the daily incident PAR energy for a clear sky increases from 8.15 to 13.75 MJ m^{-2}, between winter and summer.

At the open ocean site, algal biomass in the upper layer (0–60 m) was low, with chlorophyll *a* concentration ranging between 0.045 and 0.070 mg m^{-3}. Concentration increased with depth and formed a deep maximum at about 125 m. Nitrate and nitrite were undetectable at nanomolar level down to ca. 130 m (Raimbault et al., 1999). The euphotic depth was 125 m [K_d(PAR) ~ 0.037 m^{-1}].

Fig. 1 shows typical downwelling irradiance spectra from the outer reef slope and the oceanic site. The PAR vertical profiles are also plotted. The irradiance spectra from the two locations are very close, with the oceanic waters being slightly clearer, particularly in the blue region. PAR is reduced to 10% of its surface value at ca. 45 or 60 m near Tahiti and in the open ocean, respectively. At equivalent optical depth, the spectral composition of the downwelling irradiance in both locations is similar, with the dominance of blue radiation typical of oligotrophic environments.

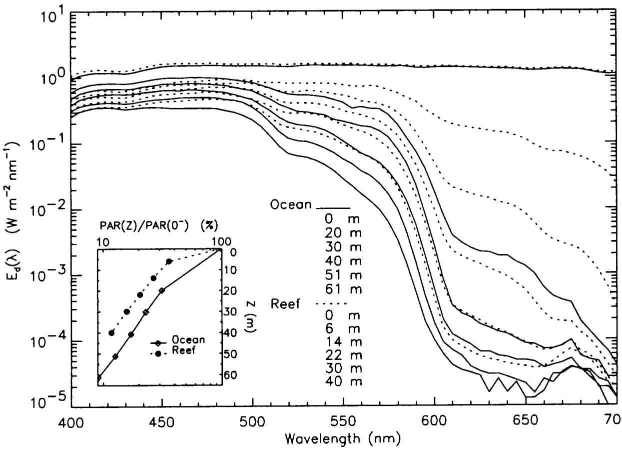

Fig. 1. Example of downwelling irradiance spectra on the outer reef slope (dotted lines) and at the oceanic site (solid line). The depth of each spectrum is indicated. The depth profile of photosynthetically available radiation (PAR, relative units) is plotted in the inset.

3.2. *Pigments per unit area in corals*

For the three pigment groups, the concentration per unit area did not exhibit marked patterns, with a rather uniform vertical distribution in both seasons (Fig. 2a, c, e). The chlorophyll *a* and PP areal concentrations were both around 3 µg cm^{-2} (Fig. 2a, c); whereas NPP was generally slightly below 1 µg cm^{-2} except close to the surface in summer, where higher values were observed (Fig. 2e). However, the ANOVAs on the summer data revealed no significant differences between depths for all three pigment groups (Table 1). The winter profiles suggest a maximum concentration per square centimeter at 10 m in the three groups of pigments, but the statistical analysis revealed no differences among depths (Table 1). The comparison of the two sampling periods for depths common to the winter and summer experiments revealed significant differences with the season only for NPP, with no significant interaction between depth and season (Table 2).

3.3. *Pigments per zooxanthella cell*

The profiles of the pigment contents per cell generally increased from the shallowest depths (1–5 m) to 10–15 m and showed less marked features at greater depths (Fig. 2b, d, f). While this kind of profile was observed for both seasons, differences among the depths were significant only for the winter experiment (Table 1), with the 5 m samples having significantly lower intracellular concentration than at 15 and 30 m deep for chl *a* and PP, and than 15 m for NPP (Table 1). Seasonal differences were significant for chl *a* and PP (Table 2), with no interaction between depth and season.

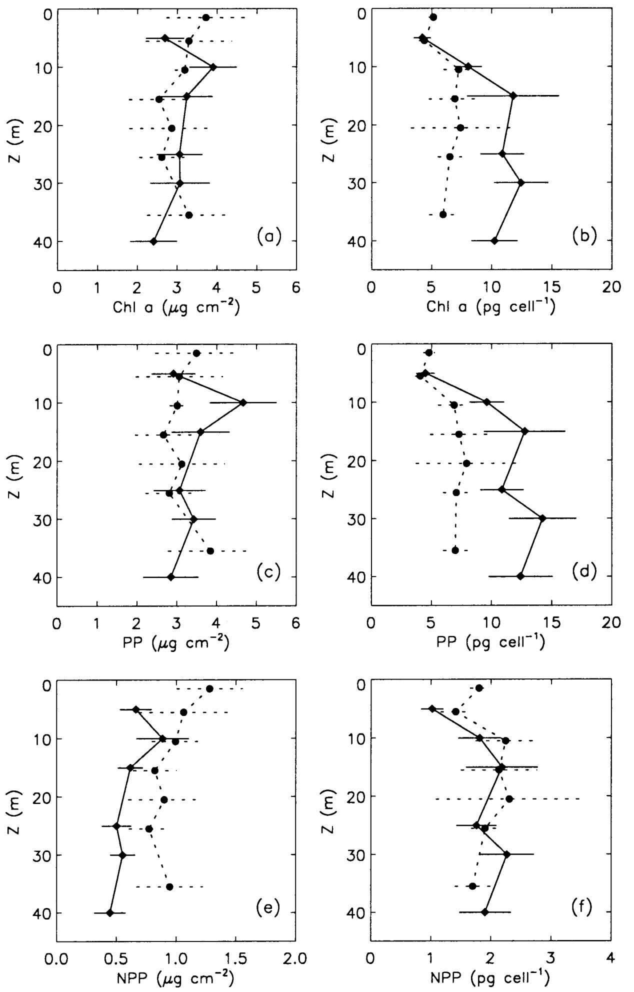

Fig. 2. Depth profiles of mean concentrations of chl *a* per unit area (a) and per cell (b), PP (= chl *c* + peridinin) per unit area (c) and per cell (d), NPP (photoprotectant carotenoids) per unit area (e) and per cell (f) for the winter (solid line, diamonds) and summer (dotted lines, circles) data. The ±1 standard deviation at each depth is also plotted.

3.4. Pigment ratios

The variations of the PP/chl *a* ratio with depth were small for the zooxanthellae (Fig. 3a) and were significant only in summer, during which the ratio was lower at 1 m ($\zeta = 0.17$) compared to the deepest depth (35 m; $\zeta = 1.98$) (Table 1). In contrast, variations with depth were stronger in winter for the NPP/chl *a* ratio (Fig. 3b), with the surface samples (5 m; $\zeta = 0.837$) having significantly higher ratio (Table 1) than at 25 m ($\zeta = 1.67$). As a result, the PP/NPP ratio increased significantly with depth in both seasons (Fig. 3c and Table 1), when comparing the shallowest samples to those at depths >30 m ($\zeta > 1.7$). All three ratios were significantly different with regard to season, with significant interaction between depth and season for the PP/chl *a* only (Table 2).

For the phytoplankton data, the three ratios (PP/chl *a*, NPP/chl *a*, and PP/NPP) showed significant differences with depth (Fig. 3 and Table 2), reflecting a significant increase in PP/chl *a* and PP/NPP with depth and an inverse pattern for the NPP/chl *a* ratio.

3.5. Specific absorption

The chl *a*-specific absorption spectra of zooxanthellae are typical of large algal cells and do not show any strongly marked feature. Compared to the chlorophyll *a* red peak at 674 nm, the blue peak is rather flat between ca. 420 and 435 nm. A shoulder originating from chlorophyll *c* (and maybe NPP) is also detected around 460 nm (Fig. 4). Chlorophyll *c* is also likely responsible for the two small features, around 585 nm, and in the 630–650 nm range. The convexity around 550 nm originates from the presence of peridinin (Kühl et al., 1995). A small difference occurred in the violet–near-UV part of the spectrum, where the decrease from the blue peak toward shorter wavelengths is less steep for the summer samples than for the winter ones. Indeed, the slopes of linear regressions between wavelength and the mean $a^*(\lambda)$ data in the 425–365 nm domain are 0.052, 0.051 and 0.07 at 5, 15 and 35 m, respectively, in summer, whereas, for the same depths, the slopes are equal to 0.078, 0.14 and 0.089 in winter (Fig. 4). This difference may result from the higher NPP/chl *a* ratio in summer and/or the presence of UV-absorbing compounds such as mycosporine-like amino acids (Dunlap and Shick, 1998). Importantly, the $a^*(\lambda)$ values are much lower (by a factor 3–5 for the blue maximum) than those typical of picoplanktonic species inhabiting the upper layers at the open ocean site (Allali et al., 1997).

Fig. 5 shows that, for both the depth and the season, the absorption coefficient of zooxanthellae at 440 and 675 nm had little variation and spans similar ranges. No differences among depths were observed in summer, whereas $a^*(440)$ was significantly higher at 15 m compared to 30 m in winter (Table 1). The seasonal comparison for samples collected at identical depths revealed no significant differences (Table 2). Because of the relative stability in pigment composition and cell size, no regular change in the shape and magnitude of the absorption spectrum was observed with either depth or season.

3.6. Photosynthetic parameters

Fig. 6 displays the vertical profiles of the photosynthetic parameters α^B, $P^B{}_{max}$, $\phi_{C\ max}$ and E_k for zooxanthellae. They all (except E_k) exhibit a minimum at 10–15 m (ζ around 1.25). Because of similar vertical trends in α^B and $P^B{}_{max}$, E_k remains roughly constant around a rather low mean value (about 85 µmol quanta m^{-2} s^{-1}). Only $P^B{}_{max}$ and E_k were significantly different among depths, with $P^B{}_{max}$ significantly higher at 1 m ($\zeta \approx 0.17$) compared to 10 m and E_k significantly higher at the shallowest depth (1 m; $\zeta \approx 0.17$) compared to the deepest (35 m; $\zeta \approx 1.98$) (Table 1). For the pelagic phytoplankton community, the α^B and ϕ_C values are similar to those of zooxanthellae over the

Table 1
Results of the Kruskal-Wallis one-way Anovas for depths comparisons. When an Anova revealed signifiant differences among depths ($p < 0.05$, indicated by an asterisk, (*)), a Noether's test was applied to identify which depths were different. These depths are indicated along with the Noether's calculated value (Z_H) and the limit value over which a difference is considered significant at the 5% level

	Winter		Summer	
	Anova	Noether's test	Anova	Noether's test
Zooxanthellae	*p*	Z_H	*p*	Z_H
chl $a\cdot$cm^{-2}	0.193	–	0.720	–
PP$\cdot$cm^{-2}	0.133	–	0.892	–
NPP$\cdot$cm^{-2}	0.051	–	0.439	–
chl $a\cdot$cell^{-1}	0.007(*)	5-15m – 3.27 > 3.14 5-30m – 3.31 > 3.14	0.169	–
PP$\cdot$cell^{-1}	0.008(*)	5-15m – 3.31 > 3.14 5-30m – 3.23 > 3.14	0.052	–
NPP$\cdot$cell^{-1}	0.021(*)	5-15m – 3.19 > 3.14	0.466	–
PP/chl *a*	0.074	–	0.008(*)	1-35m – 3.1 > 3.04
NPP/chl *a*	0.002(*)	5-25m – 3.81 > 3.14	0.085	–
PP/NPP	0.002(*)	5-40m – 3.84 > 3.24	0.016(*)	1-35m – 3.33 > 3.24
a* (440)	0.014(*)	15-30m – 3.1 > 2.87	0.081	–
α^B	–	–	0.053	–
$P^{B}_{\ max}$	–	–	0.016(*)	1-10m – 3.22 > 3.04
E_K	–	–	0.019(*)	1-35m – 3.09 > 3.04
Ø$_{max}$	–	–	0.0829	–
Phytoplankton				
PP/chl *a*	–	–	0.010(*)	5-70m –3.3>2.87
NPP/chl *a*	–	–	0.003(*)	5-50m – 3.2>2.87
PP/NPP	–	–	0.004(*)	5-70m –3.3>2.87

same irradiance range, and regularly increasing with depth down to $\zeta \approx 2$; in contrast, P^{B}_{max} remains steadily around 2.5 mg C (mg chl a)$^{-1}$ h^{-1}, a value at least twice higher than that of the zooxanthellae. As a consequence, E_k for free living phytoplankton decreases markedly with depth (Lindley et al., 1995) from an upper layer value about three times larger than that of fixed algae.

4. Discussion

Most parameters measured on the zooxanthellae showed variations with depth and/or season. However, and some what unexpectedly, the variations with depth were of limited amplitude, and seasonal differences were sometimes larger than the vertical variations at a given season. The increase with depth of the PP/NPP ratio is the most obvious sign of photoacclimatization observed on the zooxanthellae, and

Table 2
Results of the two-way Anovas for seasonal comparisons of zooxanthellae samples collected at similar depths. Significant disserences ($p < 0.05$) are indicated by an asterisk. Depth-season interactions are indicated in the right-end column

	p season	*p* depth-season interaction
chl $a\cdot$cm^{-2}	0.595	0.285
PP$\cdot$cm^{-2}	0.153	0.085
NPP$\cdot$cm^{-2}	0.0004(*)	0.511
chl $a\cdot$cell^{-1}	0.0005(*)	0.071
PP$\cdot$cell^{-1}	> 0.0001(*)	0.123
NPP$\cdot$cell^{-1}	0.455	0.455
PP/chl *a*	0.033(*)	0.034(*)
NPP/chl *a*	> 0.0001(*)	0.208
PP/NPP	> 0.0001(*)	0.281
a* (440)	0.271	0.157

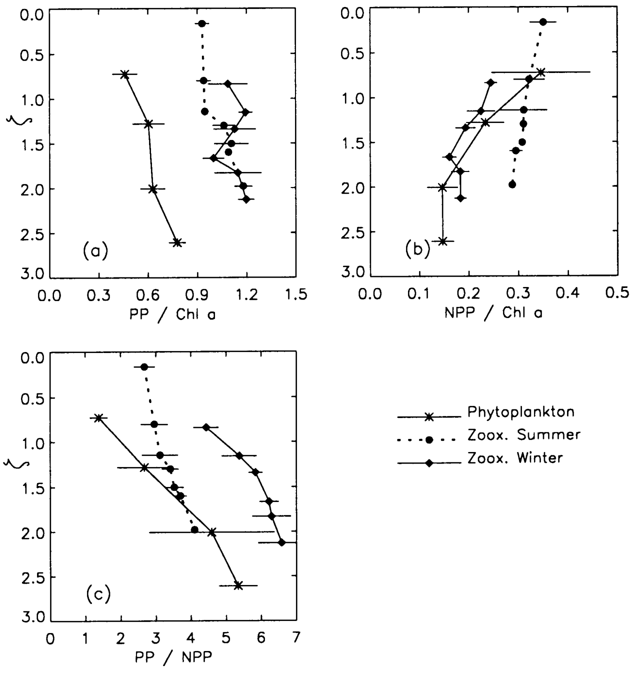

Fig. 3. Depth profiles of pigment ratios (mean ± 1 standard deviation) of zooxanthellae for the winter (diamonds, solid lines) and summer (circles, dotted lines) experiments. The same ratios are plotted for the phytoplankton assemblage (solid line, stars). Geometrical depths have been converted to optical depths (see text).

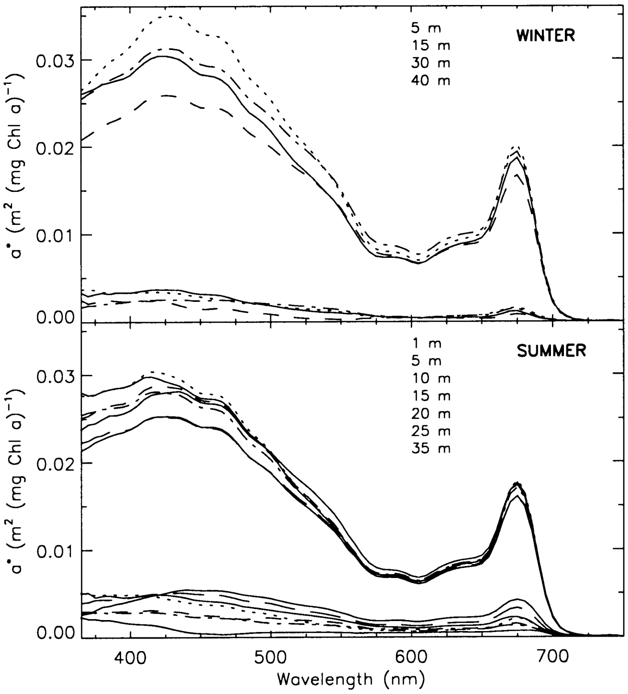

Fig. 4. Mean spectra and standard deviation of the chl *a*-specific absorption coefficients, $a^*(\lambda)$, for both sampling seasons. Each depth is represented by a different line pattern. Mean and standard deviation (i.e. lower curves) from the same depth have identical patterns. Depths are listed by decreasing value at 440 nm.

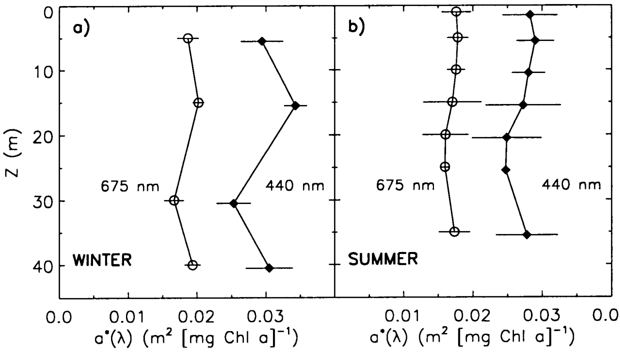

Fig. 5. Depth profiles of the chl *a*-specific absorption coefficients (mean ± 1 standard deviation) at 440 and 675 nm for the winter (a) and summer data (b).

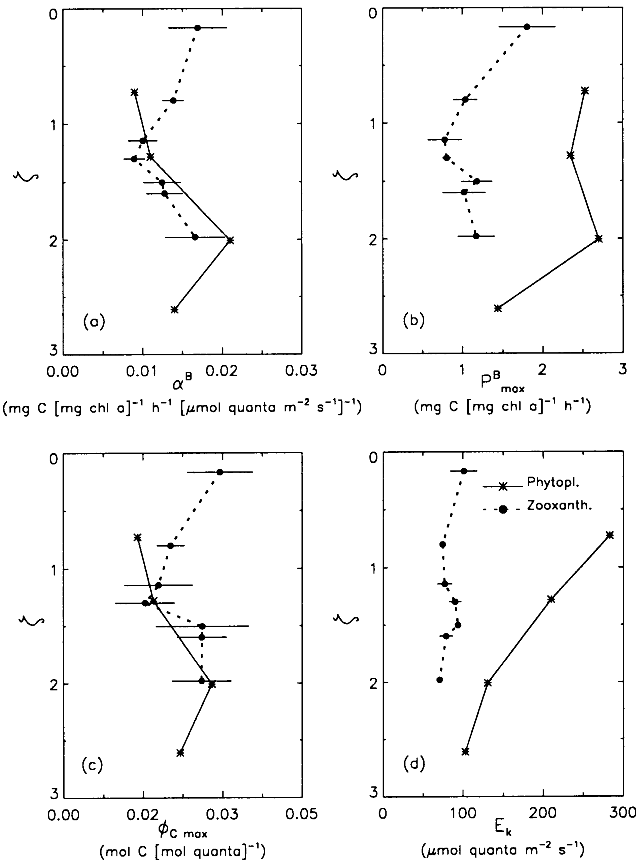

Fig. 6. Depth profiles of photosynthetic parameters data (mean ± 1 standard deviation) for the zooxanthellae summer experiment (dotted line, circles) and for the pelagic algal community (solid line, stars). Geometrical depths have been converted to optical depths (see text). (a) α^B, (b) $P^B{}_{\max}$, (c) $\phi_{C\ \max}$, and (d) E_k.

PP/NPP is the only parameter that showed significant vertical differences in both seasons. The PP/NPP ratio was higher in winter, presumably as a response to lower daily irradiation (by a factor of 0.6). Consistent with the above scheme, the intracellular concentrations of chl *a* and PP were generally higher in winter for deep samples. While previous studies on various coral species, including *P. verrucosa*, generally show an increase of the intracellular pigment concentration with diminishing light, this feature was significant only for the winter data in the present study. For *P. verrucosa*, Titlyanov et al. (1980) found shaded samples to have higher intracellular concentrations than sun-exposed samples (for all pigments except β-carotene), but they also described an opposite behavior for all pigments (except again for β-carotene) in their comparison of sun-exposed samples at 45 and 15 m.

Beside the PP/NPP ratio, several other parameters showed seasonal differences. These differences are likely caused by variations in the incident light, as other factors that may influence seasonal patterns such as temperature have limited seasonal variation in French Polynesian waters (e.g. water temperature has a typical 3 °C difference between winter and summer). In recent years, several studies have documented seasonal variations in physiological parameters in corals and zooxanthellae (Fagoonee et al., 1999; Brown et al., 1999). The seasonal variations reported here should, however, be considered with caution, because the winter sampling took place after a severe coral bleaching period which affected Tahitian reefs at the beginning of 1994. It is likely that part of the variability we observed in the winter data is a consequence of the bleaching, as corals were still in a recovery phase. Another reason for caution is that seasonal variations are better evaluated with time-series allowing multi-year analyses.

While the PP/NPP ratio of zooxanthellae show some significant vertical variations, the comparison with the results of the profile for the pelagic eukaryotes revealed some important differences (Fig. 3c). Although the ratios for the two populations are not directly comparable, the trends can be interpreted as a possible expression of photoacclimatization. It is clear (Fig. 3c) that, over approximately the same PAR range, the PP/NPP ratio increased weakly (ca. 30%) with depth for the zooxanthellae, while it increased by more than a factor of three for the stable pelagic algal assemblage (recall that the density gradient ensures the stable layering of these algae). This observation, in favor of an efficient photoacclimatory response of the pelagic community (also supported by other pigment changes inside the prokaryotic group), tends a contrario to demonstrate that a more limited photoacclimatization occurs in zooxanthellae with respect to depth.

The light absorption data are another indication of a weak photoacclimatory response by the zooxanthellae throughout the water column, as only small (and generally not significant) variations were observed. The relatively low value of the chl *a*-specific absorption, at the blue maximum in particular, as well as the reduced contrast between the two absorption peaks (the mean $a^*(440)/a^*(675)$ ratio is about 1.6), is indicative of a strong "package effect" (Kirk, 1975). This is not surprising, as the relatively large size of the zooxanthellae (around 10 µm) and their high intracellular pigment concentration (about 20 kg m^{-3} for the sole chlorophyll *a*) both contribute to lower absorption. The actual package effect, when the cells are contiguously packed within the coral tissues, is certainly even higher.

Until recently, the only available data of spectral absorption by zooxanthellae were those from Leletkin and Zvalinsky (1981) and Dustan (1982). In these studies, absorption was shown to increase with depth. However, the reality of such an increase is difficult to assess, as absorption measurements were not normalized in the Leletkin and Zvalinsky (1981) work, whereas they were normalized by cell counts in Dustan (1982). It should be mentioned that, when normalized by cell densities, our light absorption data exhibit a trend similar to that observed by Dustan (1982). Wyman et al. (1987) did not measure spectra of the specific absorption coefficient but an averaged value over the visible spectrum (named k_c or k_c in their paper), which can, however, be compared to the present data. These authors observed weak variations of the absorption with depth (in the 1–50 m range), with values generally slightly higher toward the surface and no significant difference among species. The magnitude and spectral shape of our measurements match well those recently reported by Lesser et al. (2000) for their shallowest samples (10 m). In contrast with our results, these authors reported a dramatic drop in absorption in zooxanthellae collected 8 m deeper. This important difference (in pigment content and absorption) between samples collected 8 m apart in depth, while only very weak variations over a ~40 depth range were observed in the present study, is actually not surprising when the optical properties of the water in each study are examined. As mentioned in their paper, Lesser et al. (2000) sampled in turbid waters, as evidenced by the shape of their diffuse attenuation spectra (K_d, in their Fig. 1), with a minimum value around 500 nm and a marked increase toward shorter wavelength. From their PAR data, approximately 20% of the visible light energy incident on the surface remained at 10 m and only ~1% at 18 m. These waters are obviously very turbid compared to those documented here (Fig. 1), where the 1% light level is typically deeper than 80 m.

A common vertical pattern is clearly observed for the α^B, $P^B{}_{max}$ and $\phi_{C\ max}$ parameters in zooxanthella populations (Fig. 6). Such patterns could be interpreted as the result of an "optimization" process, with the highest $P^B{}_{max}$ value close to surface, and with increasing α^B values at depths beyond 15 m. However, the non-significant variations of α^B and the relative vertical stability in E_k do not support the hypothesis of such a photoacclimatory process. Indeed, changes in E_k reflect changes in the effective absorption cross section of the photosystems (σ) and in the minimal turnover time for carbon reduction (τ) (Kolber and Falkowski, 1993). All biochemical and biophysical processes that contribute to photoacclimatization affect one of these two characteristics of the photosynthetic apparatus. Moreover, photoacclimatization implies that σ and τ never compensate each other. Hence, E_k is certainly the most reliable indicator of photoacclimatization, and although significant, its weak difference between the surface and 35 m is clearly in contradiction with the occurrence of a strong photoacclimatory process.

Sometimes, α^B and $P^B{}_{max}$ may covary when the size and/or the number of photosynthetic units per cell change (Richardson et al., 1983). Such changes would result in modification of the accessory pigment proportions and the packaging effect, and would therefore alter the chl *a*-specific absorption coefficient. While the PP/chl *a* ratio was significantly higher at 35 m than at 1 m (Fig. 3a), the absence of significant variations in $a^*(\lambda)$ (Figs. 4 and 5) tends to invalidate the hypothesis of a covariation in α^B and $P^B{}_{max}$ resulting from changes in size or in number of photosynthetic units per cell. In addition, the $\phi_{C\ max}$ vertical pattern is unexplainable in terms of photoacclimatization and photoprotection strategy, as smallest values are to be expected at the surface. The much lower $P^B{}_{max}$ and E_k values for the zooxanthellae, compared to those of the pelagic community, suggest that they behave as if they were in a low-light environment, possibly because of the presence of the animal host. The small differences in E_k also suggest a limited influence of the vertical variations in irradiance. In the zooxanthellae, with E_k close to 85 µmol quanta m^{-2} s^{-1} on average and considering the latitude, the rate of carbon fixation is saturating over ca. 11–9.5 h per day at 1 and 35 m, respectively, during austral summer. Of course, one has to consider the actual light field experienced by the coral, which may be quite different from that measured in the water column with a flat collector. However, remember that only sun-exposed branches were collected for the present study.

The extent of photoacclimatization in zooxanthellae may also be regulated by nutrient limitation. Indeed, zooxanthella $\phi_{\mathrm{C\ max}}$ values are very similar to those observed at the oceanic site where the surface layer is nutrient depleted (Raimbault et al., 1999). It has been shown that the coral host animal would keep zooxanthellae in a near-permanent nitrogen limitation to ensure unbalanced growth and thereby induce glycerol synthesis and excretion from the algae (Falkowski et al., 1993). The low zooxanthella $\phi_{\mathrm{C\ max}}$ values observed in the present study support this hypothesis in the case of *P. verrucosa*. As far as the initial slope and the quantum yield are concerned, similar vertical trends (but different magnitudes) between the zooxanthellae and phytoplankton were also observed. In contrast, compared to that of pelagic algae, the maximum photosynthetic rate in zooxanthellae is markedly lowered, and consequently, a much lower and vertically constant saturation parameter (E_k) is observed.

The aim of this study was to investigate the photoacclimatization of zooxanthellae isolated from the coral *P. verrucosa*, within the depth range of their natural habitat, through the determination of pigments, light absorption and photosynthetic parameters. The variations of these parameters with depth and season were generally small, and while their vertical distributions were often similar to those observed for free living phytoplankton, they were of much lower amplitude. Most of our observations suggest that, between the surface and ~40 m, the amount of radiant energy available for the zooxanthellae was not greatly changed, at least not enough to have caused a drastic photoacclimatization response. In the clear waters of the Tahitian reef, an attenuation of radiant energy by a factor of 10 is typical between 0 and 40 m. The limited signs of photoacclimatization seem to indicate that algal cells are living in a highly “buffered” light environment, possibly caused by the coral itself. The assumption of an optical role of the animal host is supported by the work of Kühl et al. (1995). These authors, using microprobes positioned within the coral tissues, demonstrated that the coral skeleton and tissues contribute through scattering and internal reflection to regulate the photon flux and limit the loss of incident photons not immediately absorbed by the algal cells or animal tissues. These observations may explain why, in previous works on photoacclimatization by the zooxanthellae, clearer trends were generally observed in experiments with dramatically different light regimes such as in-light versus shade comparisons (Falkowski and Dubinsky, 1981) or in waters with strong light attenuation (Lesser et al., 2000). In contrast, for samples collected in the 0 to ~40 m depth range in clear waters (Wyman et al., 1987, or in the present study), the changes in light energy available for the zooxanthellae (possibly modulated by the presence of the host) do not seem sufficient to have induced major modifications of the photosynthetic apparatus.

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