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# *Trichodesmium* and other planktonic cyanobacteria in New Caledonian waters (SW tropical Pacific) during an El Niño episode

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ABSTRACT: Contributions of filamentous and picoplanktonic cyanobacteria to the phytoplankton community structure were examined in New Caledonian waters during the 2001–2003 El Niño period at 2 ocean stations (Loyalty Channel and Santal Bay) and 1 coral-reef lagoon station (Ouinne). Morphometric characteristics of diazotrophic filamentous cyanobacteria are given, as well as the seasonal and inter-annual variations of their surface areas and integrated abundances. *Trichodesmium tenue* and *T. thiebautii* were the dominant species followed by *T. erythraeum*, altogether accounting for more than 51–80% of the biomass of the free-living filamentous cyanobacteria. *Katagnymene* spp. accounted for a smaller percentage (<13.8% at ocean stations, <3.6% in the lagoon). *Richelia intracellularis* biomass was relatively small (<1% of total surface area and volume of *Trichodesmium* trichomes), with the highest concentration observed in summer (735 trichomes $l^{-1}$). Colonies of unidentified cyanobacteria composed of spherical cells accounted on average for <1% of the *Trichodesmium* biomass, with maximum values exceeding 4000 cells $l^{-1}$. Abundance of filamentous cyanobacteria varied according to environmental factors; summer 2001–2002 was characterized by low filamentous diazotroph abundance, and summer 2003, at the peak of the 2001–2003 El Niño, was particularly rich in filamentous cyanobacteria (with a maximum *Trichodesmium* spp. abundance of 4500 trichomes $l^{-1}$ in the Loyalty Channel). A similar variability pattern was observed for large diatoms and dinoflagellates, and for all picoeukaryotic populations. Different biomass estimators are provided, including cell abundances, pigment concentrations including chlorophylls and phycoerythrin, and carbon content.

KEY WORDS: *Trichodesmium* · Filamentous cyanobacteria · Community structure · Microscopy · Picoplankton · Pigments · Loyalty Islands · Coral-reef lagoon · El Niño



## INTRODUCTION

In the oceans, most of the subtropical and tropical pelagic areas are dominated by oligotrophic conditions and picophytoplankton, which are best adapted to nutrient-poor environments (Luo et al. 2012, Buitenhuis et al. 2013). Unicellular picoplanktonic cyanobacteria are the most abundant photosynthetic group and are distributed throughout the tropical Pacific Ocean (Le Bouteiller et al. 1992, Ishizaka et al. 1994, Buitenhuis et al. 2012b). Other marine cyanobacteria, comparatively patchier and less abundant, also play a pivotal role in

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N-limited ecosystems, combining primary production and dissolved $N_2$ fixation (diazotrophy). This cyanobacterial diazotrophic community includes unicellular, filamentous, and symbiotic forms (Luo et al. 2012, Bergman et al. 2013). Pelagic filamentous cyanobacteria consist mainly of the genus *Trichodesmium* with 2 more frequently reported and quantified species: *T. erythraeum* Ehrenberg and *T. thiebautii* Gomont. Three other *Trichodesmium* species (*T. tenue* Wille, *T. contortum* Wille and *T. hildebrandtii* Gomont), and 2 *Katagnymene* species (*K. pelagica* and *K. spiralis* Lemmerman) are seldom reported. Furthermore, *Richelia intracellularis* has been regarded as one of the main tropical filamentous diazotrophs in marine pelagic waters, forming large blooms in some regions as parts of diatom–diazotrophic associations (DDAs). This heterocystous cyanobacterium is usually observed as an endosymbiont of the *Rhizosolenia* (sometimes referred as Het1 or RR) and *Hemiaulus* (Het-2 or HR) (Venrick 1974, Janson et al. 1999) diatoms and has also been reported as an epiphyte of *Chaetoceros* spp. (Gómez et al. 2005) or as a free-living filament. The epiphytic form was identified as a separate species, *Calothrix rhizosoleniae*, based on genetic characterization (Janson et al. 1999, Foster et al. 2010). It has also been reported in cohabitation with *Trichodesmium* puff-form colonies (Jabir et al. 2013, Momper et al. 2015, Girault et al. 2016). Besides *Trichodesmium* and other filamentous cyanobacteria, free-living unicellular cyanobacteria (UCYN-A, -B, and -C) are also recognized as possible major diazotrophs in tropical and subtropical areas (Zehr & Bombar 2015), occupying different ecological niches.

$N_2$ fixation by filamentous cyanobacteria in the oceans is estimated at around 80 Tg N $yr^{-1}$ (Capone et al. 1997) for a global oceanic $N_2$ fixation of 100–200 Tg N $yr^{-1}$ (Karl et al. 2002) and accounts for more than 70% of global marine $N_2$ fixation (Luo et al. 2012, Bergman et al. 2013). The southern Tropical Pacific is a region known for high $N_2$ fixation, as measured between New Caledonia and Vanuatu (Garcia et al. 2007, Masotti et al. 2007, Biegala et al. 2014), over a longitudinal transect at 17° S (Shiozaki et al. 2014), and in the New Caledonia southwest lagoon (Biegala & Raimbault 2008, Bonnet et al. 2016). Surface blooms are frequently observed in the region, mainly composed of filamentous cyanobacteria (Dupouy et al. 2011).

Identification and counts of filamentous cyanobacteria at the species level are nevertheless scarce (Campbell et al. 2005, Luo et al. 2012), as recent work has relied only on *Nifh* gene counts (Moisander et al. 2010, Bonnet et al. 2015, 2016). Moreover, few studies have addressed their temporal variability as reported from the Hawaiian Ocean Time-Series (HOT) in the North Pacific (Letelier & Karl 1996), the Bermuda Atlantic Time-Series (BATS) in the North Atlantic (Orcutt et al. 2001), and the Indian Ocean (Lugomela et al. 2002).

By quantifying the different species abundances and their specific relationship with nutrient and physical environmental conditions, it should be possible to more precisely identify the main factors stimulating filamentous cyanobacterial growth and how they affect community structure. Such data are essential for estimating global $N_2$ fixation more accurately, improving our understanding of how $N_2$-fixing species respond to their environment, and anticipating how the phytoplankton community will develop in the context of global climate change (Dutkiewicz et al. 2015, Gruber 2016). *In situ* abundance of filamentous cyanobacteria and their pigment content are also needed to calibrate recent bio-optical models aiming to determine their abundance from light reflectance of surface mats (Dupouy et al. 2011, Gower et al. 2014, McKinna 2015) or medium concentration blooms (Westberry & Siegel 2006, De Boissieu et al. 2014).

A careful study of the seasonal and inter-annual variations of different filamentous cyanobacterial species abundances was undertaken in 2001–2003 (as part of the Diapalis program) in New Caledonian waters and tentatively analyzed based on changes in environmental conditions and in the abundance of other components of the autotrophic community, particularly picoplankton. For this paper, data were obtained at 3 stations: an open-ocean station (Loyalty Channel); a large, deep, and open bay (Santal Bay, Lifou Island); and a lagoon station (Ouinne, southeastern coral reef lagoon). Taxonomic identification, measurements, and counts of filamentous species were carried out by microscopy, while data on picoplankton were analyzed by flow cytometry. The various estimators for determining filamentous cyanobacterial biomass were also compared. Nutrient data (Van Den Broeck et al. 2004, Moutin et al. 2005) and all $N_2$ fixation rates measured under the Diapalis program have already been published (Garcia et al. 2007).

## MATERIALS AND METHODS

### Sampling and environmental data

Nine oceanographic cruises (Diapalis program cruises D1 to D9), each lasting about 1 wk, were con-

ducted between October 2001 and October 2003 aboard IRD's (Institut de Recherche pour le Développement) RV 'Alis' between 165° and 168° E and between 20° and 23° S in the Eastern Coral Sea (Table 1, Fig. 1). Samples were taken at 3 stations: the first at 20° 51′ S, 167° 04′ E in the deep Santal Bay, which is broadly exposed to the ocean, on Lifou Island's west coast (bottom depth: 1050 m, Stn 1); the second in open-ocean waters in the middle of the Loyalty Channel at 21° 30′ S, 167° 00′ E (bottom depth: 2000 m, Stn 2); and the third (21° 57′ S, 166° 45′ E) in the southeastern coral-reef lagoon of New Caledonia (bottom depth: 40 m, Stn 3). The sampling covered different seasons (Table 1): summers 2002 (D2–D4) to 2003 (D7), winters 2002 (D6) to 2003 (D8), and transition periods (D1, D5, D9). A sample was also collected from a surface accumulation of *Trichodesmium erythraeum* located in New Caledonia's southwestern lagoon.

During cruises, CTD casts were carried out at each station with water sampling down to 200 m for the deepest stations and down to 30 m in the lagoon. Temperature (°C) and salinity were measured with Sea-Bird SBE 911, and water samples were collected with 8 l Niskin bottles attached to a CTD-rosette system. Nitrate + nitrite ($NO_x$) and phosphate ($PO_4^{3-}$) were preserved in Nalgene bottles with $HgCl_2$ prior to colorimetric assays on a Technicon autoanalyzer II (Raimbault et al. 1990). Ammonium was measured immediately on board by fluorometry (Turner Design TD-700) according to the procedure described by Holmes et al. (1999). More details on the methods and the full Diapalis data set are available at www.obs-vlfr.fr/proof/vt/op/ec/diapazon/dia.htm. The mixed layer depth (MLD) was estimated by taking the first depth where potential density (sigma-*t*) was 0.03 kg $m^{-3}$ greater than the density at 10 m, based on Montegut et al. (2004).

Table 1. Dates of the 9 Diapalis cruises

| Cruise | Dates | Season |
|---|---|---|
| D1 | 22–31 October 2001 | Transition period |
| D2 | 10–22 December 2001 | Austral summer |
| D3 | 15–22 January 2002 | Austral summer |
| D4 | 2–9 April 2002 | Austral summer |
| D5 | 21–28 May 2002 | Transition period |
| D6 | 6–13 August 2002 | Austral winter |
| D7 | 1–10 February 2003 | Austral summer |
| D8 | 10–14 June 2003 | Austral winter |
| D9 | 7–16 October 2003 | Transition period |

### Filamentous cyanobacteria counting and morphometric characteristics

Filamentous cyanobacteria were recovered from water samples taken at 4–6 depths down to 80 m, with a rosette of 8 l Niskin bottles. Organisms were collected by filtering the entire content of each water bottle through a 10 µm Nuclepore polycarbonate membrane. The filter was then placed in a 20 ml

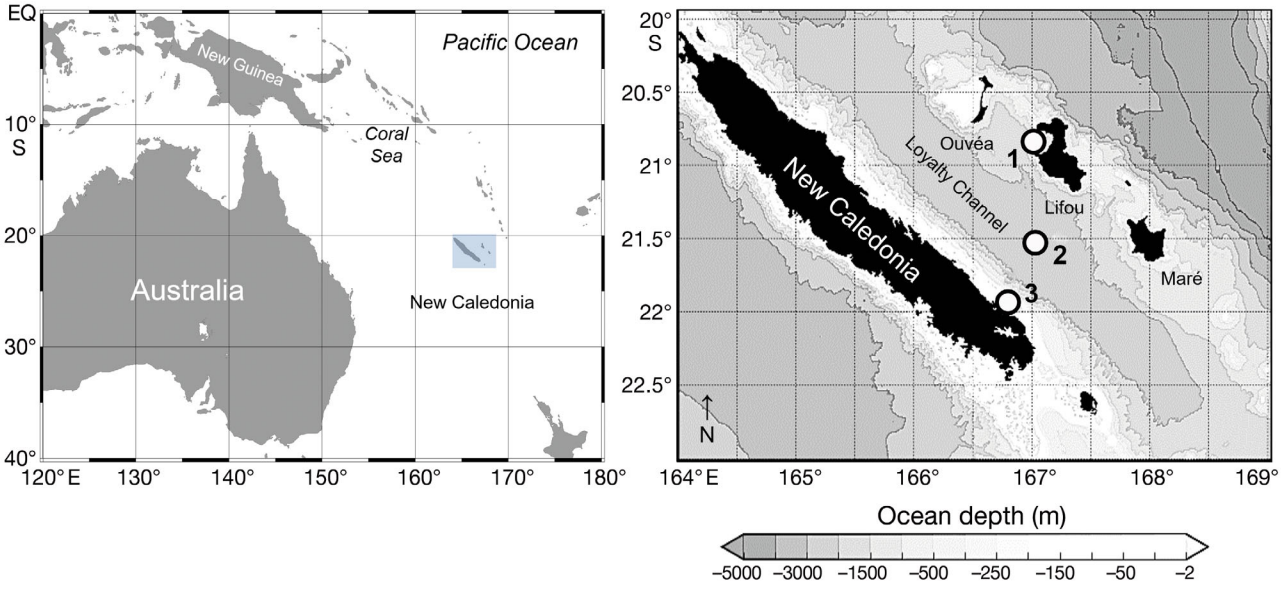


Fig. 1. Location of the 3 main stations sampled during the 9 Diapalis cruises in New Caledonian waters: Stn 1 (Santal Bay) on Lifou Island (20° 51′ S, 167° 04′ E, 1050 m depth), Stn 2 (Loyalty Channel, 21° 30′ S, 167° 00′ E, 2000 m depth), and Stn 3 (Ouinne station) in the east coast lagoon (21° 57′ S, 166° 45′ E, 40 m depth). Figure courtesy of J. Lefèvre

glass bottle containing a 4% formalin solution to preserve photosynthetic organisms. In the laboratory, the filter was rinsed with a plastic wash bottle containing filtered seawater in order to remove all organisms from the filter and collect them in a 100 ml glass cylinder. After processing, the filter was checked for any remaining photosynthetic organisms. A suitable quantity of an acidic formalin solution (1/1 mixture of 40% formalin and acetic acid) was then added to obtain organisms in a final 0.4% formalin solution. From this solution, filamentous cyanobacteria and other large phytoplanktonic species were measured and enumerated with an inverted microscope (OLYMPUS IM., 100× magnification) using a standard sedimentation technique (Utermöhl 1931). According to Cronberg et al. (2004), the acetic acid breaks the gas vesicles, facilitating the sedimentation of filaments in the sedimentation chambers. Sedimentation time was 24 h in 5 and 10 ml chambers and at least 48 h for the 25 and 50 ml chambers, depending on cell and filament abundances. A total of 88 samples were examined for the 9 cruises. For surface accumulations of *T. erythraeum*, samples were collected directly by hand with a small bottle (500 ml), which was then processed using the same method as described above.

Identification of the filamentous *Trichodesmium, Katagnymene* (see review by Bergman et al. 2013), and *Richelia* (Foster et al. 2010) was carried out based on their morphological characteristics. Cell shape and dimensions (particularly cell diameter) were the main criteria used for classifying and identifying them. The sample processing method used to facilitate enumeration and morphometric examination at the trichome level could not be used for observations at the colony level, because most of the colonies had disintegrated. Trichome surface and volume were then calculated on the assumption that they had an elongated cylindrical morphology. For *T. erythraeum* and *T. thiebautii*, identification was confirmed by molecular biology studies (Trottet 2003). Colonies of unidentified cyanobacteria composed of spherical cells were also observed and enumerated.

### Photosynthetic pigment analysis by spectrofluorometry

Chlorophylls including divinyl-chl *a* and *b* for *Prochlorococcus* were measured by spectrofluorometry (F4500 HITACHI spectrofluorometer) based on Neveux & Lantoine (1993) and Tenório et al. (2005). From 0.25 to 0.5 l, water samples were filtered on 25 mm GF/F for the chlorophyll analysis in the total community. Chlorophylls were extracted by grinding the GF/F filter in 90% acetone. Measurements in the >10 µm fraction of the community were also carried out by filtering the entire content of an 8 l Niskin bottle through 47 mm Nuclepore polycarbonate membranes. In the latter case, chlorophylls were extracted in 90% dimethylformamide without grinding the membrane. For convenience, the abbreviations chl *a*, DV-chl *a*, and Tchl *a* are used for monovinyl-chl *a*, divinyl-chl *a*, and the sum of these 2 pigments (total chl *a*), respectively. Concentrations of cyanobacterial phycoerythrin (PE) were assessed by spectrofluorometry both on the overall and >10 µm community, after extraction/cell resuspension in a 50/50 mixture of glycerol/phosphate buffer based on Wyman (1992). Water samples (1–3 l) were filtered through 47 mm 0.4 µm polycarbonate membranes for total PE, and samples of 8 l were filtered through 10 µm membranes for size-fractionated PE. Details of PE spectrofluorometric analysis are given by Lantoine & Neveux (1997) and Neveux et al. (1999, 2006).

### Pico- and nanoplankton analysis by flow cytometry

Subsamples (1 ml) were stored in cryovials and fixed with paraformaldehyde (0.2% final concentration, Campbell & Vaulot 1993) and frozen in liquid nitrogen for a period of <3 mo before flow cytometry analyses. Samples were counted on a Becton Dickinson FACSCan flow cytometer. For each cell, forward and side light scattering as well as red and orange fluorescence were quantified. The light signals were calibrated using 1.002 µm fluorescent beads (Polysciences) as a reference. Four prevalent populations were distinguished and enumerated according to their scattering and fluorescence properties. Two of them belonged to picoplanktonic cyanobacteria, *Prochlorococcus* and *Synechococcus,* and 2 were eukaryotic groups, the smallest being deemed picoeukaryotes. Unicellular nanoplanktonic cyanobacteria such as *Crocosphaera*, easily recognized by the fluorescence excitation spectra of their PE and by their flow cytometric signature (Neveux et al. 1999), were not observed during the Diapalis cruises.

### Carbon biomass estimation

Information on the relative size of each population within the total autotrophic biomass of the ecosystem was obtained by converting cell numbers into carbon

units. Conversion factors (fg C cell$^{-1}$) for *Prochlorococcus* (36), *Synechococcus* (255), and picoeukaryotes (2590) were used according to Buitenhuis et al. (2012b) and for *Richelia* (10) from Luo et al. (2012). For all filamentous cyanobacteria, the carbon conversion factor determined in this study on *T. erythraeum* was applied (17.22 ng C trichome$^{-1}$; see Table 7). The integrated carbon content of each group and their relative percentages were calculated for the upper layer (0–60 m at Stns 1 and 2; 0–30 m at Stn 3). Note that diatoms and dinoflagellates in the >10 µm fraction were not included in the analysis, since mean cell size or cell volume was not determined.

## RESULTS

### Environmental conditions during the 9 Diapalis cruises

The New Caledonia region (Fig. 1) is typical of the tropical regime in the SW Tropical Pacific, with clear seasonality in air temperature and precipitation (Fig. 2). The Diapalis period (2001–2003) coincided with the 2002–2003 El Niño build-up, with a first period (2001) marked by a neutral Multivariate El Niño Index (MEI) and the second (2003) by a stronger positive MEI, indicative of a strong Central Pacific El Niño during D7–D9 (Fig. 2). On New Caledonia's east coast, precipitation was more abundant during summer 2001–2002, with a peak of cumulative rain in January–March 2002, while summer 2003 was drier, except for the passage of tropical storm Beni, which crossed the area just before the February 2003 cruise. Beni reached peak intensity on 29 January, with winds of 205 km h$^{-1}$, before rapidly weakening, and dissipating on 5 February. It brought heavy precipitation and floods lasting 6 d.

During the austral summer, seawater temperature increased to 27°C at Stns 1 and 2 and up to 28°C in the lagoon at Stn 3 (Fig. 3), leading to a vertical stratification and the formation of a pronounced thermocline. During the winter, cooling of surface waters induced vertical mixing in the water column at least down to the deepest sampling level, i.e. 90–100 m in August 2002 at Stn 2. Minimum temperatures in the upper layer were 23.4°C at Stns 1 and 2 and 22.8°C at Stn 3. The highest salinities (around 35.4) were measured in October 2003 at all 3 study sites, and the lowest during the wet summer season, in April 2002 (from 34.47 at Stn 3, close to the mouth of the Ouinne River to 34.86 at Stn 1). Mean salinities were similar at Stns 1 and 2, but lower in summer 2002 than in summer 2003, due to different precipitation regimes linked to the El Niño episode (Fig. 2). Mean phosphate concentrations in the upper layer (Fig. 3) were weak throughout the sampling period, reaching maximum values of 0.06–0.12 µmol l$^{-1}$ in October 2001 and October 2003 during the transition periods and the minimum of 0.02 µmol l$^{-1}$ in summer. A small increase was observed in winter in August 2002 due to a deep MLD (Fig. 4). In wide Santal Bay (Stn 1), $NO_x$ concentrations were also very low, except in August 2002 when they reached 0.08 µmol l$^{-1}$, which was linked to the deep winter mixing. The upper layer waters at Stn 2, the oceanic station, were $NO_x$-poor with concentrations always <0.06 µmol l$^{-1}$ (Fig. 3), even during deep vertical winter mixing as in August 2002. Small maxima (0.05 µmol l$^{-1}$) were observed in October and February 2003 despite warmer, saltier waters. At Stn 3, the lagoon station, the $NO_x$ concentrations varied from 0.01 to a maximum of 0.07 µmol l$^{-1}$ in January 2002. $NH_4^+$ concentrations (data not shown) at Stns 1 and 2 were generally low (<0.04 µmol l$^{-1}$) and often close to the detection limit of 0.01 µmol l$^{-1}$. Higher concentrations were observed

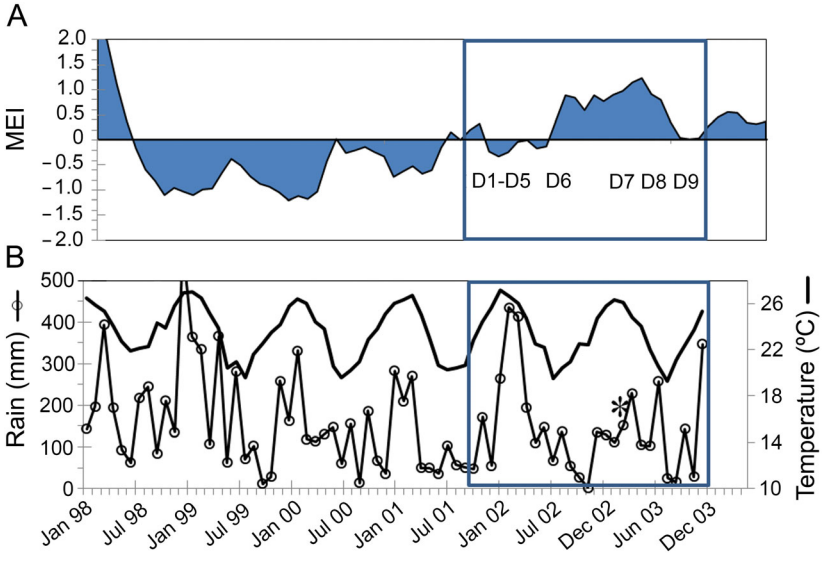


Fig. 2. (A) Multivariate El Niño Index (MEI), (B) air temperature (°C), and monthly cumulative precipitation (mm) for 1998–2003 on New Caledonia's east coast (Thio station). The framed area represents the Diapalis sampling period. Tropical Storm Beni (29 January to 5 February 2003) is indicated as (*). See Table 1 for the dates of Diapalis cruises D1–D9

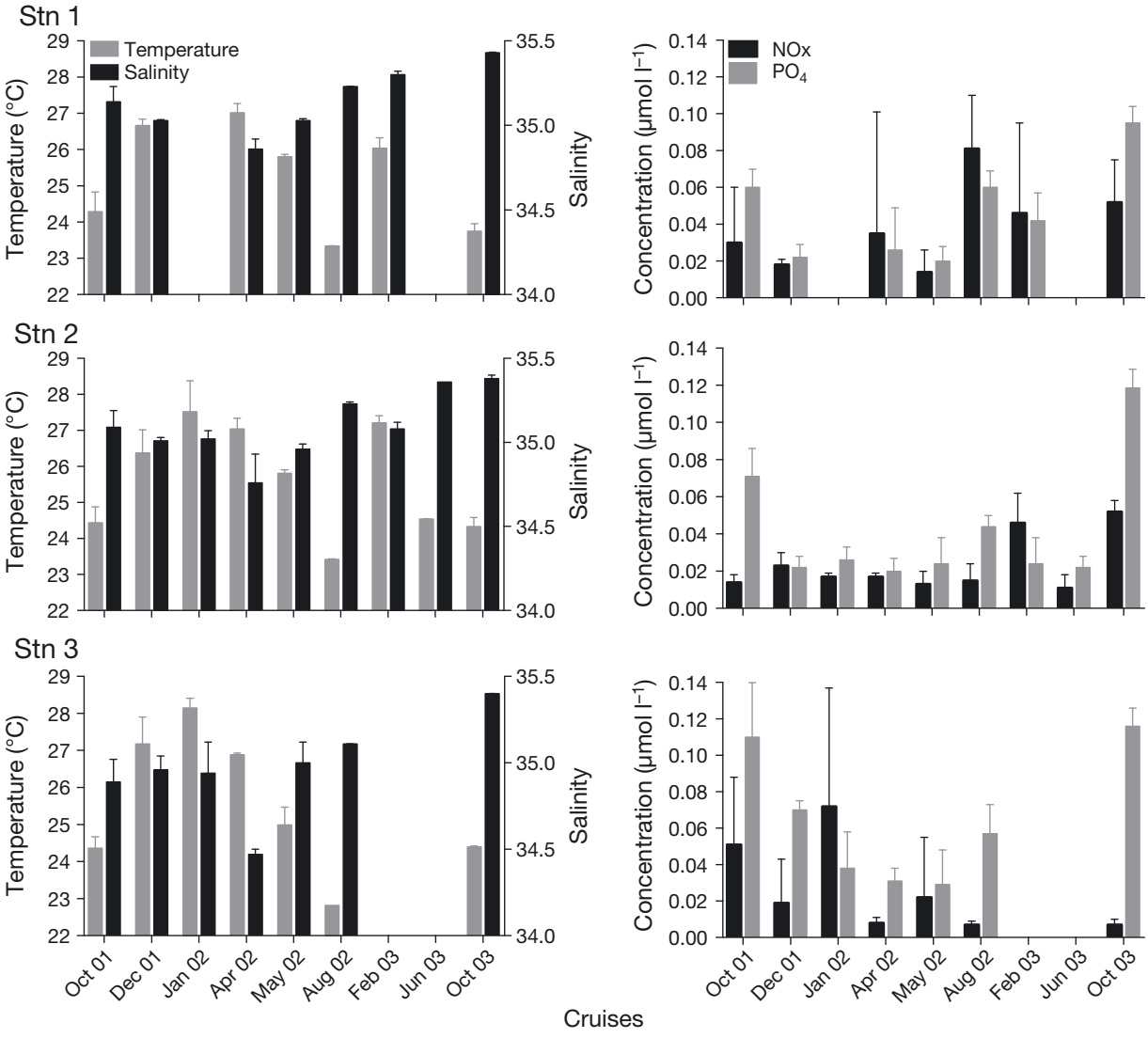


Fig. 3. Mean temperature, salinity (left column), and nutrients ($NO_x$, $PO_4^{3-}$) (right column) during the Diapalis cruises. Values are averaged from 0–60 m at Stns 1 and 2, and 0–30 m at Stn 3. Stn 3 was not sampled in February or June of 2003. Error bars represent standard deviation

only in February 2003 at both stations (0.11 and 0.19 µmol $l^{-1}$ at Stns 1 and 2, respectively). In the lagoon, concentrations were on average slightly higher, up to 0.30 µmol $l^{-1}$.

## Filamentous cyanobacteria

### Specific composition

Five species of *Trichodesmium* were observed (Figs. 5 & 6) and identified by their morphology and morphometric characteristics (Table 2). Three of them, *T. erythraeum*, *T. thiebautii*, and *T. tenue* (Fig. 5A,B,H), were the most frequently observed at all 3 sampling sites. *T. contortum* was seldom observed (3 samples). The genera *Katagnymene* (Fig. 5E–G) and *Richelia*/*Calothrix* (Fig. 6) were common (in 50 and 75% of samples, respectively), but generally in low abundance. *Richelia* was observed as an endosymbiont of *Rhizosolenia* and *Hemiaulus*, and *Calothrix* was found as an epibiont on *Chaetoceros* sp. During the May 2002 cruise, west of Lifou, we observed unidentified filaments very similar to *Trichodesmium,* but differing in their very small cellular diameter (2.7–3.0 µm). We also observed colonies of unidentified cyanobacteria (Fig. 5C,D), which formed clusters sometimes made up of several dozen cells. All filamentous species during Diapalis had dimensions (cell length and diameter) within the

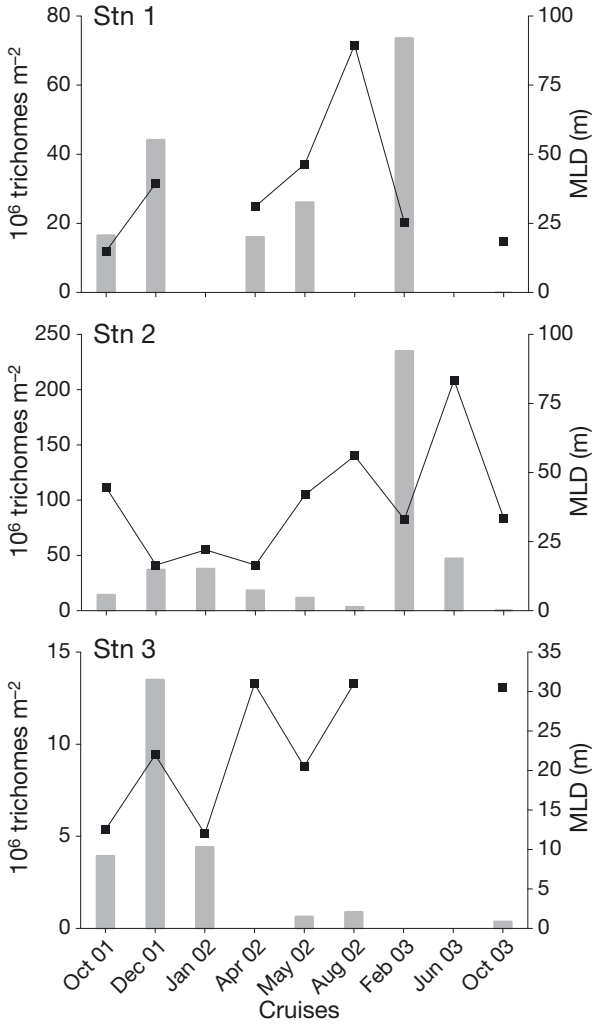


Fig. 4. Integrated concentrations of the total filamentous cyanobacteria (trichomes $m^{-2}$) in the >10 µm fraction (*Richelia intracellularis* and *Calothrix rhizosoleniae* not included) (bars) and the mixed layer depth (MLD) (lines) during the Diapalis cruises. Integration depths: 0–60 m at Stns 1 and 2, and 0–30 m at Stn 3

range published in the literature (Fig. 7 and see Table 7), and the sizes of *T. thiebautii* and *T. erythraeum* were at the low end of the size range.

### *Trichodesmium* and *Katagnymene* abundance

At Stns 1 and 2, the highest abundances of filamentous cyanobacteria in the 0–60 m upper layer were noted in summer (February 2003; Fig. 8), i.e. 3000–4500 trichomes $l^{-1}$ and 1000–3000 trichomes $l^{-1}$ between 5 and 10 m, respectively. However, the concentrations and vertical distribution could be highly variable from one day to another, as shown at Stn 1 in February 2003 (Fig. 8). Some vertical profiles showed maximum concentrations at 40 m, as at Stn 2 (mostly due to *T. erythraeum*). Vertical distributions were quite homogeneous during the period of low abundance, i.e. in the austral winter (August 2002 and June 2003). At Stn 3 (Fig. 8), concentrations did not exceed 1000 trichomes $l^{-1}$. Tables 3–5 give general statistics on the abundances of the different filamentous cyanobacteria enumerated by inverted microscopy during Diapalis cruises 1–9 for each station. Filamentous cyanobacteria were extremely rare in October 2003 regardless of depth.

Assuming that *Trichodesmium* can migrate vertically within 1 d (Villareal & Carpenter 2003), using integrated abundance seemed more appropriate for gauging their seasonal variations in the water column (Fig. 4). Thus, at Stn 2, 0–60 m integrated abundance showed a 500-fold variation between the minimum in October 2003 ($<0.5 \times 10^6$ trichomes $m^{-2}$) during the transition periods and the maximum in summer (February 2003: $235 \times 10^6$ trichomes $m^{-2}$). At Stn 1, the seasonal variations appeared similar, but the variation range (×150) and the maximum summer

Table 2. Morphometric characteristics of filamentous cyanobacteria, in the >10 µm size fraction, encountered during the Diapalis cruises. NDO: not distinctly observed

| Species | Cell diameter (µm) | Cell length (µm) | Cell shape |
|---|---|---|---|
| *Trichodesmium erythraeum* | 8–13.6 | 4–7 | As wide as or up to twice as wide as long |
| *T. thiebautii* | 6–14 | 7–19 | As long as or up to twice as long as wide |
| *T. tenue* | 4–5 | 12–20 | 3–4× longer than wide |
| *T. contortum* | 26–36 | 5–7 | 5–6× wider than long |
| *T. hildebrandtii* | 18–20 | 5–6 | 2.7–3× wider than long |
| Unidentified filaments | 2.7–3 | NDO | NDO |
| *Katagnymene spiralis* | 14–28 | 3–8 | 2.2–4.8× wider than long |
| *K. pelagica* | 17–28 | 2.5–3.5 | 4–14× wider than long |
| *Richelia intracellularis*/ *Calothrix rhizosoleniae* group | Vegetative cell 2–7<br>Heterocyst 3–10 | 2–7 | Diametric cells or slightly shorter than wide<br>Spherical |
| Colonies of unidentified cyanobacteria | Type 1: 2.7–3<br>Type 2: 6 | | Spherical |

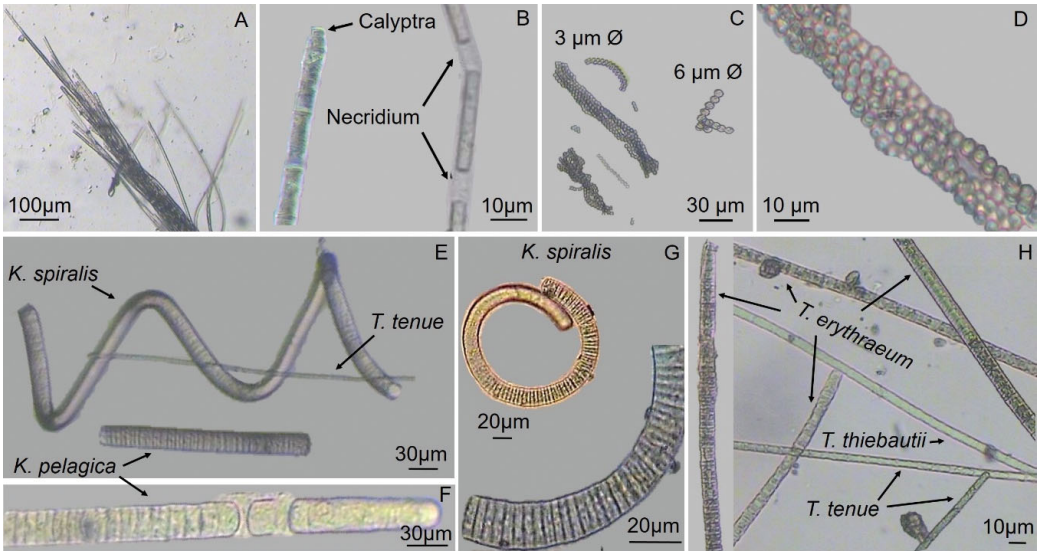


Fig. 5. Photomicrographs of filamentous cyanobacteria obtained from the Diapalis cruises. (A) *Trichodesmium tenue*. (B) Details of *T. tenue* trichomes: the calyptra (formation at the tip of the trichome) and the necridium (structure formed from a dead cell which constitutes a fracture point of the filament) note that cells are about 3 times longer than wide. (C) Colonies of unidentified cyanobacteria composed of round cells. Type 1, 3 µm ∅ and Type 2, 6 µm ∅. (D) details of Type 1. (E–G) *Katagnymene spiralis* and *K. pelagica*. Note the size difference between *Katagnymene* spp. and *T. tenue*. (H) Mix of major species (*T. erythraeum*, *T, tenue*, *T. thiebautii*)

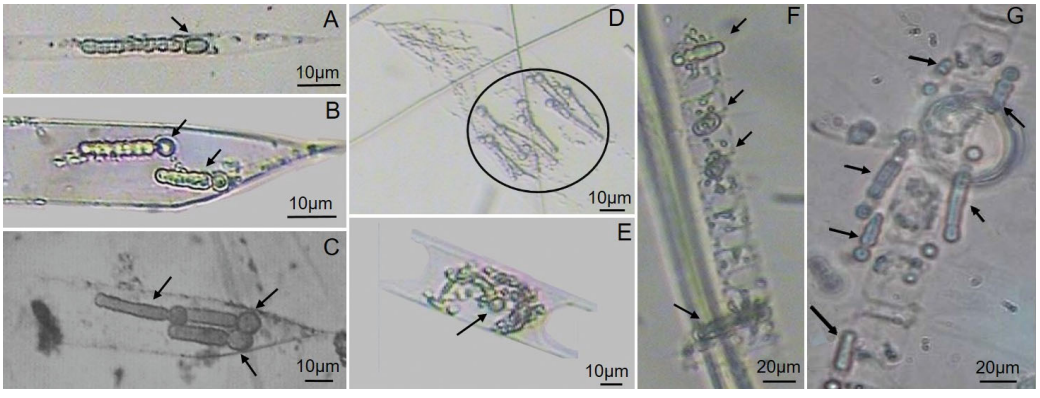


Fig. 6. Diatom–diazotrophic cyanobacteria associations. (A–D) *Richelia intracellularis* symbiotic with *Rhizosolenia* spp. and (E) *Hemiaulus* sp.; (F,G) *Calothrix rhizosoleniae* epiphytes on *Chaetoceros* sp. Size and number of *Richelia* vary based on the size of the host. Photomicrographs obtained from the Diapalis cruises. Arrows indicate the position of the cyanobacteria *Richelia* and *Calothrix*

concentrations (73 × $10^6$ trichomes $m^{-2}$) were lower than at Stn 2. At both stations, maximum abundance in summer 2003 was coincident with a shallow MLD, but low abundances were also observed in summer 2002 with a similar thermohaline structure. At Stn 3 (Fig. 4), integrated concentrations (0–30 m only) were 15 times lower and varied from 0.9 × $10^6$ to 13.5 × $10^6$ trichomes $m^{-2}$, although Stn 3 was not sampled in February 2003.

The 3 main *Trichodesmium* species together accounted for 85–100% of the integrated biomass of filamentous cyanobacteria in terms of trichome numbers and always more than 51% in terms of trichome volume (Fig. 9). *T. tenue* and *T. thiebautii* were generally the dominant species followed by *T. erythraeum*. Their mean cell numbers per trichome were relatively similar (Table 6). As expected, the percentage contribution of slender *T. tenue* was higher in terms of total trichome number than total volume (40–49 vs. 33%). Its percentage contribution (trichome numbers) was minimum at Stn 2 in February 2003 (23%) and maximum (>70%) in December 2001 at Stns 2 and 3. *T. thiebautii* reached >40% of the total trichome number in 65% of the samples with minimal contributions in

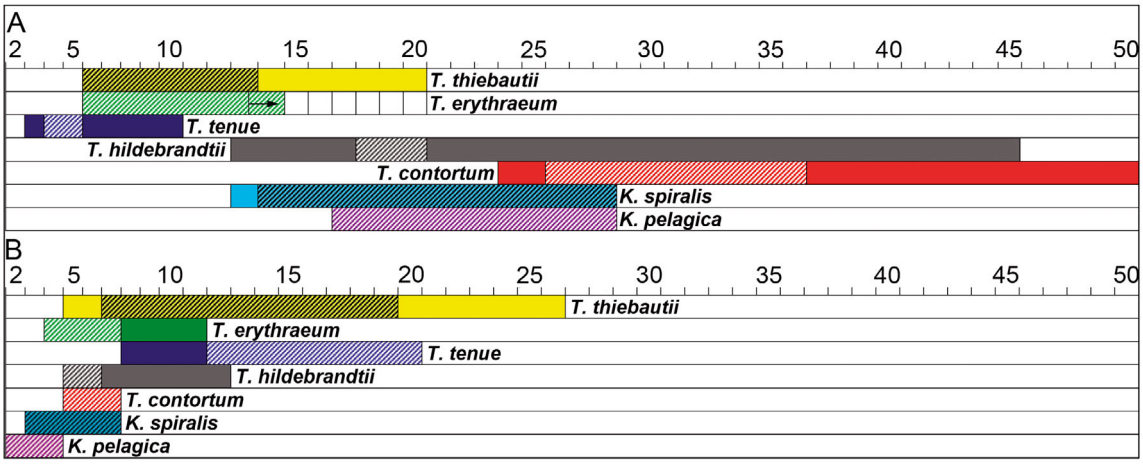


Fig. 7. (A) Cell diameter (µm) and (B) cell length (µm) of *Trichodesmium* and *Katagnymene* species. The colored areas represent size ranges published in the literature and shaded areas part of the size range observed during the Diapalis cruises in the New Caledonian Lagoon. A black arrow indicates observation of a few *T. erythraeum* trichomes with a slightly wider diameter than previously published

December 2001 and May 2002 at Stn 2 (<14 %) and a fairly stable one at Stns 1 and 3 (37–47 %).

In terms of volume, *T. thiebautii* contributions (up to 50 % at Stn 2) were more variable, and were linked to trichome length changes along the annual cycle. The third main species, *T. erythraeum*, represented <18 % of total trichomes on average, with the minimum contribution at Stn 3 (<7 %) and maximum at Stns 1 and 2 in February 2003 during the peak abundance (Tables 3–5). Its relative importance increased as expressed in volume, especially in February 2003 at the 2 open ocean stations (22–28 %). *T. contortum* and *T. hildebrandtii* (given as 'others' in Fig. 9), contributed <3 % to total trichome number or volume. The unidentified trichomes observed in May 2002 further north than Stn 2 represented <1 % of the integrated biomass.

The 2 *Katagnymene* species (*K. spiralis* and *K. pelagica*) were not numerous, but their contribution in terms of volume was significant. Each form accounted for <4 % of the total number of trichomes, except *K. spiralis* at Stn 1 in October 2001 (10 %). *K.*

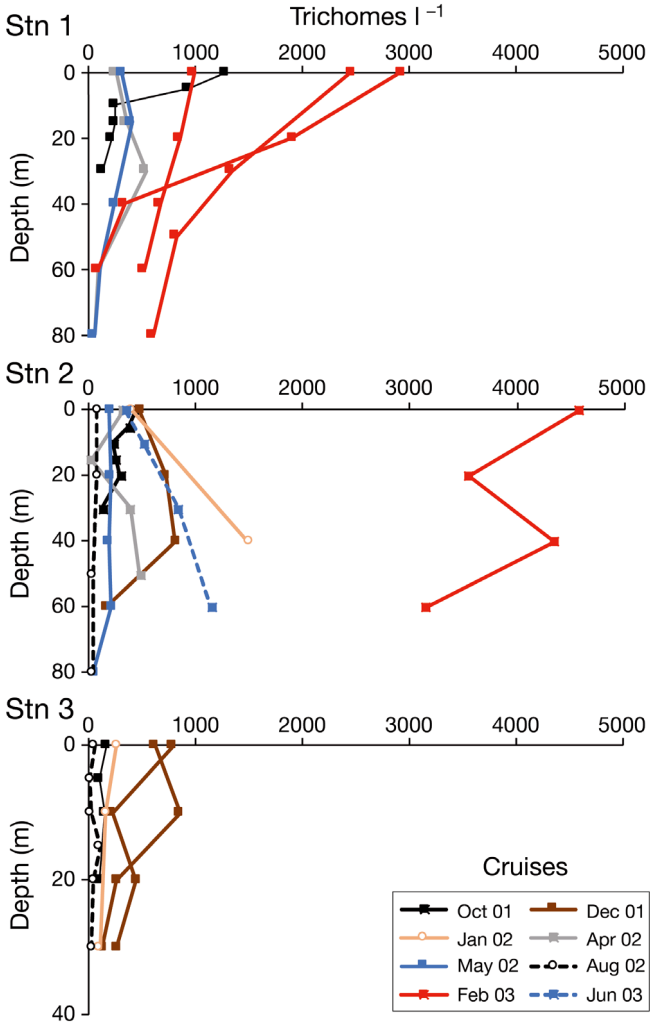


Fig. 8. Vertical profiles for filamentous cyanobacteria abundance (trichomes $l^{-1}$) (except *Richelia intracellularis* and *Calothrix rhizosoleniae*) during the Diapalis cruise stations. Stn 2 (open ocean, Loyalty Channel) is representative of the seasonal variations. Note that 2–3 profiles were sometimes established with intervals of 24 h (at Stn 1 in February 2003) and 48 h (at Stn 3 in December 2001). In May 2002 (at Stn 3) and October 2003 (all stations), the abundances never exceeded 27 trichomes $l^{-1}$ (data not shown). During some cruises Stns 1 and 3 were not sampled

*spiralis* was observed in 50% of samples. Its highest contributions to the total volume of trichomes occurred at Stn 1 in October 2001 (28%) and February 2003 at Stn 2 (12%). The contribution of *K. pelagica* was around 20% of the total volume in February 2003 at Stns 1 and 2, 15% at Stn 2 in October 2001, and was not observed at Stn 3. The relative abundance of species could change along depth. For example, during cruises D4 and D5 (April–May 2002), *T. thiebautii* and *T. tenue* were numerous in the upper layer (60%) and disappeared at 50 m, while *T. erythraeum* peaked at this depth. Obviously, the relative abundance depends on the biomass criteria considered. For example, at Stn 2 in April 2002, *T. erythraeum* contributions at 30 to 50 m decreased in terms of trichome number (6%) but increased (41%) in terms of total volume, because trichome size increased. The opposite trend was observed for *T. thiebautii*.

Table 3. Statistical data for the different groups enumerated by inverted microscopy in the >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for identified filamentous cyanobacteria and cell number for all other groups and the colonies of unidentified cyanobacteria with spherical cells), at Stn 1, the Santal Bay station, during the 9 Diapalis cruises (2001–2003; see Table 1 for cruise dates). Concentrations taken at 4–6 depths down to 60 m ($l^{-1}$) and integrated values ($m^{-2}$) in the 0–60 m layer. Trichomes: sum of *Trichodesmium* and *Katagnymene* trichomes

| Stn 1 | Min. | Max. | Mean | SD | n |
|---|---|---|---|---|---|
| Trichomes $l^{-1}$ | 1 | 2929 | 651 | 648 | 50 |
| $10^6$ trichomes $m^{-2}$ | 0.06 | 89 | 38 | 30 | 12 |
| $10^6$ µm$^3$ trichomes $l^{-1}$ | 0.59 | 49.22 | 11.46 | 12.70 | 25 |
| $10^9$ µm$^3$ trichomes $m^{-2}$ | 0.16 | 1.15 | 0.52 | 0.37 | 7 |
| *Richelia intracellularis/Calothrix rhizosoleniae* group $l^{-1}$ | 0 | 64 | 8 | 16 | 25 |
| $10^3$ µm$^3$ *R. intracellularis/C. rhizosoleniae* group $l^{-1}$ | 0.00 | 12.76 | 1.13 | 2.74 | 24 |
| Unidentified cyanobacteria with spherical cells (cells $l^{-1}$) | 0 | 20 | 1 | 5 | 25 |
| Dinoflagellates $l^{-1}$ | 22 | 1927 | 322 | 405 | 31 |
| Diatoms $l^{-1}$ | 14 | 4516 | 640 | 1096 | 31 |
| *Trichodesmium tenue* $l^{-1}$ | 20 | 947 | 285 | 255 | 28 |
| *T. erythraeum* $l^{-1}$ | 3 | 621 | 145 | 159 | 28 |
| *T. thiebautii* $l^{-1}$ | 18 | 1351 | 335 | 353 | 28 |
| *Katagnymene spiralis* $l^{-1}$ | 0 | 199 | 29 | 49 | 28 |
| *K. pelagica* $l^{-1}$ | 0 | 61 | 6 | 14 | 28 |
| $10^6$ µm$^3$ *T. tenue* $l^{-1}$ | 0.09 | 7.82 | 2.20 | 2.25 | 27 |
| $10^6$ µm$^3$ *T. erythraeum* $l^{-1}$ | 0.02 | 15.41 | 2.46 | 3.66 | 27 |
| $10^6$ µm$^3$ *T. thiebautii* $l^{-1}$ | 0.18 | 13.90 | 3.67 | 3.64 | 27 |
| $10^6$ µm$^3$ *K. spiralis* $l^{-1}$ | 0.00 | 6.90 | 1.33 | 1.98 | 27 |
| $10^6$ µm$^3$ *K. pelagica* $l^{-1}$ | 0.00 | 31.40 | 1.54 | 6.04 | 27 |
| $10^4$ *Prochlorococcus* $ml^{-1}$ | 3.73 | 46.21 | 13.37 | 8.13 | 76 |
| $10^4$ *Synechococcus* $ml^{-1}$ | 0.22 | 3.58 | 1.50 | 0.86 | 76 |
| $10^4$ Picoeukaryotes $ml^{-1}$ | 0.06 | 0.47 | 0.18 | 0.08 | 76 |
| $10^{12}$ *Prochlorococcus* $m^{-2}$ | 3.41 | 14.80 | 8.76 | 3.96 | 11 |
| $10^{12}$ *Synechococcus* $m^{-2}$ | 0.19 | 1.75 | 0.94 | 0.49 | 11 |
| $10^{12}$ Picoeukaryotes $m^{-2}$ | 0.06 | 0.17 | 0.11 | 0.04 | 11 |

### *Richelia intracellularis/Calothrix rhizosolenia* (*RC*) from DDAs and unidentified cyanobacteria abundance

*RC* (Tables 3–5) were observed in 75% of samples, but their abundance was never very high. The highest *RC* abundances were recorded in February 2003 at both Stns 1 and 2 (64 and up to 735 trichomes $l^{-1}$, respectively), accounting for <1% of total *Trichodesmium* trichome surface area and volume. Conversely, at Stn 3, peak abundance was observed for *R. intracellularis* in October 2001 (up to 117 trichomes $l^{-1}$ at the surface), equivalent to 4.3% of *Trichodesmium* trichome volume. In our samples, *RC* in DDAs experienced a considerable variation in filament length (10–88 µm) and cell diameter (vegetative cells 2–7 µm; heterocyst 3–10 µm) or trichomes per host cell (1–22). We noted that in most cases, the host cell was almost empty. Apart from *Richelia*, other symbiotic coccoid-form cyanobacteria (not necessarily diazotrophs), namely *Climacodium* sp. and *Ornithocercus* sp., were also observed, albeit only in the New Caledonian lagoon.

Colonies of unidentified cyanobacteria composed of round cells observed in some samples reached <1% of *Trichodesmium* biomass on average (Tables 3–5, Fig. 5C,D). The highest concentrations were observed at Stn 2 during January and May 2002 with values exceeding 4000 cells $l^{-1}$, which was equivalent to <1.6% of *Trichodesmium* trichome volume. At Stn 1, these organisms were rarely observed and only at very low concentrations, i.e. <20 cells $l^{-1}$. At Stn 3, they were observed only in January 2002, with a maximum value at 10 m of 429 cells $l^{-1}$. Tracking *Crocosphaera* (2–10 µm cell size) by flow cytometric and PE signatures was unsuccessful during Diapalis, although it has been reported in the region (Sato et al. 2010 and references therein). However, Diapalis samples showed relatively high $N_2$ fixation in the <10 µm fraction, particularly in October 2003 (Garcia et al. 2007), but it was not possible to link this fixation to specific organisms or biomass indicators.

Table 4. As in Table 3, but for Stn 2, the Loyalty Channel station

| Stn 2 | Min. | Max. | Mean | SD | n |
|---|---|---|---|---|---|
| Trichomes $l^{-1}$ | 4 | 4578 | 676 | 1089 | 45 |
| $10^6$ trichomes $m^{-2}$ | 0.46 | 235 | 44 | 66 | 11 |
| $10^6$ µm$^3$ trichomes $l^{-1}$ | 0.03 | 62.22 | 9.53 | 15.29 | 36 |
| $10^9$ µm$^3$ trichomes $m^{-2}$ | 0.16 | 3.05 | 0.63 | 0.92 | 9 |
| *R. intracellularis*/ *C. rhizosoleniae* group $l^{-1}$ | 0 | 735 | 36 | 121 | 41 |
| $10^3$ µm$^3$ *R. intracellularis*/ *C. rhizosoleniae* group $l^{-1}$ | 0.00 | 117.33 | 7.22 | 21.64 | 30 |
| Unidentified cyanobacteria with spherical cells (cells $l^{-1}$) | 0 | 4934 | 224 | 833 | 40 |
| Dinoflagellates $l^{-1}$ | 8 | 1205 | 289 | 306 | 40 |
| Diatoms $l^{-1}$ | 5 | 9969 | 834 | 2190 | 40 |
| *T. tenue* $l^{-1}$ | 13 | 1053 | 209 | 250 | 36 |
| *T. erythraeum* $l^{-1}$ | 2 | 417 | 50 | 78 | 36 |
| *T. thiebautii* $l^{-1}$ | 3 | 635 | 136 | 150 | 36 |
| *K. spiralis* $l^{-1}$ | 0 | 31 | 3 | 6 | 36 |
| *K. pelagica* $l^{-1}$ | 0 | 11 | 2 | 3 | 36 |
| $10^6$ µm$^3$ *T. tenue* $l^{-1}$ | 0.04 | 17.28 | 2.88 | 3.49 | 32 |
| $10^6$ µm$^3$ *T. erythraeum* $l^{-1}$ | 0.01 | 17.97 | 1.99 | 3.88 | 32 |
| $10^6$ µm$^3$ *T. thiebautii* $l^{-1}$ | 0.13 | 21.03 | 2.92 | 4.66 | 32 |
| $10^6$ µm$^3$ *K. spiralis* $l^{-1}$ | 0.00 | 15.89 | 0.82 | 2.90 | 32 |
| $10^6$ µm$^3$ *K. pelagica* $l^{-1}$ | 0.00 | 2.07 | 0.18 | 0.46 | 32 |
| $10^4$ *Prochlorococcus* $ml^{-1}$ | 2.59 | 29.02 | 13.05 | 5.95 | 111 |
| $10^4$ *Synechococcus* $ml^{-1}$ | 0.05 | 4.58 | 0.99 | 1.24 | 111 |
| $10^4$ Picoeukaryotes $ml^{-1}$ | 0.03 | 0.67 | 0.11 | 0.08 | 111 |
| $10^{12}$ *Prochlorococcus* $m^{-2}$ | 3.53 | 14.87 | 8.76 | 3.59 | 17 |
| $10^{12}$ *Synechococcus* $m^{-2}$ | 0.08 | 2.55 | 0.62 | 0.79 | 17 |
| $10^{12}$ Picoeukaryotes $m^{-2}$ | 0.02 | 0.15 | 0.07 | 0.04 | 17 |

age 5 to 14 times higher at the lagoon station (Stn 3) than offshore (Stns 1 and 2), except during the austral winter (August 2002) when they were 24% higher in oceanic waters at Stn 2 ($1.91 \times 10^{12}$ cells $m^{-2}$). This winter maximum was linked to thoroughly mixed cool waters down to >60 m. The maximum *Synechococcus* abundance at the oceanic stations was generally located shallower than that of *Prochlorococcus*, i.e. in the upper mixed layer or at the top of the thermocline, except during the October 2003 cruise when it peaked at the same depth (between 75 and 110 m).

Picoeukaryotes were the least abundant picoplankton organisms (Tables 3–5, Fig. 10C). Integrated abundances were more variable at Stn 2 ($0.02–0.12 \times 10^{12}$ cells $m^{-2}$) than at Stn 1 ($0.07–0.14 \times 10^{12}$ cells $m^{-2}$). At Stn 3, integrated abundances changed from 0.08 to $0.17 \times 10^{12}$ cells $m^{-2}$. Picoeukaryote maxima were consistently found below the thermocline and, therefore, deeper than the *Synechococcus* maxima, even in August 2002.

## Abundance of other phytoplankton groups

### Picoplankton abundance

*Prochlorococcus* concentrations at Stns 1 and 2 (Tables 3 & 4, Fig. 10A) were maximum ($10–13 \times 10^{12}$ cells $m^{-2}$) during the warm season (December 2001 to April 2002, and February 2003). During transition periods, concentrations were lower by a factor of 3 with $3.5–3.9 \times 10^{12}$ cells $m^{-2}$ at Stn 2 (October 2001 and October 2003, respectively), while at Stn 1 the minimum concentration ($4.5 \times 10^{12}$ cells $m^{-2}$) was observed during winter. At these 2 oceanic stations, the vertical distribution of *Prochlorococcus* was quite similar, with a marked maximum below the thermocline (40–80 m) in summer and a more homogeneous distribution down to 60–80 m in winter. At Stn 3, *Prochlorococcus* abundance was much lower than at the oceanic stations, with values $<2.3 \times 10^{12}$ cells $m^{-2}$ through 0–30 m (max. $70 \times 10^3$ cells $ml^{-1}$), except in December 2002 when they reached $4.8 \times 10^{12}$ cells $m^{-2}$ (max. $225 \times 10^6$ cells $ml^{-1}$ at depth).

Unlike *Prochlorococcus*, integrated abundance of *Synechococcus* (Tables 3–5, Fig. 10B), was on aver-

### Large eukaryotes

Large eukaryotes (>10 µm) described by microscopy and made up of dinoflagellates and diatoms (Fig. 11) were generally found together with *Trichodesmium* in open-ocean waters at Stns 1 and 2 and, to a lesser extent, at Stn 3 (coastal site). Dinoflagellates showed a maximum of $60 \times 10^6$ cells $m^{-2}$ during the maximum *Trichodesmium* abundance in February 2003 at Stns 1 and 2 and low abundance ($<30 \times 10^6$ cells $m^{-2}$) at other periods. Diatoms also showed a maximum concentration ($412 \times 10^6$ cells $m^{-2}$) in February 2003 at Stn 2 and, to a lesser extent, at Stn 1. At Stn 3, the coastal site, no data were available in February 2003, but there was a considerable maximum for both groups ($17 \times 10^6$ cells $m^{-2}$ for dinoflagellates; $240 \times 10^6$ cells $m^{-2}$ for diatoms) in winter (August 2002). The main diatoms belonged to the genera (1) *Navicula, Nitzschia*, and *Pseudo-nitzschia* at Stn 1, (2) *Chaetoceros*, *Navicula*, and *Pseudo-Nitszchia* at Stn 2, and (3) *Chaetoceros*, *Navicula*, and *Rhizosolenia* at Stn 3. *Rhizosolenia* was the main *RC* host.

Table 5. As in Table 3, but for Stn 3, the Ouinne station, Eastern Lagoon, which was only sampled down to 30 m

| Stn 3 | Min. | Max. | Mean | SD | n |
|---|---|---|---|---|---|
| Trichomes $l^{-1}$ | 10 | 854 | 172 | 219 | 32 |
| $10^3$ trichomes $m^{-2}$ | 0.38 | 15.00 | 4.74 | 5.30 | 9 |
| $10^6$ µm$^3$ trichomes $l^{-1}$ | 0.07 | 10.19 | 2.67 | 2.81 | 20 |
| $10^9$ µm$^3$ trichomes $m^{-2}$ | 0.01 | 0.18 | 0.09 | 0.07 | 5 |
| *R. intracellularis*/ *C. rhizosoleniae* group $l^{-1}$ | 0 | 117 | 15 | 25 | 25 |
| $10^3$ µm$^3$ *R. intracellularis*/ *C. rhizosoleniae* group $l^{-1}$ | 0 | 64.90 | 7.25 | 14.36 | 23 |
| Unidentified cyanobacteria with spherical cells (cells $l^{-1}$) | 0 | 429 | 22 | 88 | 24 |
| Dinoflagellates $l^{-1}$ | 82 | 1000 | 344 | 251 | 22 |
| Diatoms $l^{-1}$ | 26 | 16893 | 2458 | 4345 | 22 |
| *T. tenue* $l^{-1}$ | 5 | 868 | 136 | 228 | 29 |
| *T. erythraeum* $l^{-1}$ | 0 | 132 | 16 | 28 | 29 |
| *T. thiebautii* $l^{-1}$ | 2 | 147 | 43 | 40 | 29 |
| *K. spiralis* $l^{-1}$ | 0 | 9 | 0 | 2 | 29 |
| *K. pelagica* $l^{-1}$ | 0 | 8 | 0 | 2 | 29 |
| $10^6$ µm$^3$ *T. tenue* $l^{-1}$ | 0.08 | 6.09 | 1.61 | 2.10 | 18 |
| $10^6$ µm$^3$ *T. erythraeum* $l^{-1}$ | 0.00 | 4.79 | 0.66 | 1.14 | 18 |
| $10^6$ µm$^3$ *T. thiebautii* $l^{-1}$ | 0.02 | 2.41 | 0.89 | 0.68 | 18 |
| $10^6$ µm$^3$ *K. spiralis* $l^{-1}$ | 0.00 | 0.50 | 0.03 | 0.12 | 18 |
| $10^6$ µm$^3$ *K. pelagica* $l^{-1}$ | 0.00 | 1.42 | 0.10 | 0.35 | 18 |
| $10^4$ *Prochlorococcus* $ml^{-1}$ | 1.01 | 24.17 | 7.28 | 4.40 | 66 |
| $10^4$ *Synechococcus* $ml^{-1}$ | 1.14 | 13.82 | 6.60 | 2.91 | 66 |
| $10^4$ Picoeukaryotes $ml^{-1}$ | 0.13 | 1.74 | 0.34 | 0.26 | 66 |
| $10^{12}$ *Prochlorococcus* $m^{-2}$ | 0.48 | 4.75 | 2.13 | 1.05 | 11 |
| $10^{12}$ *Synechococcus* $m^{-2}$ | 0.82 | 3.27 | 1.92 | 0.79 | 11 |
| $10^{12}$ Picoeukaryotes $m^{-2}$ | 0.06 | 0.17 | 0.10 | 0.04 | 11 |

Among dinoflagellates, protoperidinians were represented at all stations, but we found few larger cells, such as *Ceratium* and *Amphisiolena* species at Stn 3 in October 2001.

### Chlorophyll and PE distribution

Pigment content (chl *a*, PE) per cell or per trichome (Table 7) was calculated for Diapalis samples during the February 2003 cruise at Stns 1 and 2, and for a pure sample of *T. erythraeum*. Mean chl *a* concentration per trichome was 99 pg cell$^{-1}$ for pure *T. erythraeum*, while the relationship between trichome numbers and chl *a* in the >10 µm fraction suggested an upper limit for the mean chl *a* concentration per trichome of 43 pg for the mixed community of *Trichodesmium* (Table 7). Considering the diatoms and dinoflagellates in the >10 µm fraction revealed by microscopy and by the presence of a significant quantity of chl *c*, the chl *a* per trichome would be approximately 10% lower (assuming a constant chl *c*:chl *a* ratio of 0.2 for diatoms and dinoflagellates) than this upper limit. Similarly, the trichome PE content (Table 7) was 251 pg trichome$^{-1}$ for pure *T. erythraeum* and 197 pg trichome$^{-1}$ for mixed *Trichodesmium* (Table 7).

Regarding PE, its fluorescence excitation spectra were typical of PE-cyanobacteria according to Neveux et al. (2006), with either PE-*Synechococcus* or PE-*Trichodesmium* dominating or a clear mixture of both PEs. At Stns 1 and 2, PE-*Trichodesmium* dominated the community in December 2002 and February 2003. Integrated total chl *a* change showed much less variation across cruises than trichome abundance (Fig. 12). The integrated proportion of Dv-chl *a* to Tchl *a* was generally around 20% in lagoon waters (Stn 3), 40–50% at Stn 1, and over 50% in the Loyalty Channel (Stn 2). The highest integrated values of PE at Stns 1 and 2 in February 2003 (69 mg $m^{-2}$; Fig. 12) was linked to the high abundance of *Trichodesmium* at the surface, while the high value in October 2003 at Stn 1 was related to a deep maximum of *Synechococcus*. In February 2003, PE maxima measured in the upper layer were 4 to >10 times higher than during the other cruises, ranging from 0.61 µg $l^{-1}$ at Stn 1 to 1.27 µg $l^{-1}$ at Stn 2. In the >10 µm fraction, the PE in the upper layer (data not shown) was exclusively related to filamentous cyanobacteria, mainly *Trichodesmium*. At Stn 3 (Fig. 12), PE was essentially related to *Synechococcus*, and concentrations varied between 0.10 (April 2002) and 0.62 µg $l^{-1}$ (August 2002) at the surface with fairly even vertical distribution. The integrated concentrations varied from 4.30 (April 2002) to 29.26 mg $m^{-2}$ (August 2002). The integrated $N_2$ fixa-

Table 6. Cell number per trichome of each *Trichodesmium* and *Katagnymene* species during the Diapalis cruises

| | Min. | Max. | Mean | SD | n |
|---|---|---|---|---|---|
| *T. tenue* | 3 | 206 | 54 | 36 | 584 |
| *T. thiebautii* | 3 | 209 | 55 | 38 | 469 |
| *T. erythraeum* | 4 | 235 | 64 | 45 | 322 |
| *T. hildebrandtii* | 16 | 132 | 62 | 40 | 9 |
| *T. contortum* | 19 | 62 | 40 | 22 | 3 |
| *K. spiralis* | 2 | 140 | 42 | 34 | 49 |
| *K. pelagica* | 10 | 334 | 57 | 77 | 18 |

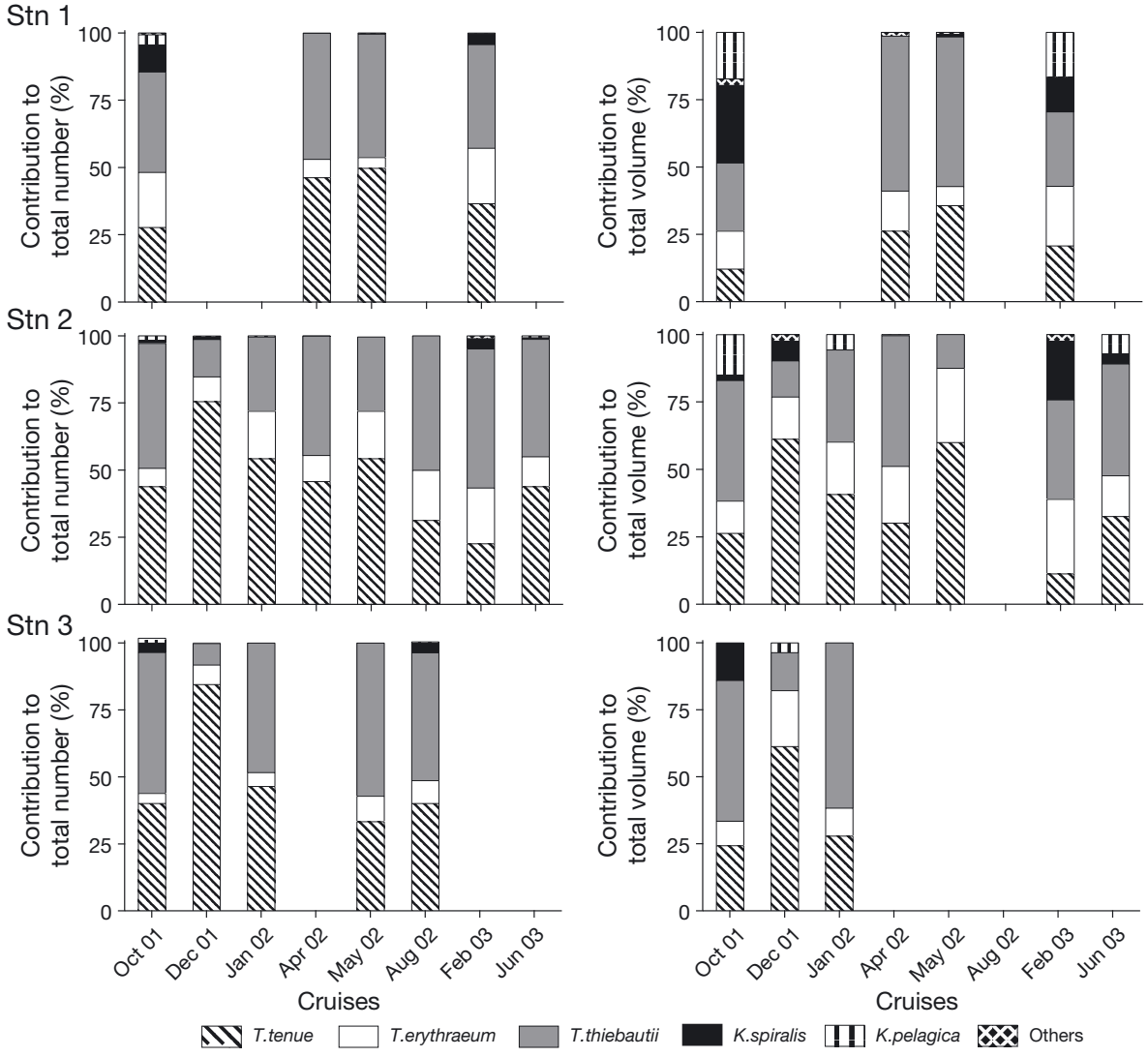


Fig. 9. Contributions of the various species (%), in terms of trichome number (left column) and trichome volume (right column), to the total community of filamentous cyanobacteria (*Richelia intracellularis* and *Calothrix rhizosoleniae* not included) at the 3 stations sampled during the Diapalis cruises. Integration depths: 0–60 m at Stns 1 and 2; 0–30 m at Stn 3. At Stn 1, 'Others' = *Trichodesmium hildebrandtii* (October 2001, May 2002, and February 2003) or *T. contortum* (October 2001). At Stn 2, 'Others' = *T. hildebrandtii.* At Stn 2, species contributions were determined at each cruise, except in August 2002 for the *Trichodesmium* volume contribution. Some cruises are missing at Stns 1 and 3. October 2003 abundance was too low for enumeration

tion rate for all species (Garcia et al. 2007) roughly followed the integrated PE pattern (Fig. 12) and was correlated with the integrated trichome concentrations ($r^2 = 0.73$, N = 11).

### C biomass distribution in the microbial community

The relative contribution of *Trichodesmium* + *Katagnymene* (*TK*) to C biomass of total picoplankton and filamentous cyanobacteria (Fig. 13) was generally high at Stn 2, with maxima in summer (80% in February 2003) and minima in winter (6% in August 2002), where *TK* was replaced by *Synechococcus* as the major contributor and in October 2003 with only a few filaments observed. Comparatively, the contribution of *TK* at Stn 1 was lower, with a maximum of ~50% in February 2003. At Stn 3, *Synechococcus* was the main contributor to C biomass and the contribution of *TK* was generally low. At all stations, the contribution of *RC* to C biomass was negligible over the whole sampling period. Picoeukaryotes may account for a significant portion of the biomass in the lagoon near Ouinne and in winter at all stations.

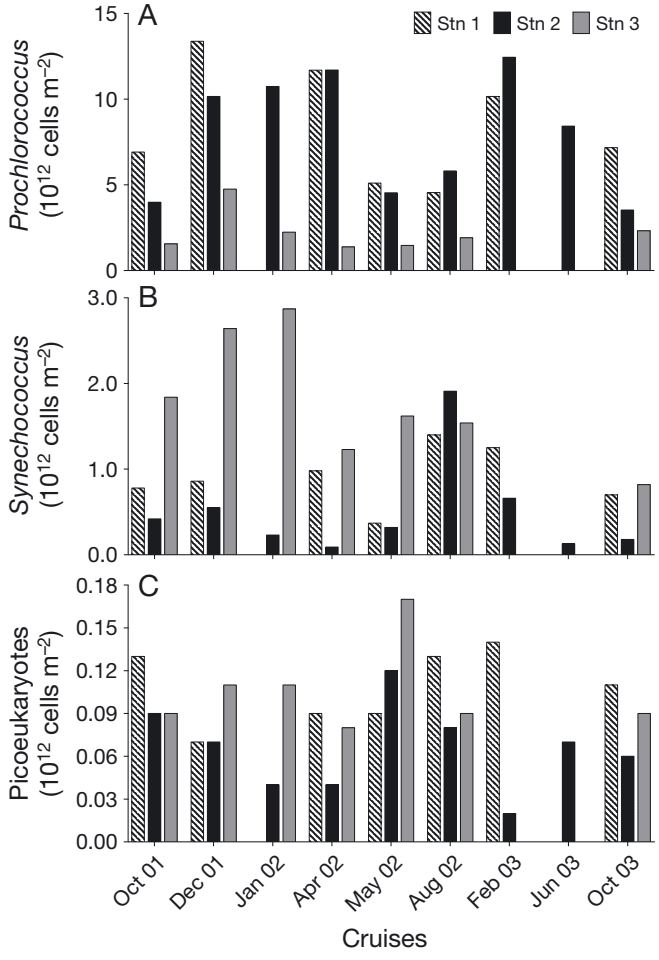


Fig. 10. Integrated concentrations of (A) *Prochlorococcus*, (B) *Synechococcus*, and (C) picoeukaryotes at the 3 stations sampled during the Diapalis cruises. Integration depths: 0–60 m at Stns 1 and 2; 0–30 m at Stn 3

### Correlations between groups and environmental variables

To determine the impact of environmental variables on the abundance variations of the different phytoplanktonic groups and species, Spearman correlations were calculated between integrated abundances of each filamentous species, integrated nutrients, and depth-averaged temperature and salinity, at each station/cruise (Table 8). Significant positive correlations were found between total trichome abundance, temperature, and ammonium and a negative correlation with phosphate, but no significant correlation with MLD (see also Fig. 4). At the species level, *RC* showed a strong positive correlation with MLD and ammonium, but *K. pelagica* only with phosphate, while *K. spiralis* was strongly correlated with all analyzed nutrients ($NO_x$, phosphate, and ammonium). Diatom and picoplanktonic species did not show any correlation with environmental parameters. Inter-species correlations revealed that *RC* was positively correlated with *T. thiebautii* and *K. spiralis,* but not with *T. tenue* (inversely correlated with *T. thiebautii*). Dinoflagellates were closely correlated with total trichome abundance and *T. erythraeum*, but inversely with *K. pelagica*. No correlations were evidenced between diatoms and the other groups. Finally, *Synechococcus* and picoeukaryotes showed a positive correlation.

## DISCUSSION

### Influence of methodology on enumeration and biomass estimates

As part of the effort of collecting biomass and metabolic rates specific to different phytoplankton functional groups, a database on diazotrophic organisms in the global pelagic upper ocean was built by Luo et

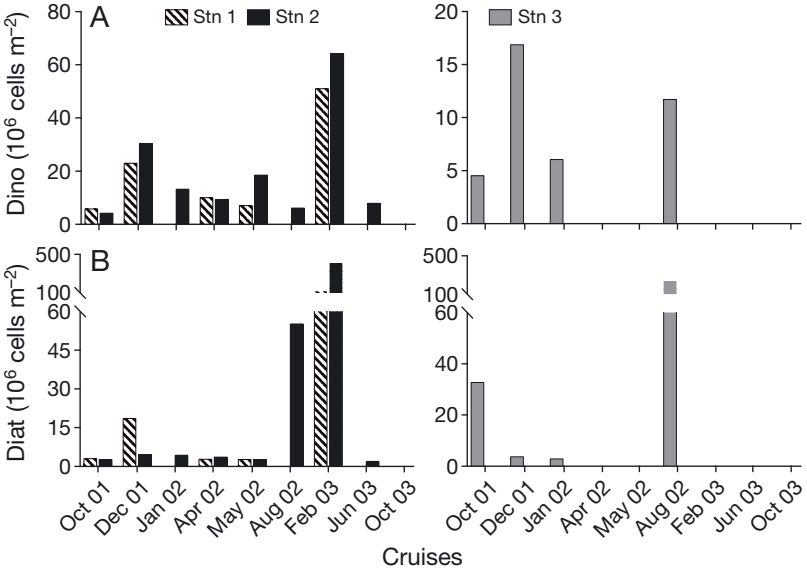


Fig. 11. Integrated concentrations of (A) dinoflagellates (Dino) and (B) diatoms (Diat) in the >10 µm fraction. Enumeration was carried out by inverted microscopy at the 3 stations sampled during Diapalis cruises. Integration depths: 0–60 m at Stns 1 and 2; 0–30 m at Stn 3. During some cruises Stns 1 and 3 were not sampled. No counts were performed in October 2003

Table 7. Morphometric characteristics of *Trichodesmium and Katagnymene* species at the *Trichodesmium* peak abundance in February 2003 (Diapalis cruise D7). Chlorophyll *a* (chl *a*), phycoerythrin (PE), and carbon (C) content per trichome and volume of trichome are shown. Comparison with cellular characteristics, pigments, and carbon content obtained with *T. erythraeum* in New Caledonian waters (west coast). *TK*: sum of *Trichodesmium* and *Katagnymene* trichomes

| | Mean | SD | No. |
|---|---|---|---|
| **D7 Cruise** | | | |
| pg chl *a* trichome$^{-1}$ | 43.23 | 18.86 | 30 |
| µg chl *a* mm$^{-3}$ trichome | 3.47 | 1.71 | 23 |
| pg PE trichome$^{-1}$ | 196.86 | 58.34 | 30 |
| µg PE mm$^{-3}$ trichome | 16.71 | 7.94 | 24 |
| *TK* trichome diameter (µm) | 6.64 | 3.32 | 1884 |
| *TK* trichome length (µm) | 360 | 302 | 1884 |
| *TK* trichome volume (µm$^3$) | 12808 | 28847 | 1884 |
| *T. contortum* trichome diameter (µm) | 33.00 | – | 2 |
| *T. contortum* trichome length (µm) | 128 | – | 2 |
| *T. erythraeum* trichome diameter (µm) | 8.01 | 1.11 | 402 |
| *T. erythraeum* trichome length (µm) | 320 | 245 | 402 |
| *T. hildebrandtii* trichome diameter (µm) | 16.00 | 2.49 | 9 |
| *T. hildebrandtii* trichome length (µm) | 269 | 308 | 9 |
| *T. tenue* trichome diameter (µm) | 4.49 | 0.57 | 530 |
| *T. tenue* trichome length (µm) | 472 | 357 | 530 |
| *T. thiebautii* trichome diameter (µm) | 6.06 | 0.28 | 735 |
| *T. thiebautii* trichome length (µm) | 327 | 273 | 735 |
| *K. pelagica* trichome diameter (µm) | 24.00 | 5.10 | 5 |
| *K. pelagica* trichome length (µm) | 403 | 558 | 5 |
| *K. spiralis* trichome diameter (µm) | 20.00 | 3.95 | 65 |
| *K. spiralis* trichome length (µm) | 170 | 189 | 65 |
| ***T. erythraeum*** | | | |
| pg chl *a* cell$^{-1}$ | 1.58 | 0.26 | 5 |
| pg chl *a* trichome$^{-1}$ | 99.00 | 14.62 | 5 |
| ng chl *a* mm$^{-3}$ trichome | 3.31 | 3.86 | 5 |
| pg PE cell$^{-1}$ | 4.01 | 0.76 | 5 |
| pg PE trichome$^{-1}$ | 251.57 | 41.26 | 5 |
| µg PE mm$^{-3}$ trichome | 8.37 | 1.21 | 5 |
| pg carbon cell$^{-1}$ | 289.89 | – | 2 |
| ng carbon trichome$^{-1}$ | 17.22 | – | 2 |
| µg carbon mm$^{-3}$ trichome | 588.06 | – | 2 |
| PE:chl *a* | 3.81 | 1.79 | 13 |
| Carbon:chl *a* | 173.21 | 55.96 | 9 |
| Carbon:PE | 71.15 | 33.54 | 6 |
| Cell diameter (µm) | 9.6 | 0.5 | 1849 |
| Cell length (µm) | 6.3 | 0.7 | 1849 |
| Cell volume (µm$^3$) | 456 | 76 | 1849 |
| Trichome length (µm) | 402 | 157 | 1849 |

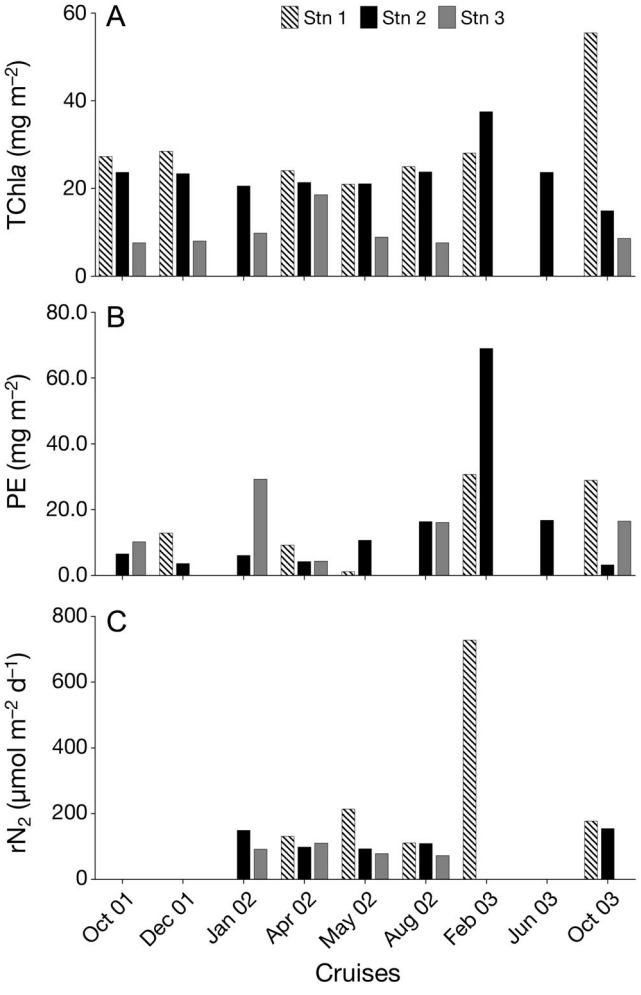


Fig. 12. Integrated (A) total chlorophyll *a* (Tchl*a*) and (B) phycoerythrin (PE) concentrations, and (C) $N_2$ fixation rates ($rN_2$) (Garcia et al. 2007) at the 3 stations sampled during the Diapalis cruises: integration depths: 0–60 m at Stns 1 and 2, and 0–30 m at Stn 3

al. (2012). Variability in counting and sampling procedures, however, hamper comparisons between studies around the world, especially for filamentous cyanobacteria. Significant error in the accuracy of low abundance measurements occurs depending on the sampling mode. In this case, the use of a plankton net increased the detection of *Trichodesmium* by a factor of 10 (0.1 vs. 1 trichome l$^{-1}$) compared to water samples collected with 2.5 L GO FLO bottles (Chang et al. 2000). When bottle sampling, it is essential to filter all of the contents to prevent uneven distribution of *Trichodesmium*, which can occur when trichomes (and colonies) migrate due to cell-controlled or uncontrolled buoyancy (Bergman et al. 2013). When tightly-packed filaments of *T. erythraeum* are observed at the surface, manual sampling within the first few millimeters of depth with narrow-necked plastic bottles provides a fairly accurate picture of trichome abundance, despite the somewhat uneven distribution.

Abundance data are usually expressed as cell, trichome, or colony numbers with or without specific information on the taxonomic composition of filamentous cyanobacteria. Conversion factors from one unit to another are, however, vague and based on the morphometric variability of filamentous diazotrophs (Luo et al. 2012), such as the number of trichomes per

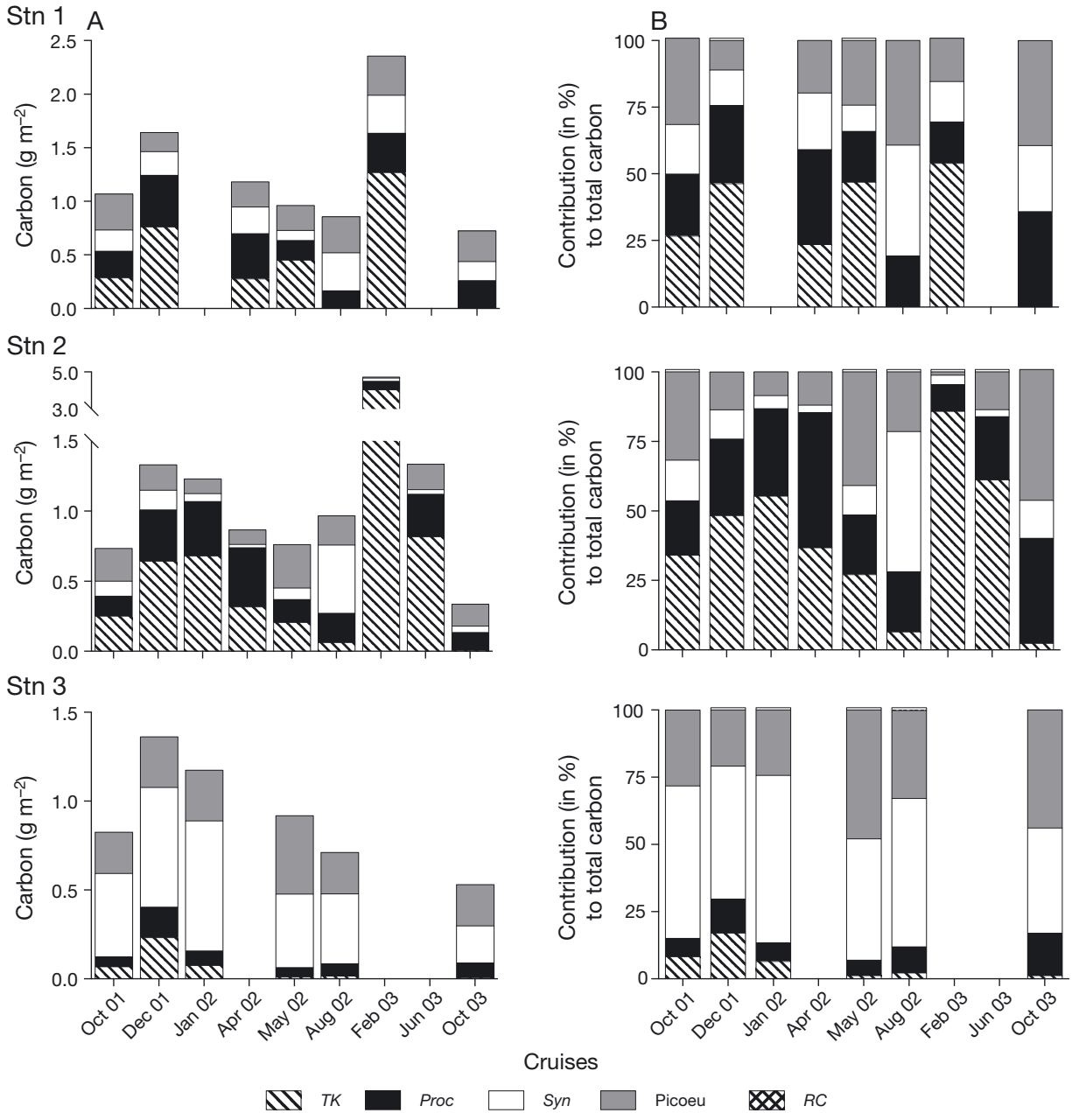


Fig. 13. (A) Carbon biomass of picoplankton and filamentous cyanobacteria at the 3 stations sampled during the Diapalis cruises. The conversion factors were (fgC cell$^{-1}$): *Prochlorococcus* (*Proc*, 36), *Synechococcus* (*Syn*, 255), picoeukaryotes (Picoeu, 2590), *Richelia* (*RC*, 10); sum of *Trichodesmium* and *Katagnymene* (*TK*, 17.22 × 10$^6$ fgC trichome$^{-1}$). (B) Contribution of each group to the total carbon shown in A

colony, cell numbers per trichome, and trichome dimensions, which are quite variable. For the Diapalis data set, trichome counts of mixed species were linearly correlated (Spearman's rank coefficient > 0.95, $p < 0.001$) with the estimates of entire surface and total volume of trichomes, as previously pointed out by Neveux et al. (2006). Therefore, replacing trichome numbers with trichome surface areas or volumes did not greatly modify the qualitative vertical distributions or seasonal trends in the filamentous biomass. This indicated a relatively constant composition of the filamentous community. Also, mean *T. erythraeum* carbon content per trichome (17.22 ng) and per cell (290 pg) were determined by our microscope counts. These values were in the upper range of the approximate cell carbon content previously

Table 8. Spearman's correlation coefficient for the relationships between integrated abundance (cell or trichomes $m^{-2}$ over 60 m for Stns 1 and 2, and 30 m for Stn 3) of autotrophic groups, the percentage of the different species of filamentous cyanobacteria, and environmental parameters (n = 28). Data from the Diapalis cruises (2001–2003; see Table 1 for cruise dates). $^{\star}p < 0.05$, $^{\star\star}p < 0.01$, ns: not significant; *TK*: sum of *Trichodesmium* and *Katagnymene* trichomes; Din: dinoflagellates; Diat: diatoms; *Rich*: *Richelia*; *Proc*: *Prochlorococcus*; *Syn*: *Synechococcus*; Peuk: Picoeukaryotes; *T.t*: *T. tenue; T. e*: *T. erythraeum*; *T. th*: *T. thiebautii*; *K. s*: *K. spiralis*; *K. p*: *K. pelagica*; MLD: mixed layer depth; T(°C): temperature; Sal: salinity

| Variables | Din | Diat | *TK* | *Rich* | *Proc* | *Syn* | Peuk | %*T. t* | %*T. e* | %*T. th* | %*K. s* | %*K. p* | MLD | T(°C) | Sal | $NO_x$ | $NH_4$ | $PO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Din | 1 | 0.46$^{\star}$ | 0.63$^{\star}$ | ns | ns | ns | ns | ns | 0.50$^{\star}$ | ns | ns | −0.57$^{\star}$ | ns | ns | ns | ns | ns | ns |
| Diat | | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| *TK* | | | 1 | ns | 0.65$^{\star\star}$ | ns | ns | ns | ns | ns | ns | ns | ns | 0.62$^{\star\star}$ | ns | ns | 0.51$^{\star}$ | −0.48$^{\star}$ |
| *Rich* | | | | 1 | ns | ns | ns | ns | ns | 0.61$^{\star\star}$ | 0.69$^{\star\star}$ | ns | 0.60$^{\star}$ | ns | ns | ns | 0.85$^{\star\star}$ | ns |
| *Proc* | | | | | 1 | −0.42$^{\star}$ | ns | ns | ns | −0.50$^{\star}$ | ns | ns | ns | ns | ns | ns | ns | ns |
| *Syn* | | | | | | 1 | 0.52$^{\star}$ | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Peuk | | | | | | | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| %*T. t* | | | | | | | | 1 | ns | −0.65$^{\star\star}$ | −0.59$^{\star}$ | ns | ns | 0.49$^{\star}$ | ns | ns | −0.57$^{\star}$ | ns |
| %*T. e* | | | | | | | | | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| %*T. th* | | | | | | | | | | 1 | ns | ns | ns | ns | ns | ns | ns | ns |
| %*K. s* | | | | | | | | | | | 1 | ns | ns | ns | ns | 0.64$^{\star}$ | 0.55$^{\star}$ | 0.59$^{\star}$ |
| %*K. p* | | | | | | | | | | | | 1 | ns | ns | ns | ns | ns | 0.63$^{\star}$ |
| MLD | | | | | | | | | | | | | 1 | ns | ns | −0.46$^{\star}$ | ns | ns |
| T(°C) | | | | | | | | | | | | | | 1 | −0.63$^{\star\star}$ | ns | ns | −0.55$^{\star\star}$ |
| Sal | | | | | | | | | | | | | | | 1 | ns | ns | 0.52$^{\star}$ |
| $NO_x$ | | | | | | | | | | | | | | | | 1 | ns | 0.48$^{\star}$ |
| $NH_4$ | | | | | | | | | | | | | | | | | 1 | ns |
| $PO_4$ | | | | | | | | | | | | | | | | | | 1 |

published for *Trichodesmium* species assuming 100 cells trichome$^{-1}$ (110–250 pg C cell$^{-1}$; Luo et al. 2012). Taking the measured value of 290 pg C cell$^{-1}$ of *T. erythraeum* (Table 7), *TK* accounted for up to 80% of C cyanobacterial biomass in summer at the oceanic station of the Loyalty Channel, which confirms the essential role of filamentous cyanobacteria for the global food chain (Bergman et al. 2013) and in the SW tropical Pacific Ocean (SWTP) (Biegala et al. 2014, Bonnet et al. 2015).

### Comparison of *Trichodesmium* abundance with other studies

During the Diapalis program, *Trichodesmium* spp. abundance in the ocean varied from 1 to 4578 trichomes $l^{-1}$ found at 0 to 60 m (average 586 trichomes $l^{-1}$, n = 88), which was much higher than previously published data in New Caledonian waters at the end of the summer (250–1000 trichomes $l^{-1}$ in April 1998; Campbell et al. 2005). The only other abundance data available for our region around New Caledonia are *nifH* gene copies (Moisander et al. 2010). In Ouinne lagoon, a maximum of 1200 trichomes $l^{-1}$ was found in summer 2007 and more than 10 000 trichomes $l^{-1}$ in accumulations (Rodier & Leborgne 2010). These concentrations are lower than measured in a surface accumulation in the Southwest Lagoon, namely up to $30 \times 10^6$ trichomes $l^{-1}$ for a chl *a* value of 3 mg $l^{-1}$ (Neveux et al. 2009), the highest values reported for the region. Such surface accumulations (>10 000 trichomes $l^{-1}$) of mixed *Trichodesmium* species were collected 98 times in summer between New Caledonia and Vanuatu (1998 to 2010: Dupouy et al. 2011, 2017). In the southernmost part of the SWTP at 28° S in the austral summer, Law et al. (2011) observed comparatively low surface *Trichodesmium* abundance, averaging 11 trichomes $l^{-1}$ in the upper 10 m to 6 trichomes $l^{-1}$ at 35–50 m. Some high values were found in the Solomon Islands, expressed as trichome number (7700 trichomes $l^{-1}$; Girault et al. 2016) or *NifH* copies (Bonnet et al. 2015). In the western North Pacific Ocean, abundance was between 1 and 400 trichomes $l^{-1}$ (Marumo & Asaoka 1974, Marumo & Nagasawa 1976, Chen et al. 2003, Shiozaki et al. 2015), with some high values in accumulations (>20 000 filaments $l^{-1}$) near the Miyako Islands (Shiozaki et al. 2015).

Comparing the Diapalis program with other time series conducted on *Trichodesmium* in subtropical waters raises a number of problems, as a different sampling strategies and sample processing methods were used. During the HOT and BATS programs, *Trichodesmium* abundance was studied at approximately monthly intervals (HOT: October 1989 to December 1992, Letelier & Karl 1996; BATS: 1995–1997, Orcutt et al. 2001), and free trichomes

(water filtration sampling) and colonies (net sampling) were counted separately. Therefore, a conversion factor of 200 trichomes colony$^{-1}$ used by Orcutt et al. (2001) was applied to HOT and BATS counting data for comparisons with the Diapalis values, as in our study, colonies were not counted (because they disintegrated during the counting) and only trichome abundance was available. This showed that trichome abundances in New Caledonian waters were generally higher than those observed at HOT and BATS stations. In fact, the integrated maxima observed in February 2003 in the Loyalty Channel ($240 \times 10^6$ trichomes m$^{-2}$) were 70 times and 400 times higher than at HOT ($3.5 \times 10^6$ trichomes m$^{-2}$) and BATS ($0.64 \times 10^6$ trichomes m$^{-2}$), respectively. Moreover, mean cell numbers per trichome were generally lower in Diapalis samples (50–60) than at HOT (100). At HOT, apical cells were counted and divided by 2 to obtain the trichome number (Letelier & Karl 1996). This precaution was not applied to Diapalis samples, leading to a possible overestimation of trichome numbers due to trichome breaking during sample processing. HOT and BATS studies have shown a maximum abundance in summer and relatively strong interannual variability, even if free trichome variations at BATS seemed identical from one year to another. At BATS, however, interannual variations, which were deduced from only 7 integrated depth profiles for 3 yr fitted by a third-degree polynomial function of days of the year (Fig. 1A in Orcutt et al. 2001), are questionable.

### Filamentous diazotroph species and their relationship with environmental factors

The SWTP around New Caledonia is a typical warm low-nutrient low-chlorophyll (LNLC) region (Ceccarelli et al. 2013), with phosphate recharged annually (presumably other nutrients as well) in the euphotic zone during the transition periods (Moutin et al. 2005). Phosphate can limit the growth of diazotroph species and consequently controls nitrogen fixation rates in the ocean (Karl et al. 2002). In LNLC, diazotroph species such as *Trichodesmium* are better adapted as they are able to use atmospheric $N_2$ (Bergman et al. 2013). Diazotrophs are likely responsible for the lower phosphate concentrations in summer in the region due to their $PO_4$ uptake (Moutin et al. 2005, Shiozaki et al. 2014), which corroborates the negative correlation between *Trichodesmium* and phosphate found in this study. During Diapalis cruises, the ammonium concentrations at ocean stations were only high in February 2003, coinciding with the highest concentrations of trichomes, PE, and cyanobacterial carbon. The ammonium concentrations were also positively correlated to *Trichodesmium* densities. Therefore, the relatively high ammonium concentrations may be attributed partially to direct release of this nutrient by the diazotrophs (Mulholland 2007, Bergman et al. 2013). We did not find any correlation between *Trichodesmium* abundance and MLD. As stated by Hood et al. (2004), increased *Trichodesmium* concentrations are linked to a shallow MLD (resulting in high mean light levels) and low dissolved inorganic nitrogen concentrations for extended periods of time. The relationship between nitrogen fixation rates in plankton net samples and MLD was significantly fitted by a hyperbolic function in the Atlantic Ocean ($r^2 = 0.31$, $p < 0.05$, $n = 18$, Agawin et al. 2013).

*T. thiebautii* and *T. tenue* were the predominant species in the oceanic waters around New Caledonia and in the open lagoon of the east coast, as previously stated by Rodier & Leborgne (2010). By contrast, and as also reported by Rodier & Leborgne (2010), *T. erythraeum* dominated in the SW lagoon of New Caledonia, which is a semi-closed and larger lagoon than the SE lagoon. Generally, *T. thiebautii* and *T. tenue* are poorly represented in coastal regions (Revelante & Gilmartin 1982). They dominate in the oligotrophic gyre of the North Pacific, western Pacific (Marumo & Nagasawa 1976), and eastern part of the China Sea (Marumo & Asaoka 1974) and are likely better adapted to low-nutrient conditions than *T. erythraeum*. In our study, *T. tenue* was inversely correlated with *T. thiebautii,* which suggests competition between the 2 species for an apparently similar environment. According to Romans et al. (1994), *T. tenue* is a common species often confused with *T. thiebautii* in optical microscopy, which can explain the lack of information on the species' distribution and quantification. The predominance of *T. tenue* observed on occasion during the Diapalis cruises is a relatively original finding. In this study around New Caledonia, *T. contortum* and *T. hildebrandtii* occur in very low densities, as in most oceans (Bergman et al. 2013).

Information on *Katagnymene* species in the field is very scarce. They have been found in the SW Pacific (Lundgren et al. 2001, Dupouy et al. 2011, Girault et al. 2016). Both *Katagnymene* species, *K. pelagica and K. spiralis,* should now be considered as 2 morphological forms of the same species (Lundgren et al. 2005). In our study, both forms were frequently observed, but always in low abundance (<284 tri-

chomes $l^{-1}$). The strongest concentrations were observed in the first 40 m, although a deeper maximum (60 m) was found in the Loyalty Channel in February 2003. At this maximum, the *K.* s*piralis* form accounts for 81% of the *Katagnymene* genus. Along the RV 'Roger Revelle' cruise track between New Caledonia and the Fiji Islands (April 1998), Lundgren et al. (2001) found both *Katagnymene* forms at 34 of the 51 stations and counted up to 400 trichomes $l^{-1}$ of *Katagnymene* in the 0–25 m layer, with 89% *K. pelagica.* It should be noted that *Katagnymene* trichomes are generally long (1 cm) and extremely fragile and can be broken up by handling before counting; hence the recommended use here of surface and total volume for biomass estimates. The significant relationship of *K. spiralis* with all nutrients, while *K. pelagica* was correlated only with phosphate, may indicate a different ecological niche for these 2 species.

Like *Trichodesmium* and *Katagnymene*, the heterocystous *Richelia intracellularis* is widespread in warm tropical and subtropical waters (Luo et al. 2012, Bonnet et al. 2015), whereas *Calothrix rhizosolenia*, as epibionts of *Chaetoceros*, have been observed in the Indian and western Pacific Oceans (Sarma 2012, Girault et al. 2016). Around New Caledonia in this study, both *Richelia* and *Calothrix* species (*RC* groups) were sometimes present as free-living organisms, but more frequently in endosymbiotic association with diatoms (mainly in *Rhizosolenia* sp.). *RC* were present in all seasons with a maximum (>700 trichomes $l^{-1}$) in the Loyalty Channel in summer (February 2003), as was *Trichodesmium*. Based only on samples where *RC* was detected, its biomass correlated with the number of diatoms in the >10 µm fraction (Spearman rank correlation between 0.52 and 0.57, $p < 0.0001$; data not shown). In endosymbiosis, the host cell provides a protective micro-environment and positive buoyancy to the symbiont, which enables the cell to remain in the euphotic layer, as, unlike *Trichodesmium,* this species does not have gas vacuoles (Janson et al. 1999). *Richelia* has a very uneven space distribution in the oceans, like *Trichodesmium*, depending on whether the environment is conducive to its growth. Both genera often inhabit the same types of ecosystems, as they have similar physiological properties, like all diazotrophs (Bergman et al. 2013 and references therein). In the case of cyanobacteria–diatom associations, environmental conditions must meet the needs of both organisms, and particularly sufficient silicate concentration for the diatom host growth. Iron and silica are not limiting factors in our study area and are provided by leaching from terrestrial soils (Shiozaki et al. 2014). In our study, *RC* was more correlated with *T. thiebautii* and *K. spiralis*, and its positive dependence on the density structure of the water column (MLD) and ammonium needs to be further explored. In the North Pacific Gyre, the greatest abundance in *R. intracellularis* is also observed in summer, when the species can form blooms of about $10^4$ filaments $l^{-1}$ (Venrick 1974), values which are thus greater than ours (max. 734 filaments $l^{-1}$). Around the Hawaiian Islands (Sohm et al. 2011), a high abundance of all 3 groups, i.e. *Trichodesmium*, *Katagnymene*, and *Richelia,* was reported in late boreal summer of 2002 (September and October) and summer 2003 (July and August), with *Richelia*–diatom associations more abundant to the south of the islands. The high variability in *Richelia* size in our samples is another reason to underline the inaccuracy of biomass measurements in terms of filament numbers rather than total filament surface area or volume. The contribution of *RC* in terms of volume (or carbon) was minor (<1%) but did not mask the great impact of this species on carbon sequestration by DDAs as shown in the northern Tropical Pacific (Dore et al. 2008, Karl 2014, Karl & Church 2014).

In our study, large diatoms did not show significant correlations with any of the other groups enumerated nor with any of the environmental variables. Dinoflagellates correlated positively with total trichome abundance. Their positive correlation with the percentage of *T. erythraeum* and their negative correlation with the percentage of *K. pelagica* suggested affiliation to more coastal than oceanic environments. The diversity of trophic status in dinoflagellates (photosynthetic, mixotroph, heterotroph) was nevertheless not considered in the analysis. In the northern Tropical Pacific, the dinoflagellate group revealed no specific ecological pattern nor any characteristic assemblages (Girault et al. 2016). During the Diapalis cruises, the picoplanktonic groups did not show any correlation with environmental parameters (Table 8), which was surprising, as *Synechococcus* and *Prochlorococcus* have distinct ecophysiology, with *Synechococcus* being better adapted to mesotrophic conditions and *Prochlorococcus* to oligotrophic conditions (Partensky et al. 1996). This could be explained for *Prochlorococcus* by the fact that their abundance varied little over the annual cycles. *Prochlorococcus* never dominated the phytoplankton C biomass, but were often the second contributor to C biomass after filamentous cyanobacteria in oceanic waters (Fig. 13). All phytoplankton share a requirement for light and nutrients. A more specific mecha-

nism underlying the covariation between *Synechococcus* and the picoeukaryotes, as opposed to *Prochlorococcus*, is that they have a larger cell size than the latter, and are able to use nitrate. This could explain why they tend to respond positively to enhanced nutrient supply as observed mostly in the lagoon station and at Santal Bay. The covariation between *Synechococcus* and the picoeukaryotes has been described before in the Atlantic subtropical gyres (Marañón et al. 2003) and in the Pacific Ocean (Worden et al. 2004). The significant positive correlation between *Synechococcus* and picoeukaryotes may also occur due to similarities in favorable light conditions (Gutiérrez-Rodríguez et al. 2016). *Synechococcus* and picoeukaryotes dominated the C biomass in the lagoon, but may also be large contributors in oceanic waters, as observed in winter (Fig. 13).

### Inter-annual variability (2001–2003)

The difference in large diazotroph abundance (and nitrogen fixation rates, Garcia et al. 2007; Fig. 12C) observed between the summers of 2001–2002 and 2002–2003 could be the result of a relatively low *in situ* sampling frequency. Satellite imagery nevertheless confirmed the very low chlorophyll values during summer 2001–2002 compared to summer 2003 (Dupouy et al. 2011). Moreover, satellite-classified *Trichodesmium* pixels in the region 5–25° S, 160° E–170° W determined using the TRICHOSAT model were clearly lower in summer 2001–2002 (La Niña years) than summer 2003 (an El Niño year). The spectral fluorescence signature of surface blooms as measured by the MERIS sensor (Gower et al. 2014) was also high during El Niño summers (2003, 2007, 2010) but low during La Niña summers. The difference in cumulative precipitation between 2001–2002 and 2003 summers is striking, with 2003 being drier despite the occurrence of 2 tropical storms, namely Beni in January 2003 and Erica in March 2003 (Neveux et al. 2009). Law et al. (2011) reported that nitrogen fixation increased owing to an iron supply from a wet-dust deposition 8 d after tropical cyclone Wati in the Tasman Sea. Australian dust peaking in 2001 and 2002 was cited as a potential factor in increasing occurrence in the seas to the east of Australia (estimated by satellite) in 2001–2005 (Mackie et al. 2008). The regional distribution of aerosols was probably also influenced by the continuous passive (non-explosive) volcanic degassing of the Melanesian Volcanic Arc, as indicated by sulfur dioxide ($SO_2$), the composition of which has been analyzed using satellite data and modeling (Lefèvre et al. 2016). Likewise, Sahara dust deposition at BATS corresponded to an increased abundance of *Trichodesmium* colonies as well as changes in their chemical composition, even though it did not enhance the $N_2$ fixation rate per colony (Orcutt et al. 2001). At BATS, inter-annual variability could also be related to the relative extent of vertical mixing from one year to another, including the effects of hurricane frequency, which would reduce *Trichodesmium* abundance (Orcutt et al. 2001). During the Diapalis program, this negative vertical-mixing effect was not confirmed, as the highest *Trichodesmium* abundance occurred just after tropical storm Beni struck New Caledonia in late January 2003. Inter-annual variability would also have been related to changes in main-current transports, i.e. a westward Sverdrup transport and trade winds enhanced during El Niño periods vs. falling Sverdrup transport and strengthening northwesterly winds during La Niña (Kessler & Cravatte 2013, Cravatte et al. 2015). The wind regime, which may impact mixed-layer formation, is known to alternate around New Caledonia with a trade-wind regime during El Niño and more westerly winds during La Niña, which may increase surface currents in the Loyalty Channel (Lefèvre et al. 2010). How such large-scale current variability or atmospheric composition influence total biomass and *Trichodesmium* abundance in New Caledonia is not clear yet.

## CONCLUSION

In 2001–2003, the seasonal and inter-annual variations in cyanobacterial abundance were investigated in New Caledonian waters during the 9 Diapalis program cruises. Three sites representative of different biotopes were sampled, i.e. open ocean (Loyalty Channel), coastal (lagoon off the Ouinne River estuary), and deep bays (Santal Bay at Lifou). Abundance was determined at the species level for diazotrophic filamentous cyanobacteria. Our description of the organisms (*RC* and diatoms) in symbiotic associations was well detailed. Filamentous cyanobacteria were almost always present throughout the year, but with relatively high variations. The highest abundances occurred during the summer seasons. The dominant species were *Trichodesmium thiebautii* and *T. tenue*, followed by *T. erythraeum*. *Katagnymene* as well as *Richelia* species (mainly endosymbiotic in diatoms) were consistently present in low abundance. Microscopic examination also revealed the presence of

unknown filamentous species formed by round cells not yet described by molecular tools. Picoplanktonic cyanobacteria (*Prochlorococcus* and *Synechococcus*) were consistently present in abundance with less pronounced seasonal variations than filamentous cyanobacteria. *Trichodesmium* dominated in terms of carbon content in the Loyalty Channel and Santal Bay in summer, while *Synechococcus* dominated at the Ouinne station and in winter at all 3 stations. The comparison of the 2002 and 2003 summers showed substantial inter-annual variability. Environmental factors that could explain this variability were not clearly identified. One hypothesis could be the effects of alternation between La Niña and El Niño, with maximum diazotrophs and *Trichodesmium* abundance during a well-established El Niño. Some hypotheses relating to the El Niño/La Niña cycle need verifying, i.e. dust inputs and links with precipitation, winds, vertical movements, and horizontal circulation within the upper 200 m layer, gyre formation in relation to wind strength and direction, and increased mesoscale circulation.

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