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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.

**Keywords** : Trichodesmium, Filamentous cyanobacteria, Community structure, Microscopy, Picoplankton, Pigments, Loyalty Islands, Coral-reef lagoon, El Nino

## 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 (Buitenhuis et al. 2012a, Luo et al. 2012). Unicellular picoplanktonic cyanobacteria are certainly the most abundant photosynthetic group and 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 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 *Trichodesmium* genus with two 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 two *Katagnymene* species (*K. pelagica* and *K. spiralis* Lemmerman) are more scarcely 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 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, Giraud 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 per year (Capone et al. 1997) for a global oceanic $N_2$ fixation of 100-200 Tg N per year (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 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 relies only on *Nifh* gene counts (Moisander et al. 2010, Bonnet et al. 2015, 2016). Moreover, few studies address their temporal variability as reported at HOT in the Northern Pacific (Letelier & Karl 1996), BATS in the Northern Atlantic (Orcutt et al. 2001), and in 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 the 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 content in pigment 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 cyanobacteria species abundances was undertaken in 2001-2003 (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 three stations: an open-ocean station (Loyalty Channel), a large, deep and open bay (Santal Bay, Lifou Island) and a lagoon station (Ouinne, southeast 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 had been published earlier (Garcia et al. 2007).

## MATERIAL AND METHODS

**Sampling and environmental data.** Nine oceanographic cruises (Diapalis program: D1 to D9) lasting about a week each were conducted between October 2001 and October 2003 aboard IRD's R.V. *Alis* between 165°E and 168 °E and between 20°S and 23°S in the Eastern Coral Sea (Table 1, Fig 1). Samples were taken at three stations: the first at 167°30'E 20°30'S in the deep Santal Bay which is broadly exposed to the ocean, on Lifou Island's west coast (bottom depth: 1050 m, ST1), the second in open ocean waters in the middle of the Loyalty Channel at 21°30'S 167°E (bottom depth: 2000 m, ST2), and the third (166°45'E 21°57'S) in the south eastern coral-reef lagoon of New Caledonia (bottom depth: 40 m, ST3). The sampling covered different seasons (Table 1): summers 2002 (D2 to D4) - 2003 (D7), winters 2002 (D6) - 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 (T°C) and salinity were measured with Sea-Bird SBE 911 and water samples were collected with 8L-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 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 in Holmes et al. (1999). More details on

131 the methods and the full data set Diapalis are available on http://www.obs-
132 vlfr.fr/proof/vt/op/ec/diapazon/dia.htm. The mixed layer depth (MLD) was estimated by taking
133 the first depth where potential density (sigma-t) was 0.03 Kg $m^{-3}$ greater than the density at 10
134 meters, based on Montegut et al. (2004).
135
136 **Filamentous cyanobacteria counting and morphometric characteristics.** Filamentous
137 cyanobacteria were recovered from water samples taken at 4-6 depths down to 80 m, with a
138 rosette of 8L Niskin bottles. Organisms were collected by filtering whole water bottles through a
139 10 µm Nuclepore polycarbonate membrane. The filter was then placed in a 20 mL glass bottle
140 containing a 4 % formalin solution for preserving photosynthetic organisms. In the laboratory,
141 the filter was rinsed with a plastic wash bottle containing filtered seawater in order to remove all
142 the organisms from the filter and collect them in a 100 mL glass cylinder. After processing, the
143 filter was checked for any remaining photosynthetic organisms. A suitable quantity of an acidic
144 formalin solution (1/1 mixture of 40 % formalin and acetic acid) was then added to obtain
145 organisms in a final 0.4 % formalin solution. From this solution, filamentous cyanobacteria and
146 other large phytoplanktonic species were measured and enumerated with an inverted microscope
147 (OLYMPUS IM., 100× magnification) using a standard sedimentation technique (Utermöhl
148 1931). According to Cronberg et al. (2004), the acetic acid breaks the gas vesicles facilitating the
149 sedimentation of filaments in the sedimentation chambers. Sedimentation time was 24 hours in 5
150 and 10 mL chambers and at least 48 hours for the 25 and 50 mL chambers, depending on cell and
151 filament abundances. A total of 88 samples were examined for the 9 cruises. For surface
152 accumulations of *Trichodesmium erythraeum*, samples were collected directly by hand with a
153 small bottle (500 mL), which was then processed using the same method as described above.
154 Identification of the filamentous *Trichodesmium, Katagnymene* (see review in Bergman et
155 al. 2013) and *Richelia* (Foster et al. 2010) was carried out based on their morphological
156 characteristics. Cell shape and dimensions (particularly cell diameter) were the main criteria used
157 for classifying and identifying them. The sample processing method used to facilitate
158 enumeration and morphometric examination at trichome level could not be used for observations
159 at colony level, because most of them had disintegrated. Trichome surface and volume were then
160 calculated on the assumption that they had an elongated cylindrical morphology. For *T.*
161 *erythraeum* and *T. thiebautii*, identification was confirmed by molecular biology studies (unp.
162 data). 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 chlorophylls analysis in the total community. Chlorophylls were extracted in 90 % acetone after grinding the GF/F filter. Measurements in the >10 µm fraction of the community were also carried out by filtering a whole 8L Niskin bottle through 47 mm Nuclepore polycarbonate membranes. In the latter case, chlorophylls were extracted in 90 % Dimethylformamide (DMF) without grinding the membrane. For convenience's sake, the abbreviations Chla, DV-Chla and TChla were used for monovinyl-chlorophyll a, divinyl-chlorophyll a and the sum of these two pigments (total chlorophyll a) respectively. Concentrations of cyanobacteria 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). 1 to 3 L water samples were filtered through 47 mm 0.4 µm polycarbonate membranes for total PE and 8L onto 10 µm membranes for size-fractionated PE. Details of the PE spectrofluorometric analysis are given in Lantoine & Neveux (1997), Neveux et al. (1999) and Neveux et al. (2006).

**Picoplankton 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 less than three months 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 Inc., Europe) as a reference. Four prevalent populations were distinguished and enumerated according to their scattering and florescence properties. Two of them belonged to picoplanktonic cyanobacteria, *Prochlorococcus* and *Synechococcus,* and two were eukaryotic groups, the smallest being deemed picoeukaryotes. Unicellular nanoplanktonic cyanobacteria such as *Crocosphaera*, easily recognized by the fluorescence excitation spectra of their phycoerythrin 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 *Trichodesmium erythraeum* was applied (17.22 ng C trichome$^{-1}$; see Table 8). The integrated carbon content of each group and their relative percentages were calculated for the upper layer (0-60 m ST1 and ST2, 0-30 m at ST3). 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 South West Tropical Pacific with clear-air temperature and precipitation seasonality (Fig. 2). The Diapalis period (2001-2003) coincided with the 2002-2003 El Nino build-up, with a first period (2001) marked by a Neutral Multivariate El Nino Index (MEI) and the second (2003) by a stronger positive MEI, indicative of a strong Central Pacific El Nino during D7-D9 (Fig. 2). On New Caledonia's east coast, precipitations were 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 January 29 with winds of 125 mph (205 km/h), before rapidly weakening, and dissipating on February 5. It brought heavy precipitation and floods lasting 6 days.

During the austral summer, seawater temperature increased to 27 °C at ST1 and ST2 and up to 28°C in the lagoon at ST3 (Fig. 3, left panel), leading to a vertical stratification and the formation of a pronounced thermocline (Fig. 4). During the winter, cooling of surface waters induced vertical mixing in the water column at least down to the deepest sampling level, e.g. 90-100 m in Aug 02 at ST2. Minimum temperatures in the upper layer were 23.4 °C at ST1 and ST2 and 22.8 °C at ST3. The highest salinities (around 35.4) were measured in Oct 03 at all three study sites, and the lowest during the wet summer season, in Apr 02 (from 34.47 at ST3, close to the mouth of the Ouinne River to 34.86 at ST1). Mean salinities were similar at ST1 and ST2 stations,

but lower in summer 2002 than summer 2003, due to different precipitation regimes linked to the El Nino episode (Fig. 2). Mean phosphate ($PO_4^-$) concentrations in the upper layer (Fig. 3, right panel) were weak throughout the sampling period, reaching maximum values of 0.06-0.12 µmol $l^{-1}$ in Oct 01 and Oct 03 during the transition periods and the minimum of 0.02 µmol $l^{-1}$ in summer. A small increase was observed in winter in Aug 02 due to a deepest MLD (Fig. 4). In wide Santal Bay (ST1), $NO_x$ concentrations were also very low, except in Aug 02, when they reached 0.08 µmol $l^{-1}$, linked to the deep winter mixing. The upper layer waters at ST2 oceanic station were $NO_x$ poor with concentrations always <0.06 µmol $l^{-1}$ (Fig. 3), even during deep vertical winter mixing as in Aug 02. Small maxima (0.05 µmol $l^{-1}$) were observed in Oct 03 as in Feb 03 despite warmer, saltier waters. At ST3 lagoon station, the $NO_x$ concentrations varied from 0.01 to a maximum of 0.07 µmol $l^{-1}$ in Jan 02. $NH_4^+$ concentrations (data not shown) at ST1 and ST2 were generally low <0.04 µmol $l^{-1}$ and often close to the detection limit 0.01 µmol $l^{-1}$. Higher concentrations were observed only in Feb 03 at both stations (0.11 and 0.19 µmol $l^{-1}$ at ST1 and ST2, respectively). In the lagoon, concentrations were on average slightly higher: up to 0.30 µmol $l^{-1}$.

**Filamentous cyanobacteria**

***Specific composition*:** Five species of the *Trichodesmium* genus were observed (Fig. 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 three sampling sites. *T. contortum* was seldom observed (3 samples). *Katagnymene* (Fig. 5E, F, G) and *Richelia/Calothrix* (Fig. 6) genera 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* as an epibiont on *Chaetoceros sp.* During the May 02 cruise, west of Lifou, we observed unidentified filaments very similar to *Trichodesmium,* but differing in their very small cellular diameter (2.7-3 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 cell diameter) within the range published in the literature (Table 3 and 8) and the sizes of *T. thiebautii* and *T. erythraeum* species were in the low part of the size range.

***Trichodesmium and Katagnymene abundance:*** At ST1 and ST2, the highest abundances of filamentous cyanobacteria in the 0-60 m upper layer were noted in summer (Feb 03; Fig. 7), i.e.

259 3000-4500 trichomes $l^{-1}$ and 1000-3000 trichomes $l^{-1}$ between 5 and 10 m, respectively. However,
260 the concentrations and vertical distribution could be highly variable from one day to another, as
261 shown at ST1 in Feb 03 (Fig. 7). Some vertical profiles showed maximum concentrations at 40
262 m as at ST2 (mostly due to *T. erythraeum*). Vertical distributions were quite homogeneous at the
263 period of low abundance, i.e. in the austral winter (Aug 02 and Jun 03). At ST3 (Fig. 7),
264 concentrations did not exceed 1000 trichomes $l^{-1}$. Tables 4 to 6 give general statistics on the
265 abundances of the different filamentous cyanobacteria enumerated by inverted microscopy
266 during Diapalis cruises 1-9 for each station. Filamentous cyanobacteria were extremely rare in
267 Oct 03 regardless of depth.

268 Assuming that *Trichodesmium* can migrate vertically within one day (Villareal & Carpenter
269 2003), using integrated abundance seemed more appropriate for gauging their seasonal variations
270 in the water column (Fig. 4). Thus, at ST2, 0-60 m integrated abundance showed a 500-fold
271 variation between minimum in Oct 03 (<0.5 $10^6$ trichomes $m^{-2}$) during the transition periods and
272 maximum in summer (Feb 03: 235 $10^6$ trichomes $m^{-2}$). At ST1, the seasonal variations appeared
273 similar but the variation range (x 150) and the maximum summer concentrations (73 $10^6$ trichomes
274 $m^{-2}$) were lower than at ST2. At both stations, maximum abundance in summer 2003 was
275 coincident with a shallow MLD, but low abundances were also observed in summer 2002 with a
276 similar thermohaline structure. At ST3 station (Fig. 4), integrated concentrations (0-30 m only)
277 were 15 times lower and varied from 0.9 $10^6$ trichomes $m^{-2}$ to 13.5 $10^6$ trichomes $m^{-2}$, although
278 ST3 was not sampled in Feb 03.

279 The three main *Trichodesmium* species together accounted for 85-100 % of the integrated
280 biomass of filamentous cyanobacteria in terms of trichome numbers and always more than 51 %
281 in terms of trichome volume (Fig. 8). *Trichodesmium tenue* and *T. thiebautii* were generally the
282 dominant species followed by *T. erythraeum*. Their mean cell numbers per trichome were
283 relatively similar (Table 7). As expected, the percentage contribution of slender *T. tenue* was
284 higher in terms of total trichome number than total volume (40-49 % vs 33 %). Its percentage
285 contribution (trichome numbers) was minimum at ST2 in Feb 03 (23 %) and maximum (>70 %)
286 in Dec 01 at ST2 and ST3. *T. thiebautii* reached >40 % of the total trichome number in 65 % of
287 the samples with minimal contributions in Dec 01 and May 02 at ST2 (<14 %) and a fairly stable
288 one at ST1 and ST3 (37-47 %).

289 In terms of volumes, *T. thiebautii* contributions (reaches 50 % at ST2) were more variable,
290 linked to a trichome length changes along the annual cycle. The third main species, *T.*

291 *erythraeum*, represented less than 18 % of total trichomes on average, with the minimum
292 contribution at ST3 (<7 %) and maximum at ST1 and ST2 in Feb 03 during the peak abundance
293 (Tables 4 to 6). Its relative importance increased as expressed in volume, especially in Feb 03 at
294 the two open ocean stations (22-28 %). *T. contortum* and *T. hildebrandtii* (given as "others" in
295 Fig. 8), contributed <3 % to total trichome number or volume. The unidentified trichomes
296 observed in May 02 further north than ST2 represented less than 1 % of the integrated biomass.
297 The two *Katagnymene* species (*K. spiralis* and *K. pelagica*) were not numerous, but their
298 contribution in terms of volume was significant. Each form accounted for less than 4 % of the
299 total number of trichomes, except *K. spiralis* at ST1 in Oct 01 (10 %). *K. spiralis* was observed
300 in 50 % of samples. Its highest contributions to the total volume of trichomes occurred at ST1 in
301 Oct 01 (28 %) and Feb 03 at ST2 (12 %). The contribution of *K. pelagica* was around 20 % of
302 the total volume in Feb 03 at ST1 and ST2, 15 % at ST2 in Oct 01, and was not observed at
303 ST3. The relative abundance of species could change along depth. For example, during cruises
304 D4 and D5 (Apr-May 02), *T. thiebautii* and *T. tenue* were numerous in the upper layer (60 %)
305 and disappeared at 50 meters while *T. erythraeum* peaked at this depth. Obviously, the relative
306 abundance depends on the biomass criteria considered. For example, at ST2 in Apr 02, *T.*
307 *erythraeum* contributions at 30 to 50 m decreased in terms of trichome number (6 %) but
308 increased (41 %) in terms of total volume, because trichome size increased. The opposite trend
309 was observed for *T. thiebautii*.
310
311 ***Richelia intracellularis/Calothrix rhizosolenia* (*RC*) *from DDAs and unidentified***
312 ***cyanobacteria abundance:*** *RC* (Tables 4 to 6) were observed in 75 % of samples, but their
313 abundance was never very high. The highest *RC* abundances were recorded in Feb 03 at both
314 ST1 and ST2 (64 trichomes $l^{-1}$ and up to 735 trichomes $l^{-1}$, respectively), accounting for less than
315 1 % of total *Trichodesmium* trichome surface area and volume. Conversely, at ST3, peak
316 abundance was observed for *R. intracellularis* in Oct 01 (up to 117 trichomes $l^{-1}$ at the surface)
317 equivalent to 4.3 % of *Trichodesmium* trichome volume. In our samples, *RC* in DDAs
318 experienced a considerable variation in filament length (10-88 μm) and cell diameter (vegetative
319 cells 2-7 μm; heterocyst 3-10 μm) or trichomes per host cell (1-22). We also noted that in most
320 cases, the host cell was almost empty. Apart from *Richelia*, other symbiotic coccoid-form
321 cyanobacteria (not necessarily diazotrophs) were also observed in *Climacodium* sp. and
322 *Ornithocercus* sp., though only in the New Caledonian lagoon.

Colonies of unidentified cyanobacteria composed of round-shaped cells observed in some samples reached less than 1 % of *Trichodesmium* biomass on average (Tables 4 to 6, Fig 5C, D). The highest concentrations were observed at ST2 during Jan 02 and May 02 with values exceeding 4000 cells $l^{-1}$, which was equivalent to less than 1.6 % of *Trichodesmium* trichome volume. At ST1, these organisms were rarely observed and only at very low concentrations, i.e. <20 cells $l^{-1}$. At ST3 they were observed only in Jan 02 with a maximum value at 10 m of 429 cells $l^{-1}$. Tracking *Crocosphaera* (2-10 µm cell size) by flow cytometric and phycoerythrin signatures was unsuccessful during Diapalis although it has been reported in the region (Saito et al. 2010 and references therein). However, Diapalis samples showed relatively high $N_2$ fixation in the <10 µm fraction, particularly in Oct 03 (Garcia et al. 2007), but it was not possible to link this fixation to specific organisms or biomass indicators.

## Abundance of other phytoplankton groups

***Picoplankton abundance:*** *Prochlorococcus* concentrations at ST1 and ST2 (Tables 4-5 and Fig. 9A) was maximum (10-13 $10^{12}$ cells $m^{-2}$) during the warm season (Dec 01-Apr 02 and in Feb 03). During transition periods, concentrations were lower by a factor of 3 with 3.5-3.9 $10^{12}$ cells $m^{-2}$ at ST2 (Oct 01 and Oct 03, respectively), while at ST1 the minimum concentration (4.5 $10^{12}$ cells $m^{-2}$) was observed during winter. At these two 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 ST3, *Prochlorococcus* abundance was much lower than at the oceanic stations, with values <2.3 $10^{12}$ cells $m^{-2}$ through 0-30 m (max 70 $10^3$ cells $ml^{-1}$), except in Dec 02 when they reached 4.8 $10^{12}$ cells $m^{-2}$ (max 225 $10^6$ cells $ml^{-1}$ at depth).

Unlike *Prochlorococcus*, integrated abundance of *Synechococcus* (Tables 4 to 6, Fig. 9B), was on average 5 to 14 times higher at the lagoon station (ST3) than offshore (ST1 and ST2), except during the austral winter (Aug 02) when they were 24 % higher in oceanic waters at ST2 (1.91 $10^{12}$ 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 Oct 03 cruise when it peaked at the same depth (between 75 and 110 m).

355 Picoeukaryotes were the least abundant picoplankton organisms (Tables 4 to 6, Fig. 9C).
356 Integrated abundances were more variable at ST2 (factor 6; 0.02 to 0.12 $10^{12}$ cells $m^{-2}$) than at
357 ST1 (0.07 to 0.14 $10^{12}$ cells $m^{-2}$). At ST3, integrated abundances changed from 0.08 to 0.17 $10^{12}$
358 cells $m^{-2}$. Picoeukaryote maxima were consistently found below the thermocline and, therefore,
359 deeper than the *Synechococcus* maxima, even in Aug 02.
360
361 ***Large eukaryotes:*** Large eukaryotes (>10 µm) described by microscopy and made up of
362 dinoflagellates and diatoms (Fig. 10) were generally found together with *Trichodesmium* in open
363 ocean waters at ST1 and ST2 and, to a lesser extent, at ST3 coastal site. Dinoflagellates showed
364 a maximum of 60 $10^6$ cells $m^{-2}$ during the maximum *Trichodesmium* abundance in Feb 03 at ST1
365 and ST2 and low abundance (<30 $10^6$ cells $m^{-2}$) at other periods. Diatoms also showed a maximum
366 concentration (412 $10^6$ cells $m^{-2}$) in Feb 03 at ST2 and, to a lesser extent, at ST1. At ST3 coastal
367 site, no data were available in Feb 03, but there was a considerable maximum for both groups (17
368 $10^6$ cells $m^{-2}$ for dinoflagellates; 240 $10^6$ cells $m^{-2}$ for diatoms) in winter (Aug 02). The main
369 diatoms belonged to 1) the *Navicula, Nitzschia* and *Pseudo-nitzschia* genera at ST1, 2) the
370 *Chaetoceros*, *Navicula* and *Pseudo-Nitszchia* genera at ST2, 3) the *Chaetoceros*, *Navicula* and
371 *Rhizosolenia* genera at ST3. *Rhizosolenia* was the main *RC* host. Among dinoflagellates,
372 protoperidinians were represented at all stations, but few larger cells were found as *Ceratium* and
373 *Amphisiolena* species at ST3 in Oct 01.

374 **Chlorophyll and phycoerythrin distribution**

375 Pigment content (Chla, PE) per cell or per trichome (Table 8) was calculated for Diapalis
376 samples during the Feb 03 cruise at ST1 and ST2, and for a pure sample of *T. erythraeum*. Mean
377 Chla concentration per trichome was 99 pg $cell^{-1}$ for pure *T. erythraeum* while the relationship
378 between trichome numbers and Chla in the >10 µm fraction suggested an upper limit for the mean
379 Chla concentration per trichome of 43 pg for the mixed community of *Trichodesmium* (Table 8).
380 Considering the diatoms and dinoflagellates in the >10 µm fraction revealed by microscopy and
381 by the presence of a significant quantity of Chlc, the Chla per trichome would be approximately
382 10 % lower (assuming a constant Chlc/Chla ratio of 0.2 for diatoms and dinoflagellates) than this
383 upper limit. Similarly, the PE per trichome (Table 8) was 251pg $trichome^{-1}$ for pure *T. erythraeum*
384 and 197 pg $trichome^{-1}$ for mixed *Trichodesmium* (Table 8).
385 Regarding phycoerythrin (PE), its fluorescence excitation spectra were typical of PE-

386 cyanobacteria according to Neveux et al. (2006) with either PE-*Synechococcus* or PE-
387 *Trichodesmium* dominating or a clear mixture of both PEs. At ST1 and ST2 stations, PE-
388 *Trichodesmium* dominated the community in Dec 02 and Feb 03. Integrated Total Chla change
389 showed much less variation across cruises than trichome abundance (Fig. 11). The integrated
390 proportion of Dv Chla to TChla was generally around 20 % in lagoon waters (ST3), 40 %-50 %
391 at ST1 and over 50 % in the Loyalty Channel (ST2). The highest integrated values of PE at ST1
392 and ST2 in Feb 03 (69 $mg.m^{-2}$, Fig. 11) was linked to the high abundance of *Trichodesmium* at
393 the surface while the high value in Oct 03 at ST1 was related to a deep maximum of
394 *Synechococcus*. In Feb 03, PE maxima measured in the upper layer were 4 to >10 times higher
395 than during the other cruises, ranging from 0.61 $\mu g\ l^{-1}$ at ST1 to 1.27 $\mu g\ l^{-1}$ at ST2. In the >10 µm
396 fraction, the PE in the upper layer (data not shown) was exclusively related to filamentous
397 cyanobacteria, mainly *Trichodesmium*. At ST3 station (Fig. 11), PE was essentially related to
398 *Synechococcus* and concentrations varied between 0.10 (Apr 02) and 0.62 $\mu g\ l^{-1}$ (Aug 02) at the
399 surface with fairly even vertical distribution. The integrated concentrations varied from 4.30 (Apr
400 02) to 29.26 mg $m^{-2}$ (Aug 02). The integrated $N_2$ fixation rate for all species (Garcia et al. 2007)
401 roughly followed the integrated PE pattern (Fig. 11) and are correlated with the integrated
402 trichome concentrations ($r^2$=0.73, N=11).
403
404 **C biomass distribution in the microbial community**
405
406 The relative contribution of *Trichodesmium* + *Katagnymene* (TK) to C biomass of total
407 picoplankton and filamentous cyanobacteria (Fig. 12) was generally high at ST2 with maxima in
408 summer (80 % in Feb 03) and minima in winter (6 % in Aug 02), where TK was replaced by
409 *Synechococcus* as the major contributor and in Oct 03 with only a few filaments observed.
410 Comparatively, the contribution of TK at ST1 was lower, with a maximum of ~ 50 % in Feb 03.
411 At ST3, *Synechococcus* was the main contributor to C biomass and the contribution of TK was
412 generally low. At all stations, the contribution of *RC* to C biomass was negligible over the whole
413 sampling period. Picoeukaryotes may account for a significant portion of the biomass in the
414 lagoon near Ouinne and in winter at all stations.
415 **Correlations between groups and environmental variables**
416

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 9). 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 the 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 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* (0.1 against 1 trichome. $l^{-1}$) by a factor of 10 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 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 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). So, 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 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 8), TK accounted for up to 80 % of C cyanobacteria 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 south western 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 meters (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, however, lower than measured in a surface accumulation in the Southwest Lagoon, namely up to 30 $10^6$ trichomes l$^{-1}$ for a Chla 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 Solomons Islands, expressed as trichome number (7700 trichomes $l^{-1}$; Giraud 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 strategy and sample processing method were used. During the Hawaiian (HOT) and Bermuda Atlantic Time-Series (BATS), *Trichodesmium* abundance was studied at approximately monthly intervals (HOT= October 1989-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. So, a conversion factor of 200 trichomes per colony used in 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 (disintegrated during the counting) and only trichome abundance is 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 Feb 03 in the Loyalty Channel (240 $10^6$ trichome $m^{-2}$) were 70 times and 400 times higher than at HOT (3.5 $10^6$ trichome $m^{-2}$) and BATS (0.64 $10^6$ trichome $m^{-2}$), 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 two 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 inter-annual variability, even if free trichome variations at BATS seemed identical from one year to another. At BATS, however, interannual variations deduced from only 7 integrated depth profiles for 3 years fitted by a third-degree polynomial function of Julian days (Fig 1A in Orcutt et al. 2001), are questionable.

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

512 The SWTP around New Caledonia is a typical warm low-nutrient low-chlorophyll
513 (LNLC) region (Ceccarelli et al. 2013), with phosphate recharged annually (presumably other
514 nutrients as well) in the euphotic zone during the transition periods (Moutin et al. 2005). The
515 phosphate can limit the growth of diazotrophs species and consequently controls nitrogen
516 fixation rates in the ocean (Karl et al. 2002). In LNLC, diazotroph species such as
517 *Trichodesmium* are better adapted as they are able to use atmospheric $N_2$ (Bergman et al. 2013).
518 Diazotrophs are likely responsible for the lower phosphate concentrations in summer in the
519 region due to their $PO_4$ uptake (Moutin et al. 2005, Shiozaki et al. 2014), which corroborates the
520 negative correlation between T*richodesmium* and phosphate found in this study. During
521 Diapalis cruises the ammonium concentrations at ocean stations were only high in February
522 2003 coinciding with the highest concentrations of trichomes, PE and cyanobacteria carbon. The
523 ammonium concentrations also were positively correlated to *Trichodesmium* densities.
524 Therefore, the relatively high ammonium concentrations may be attributed partially to direct or
525 indirect release of this nutrient by the diazotrophs (Mulholland 2007; Bergman et al. 2013). We
526 did not find any correlation between *Trichodesmium* abundance and MLD. As stated by Hood et
527 al. (2004), increased *Trichodesmium* concentrations are linked to a shallow MLD (resulting in
528 high mean light levels) and low dissolved inorganic nitrogen (DIN) concentrations for extended
529 periods of time. The relationship between nitrogen fixation rates in plankton net samples and
530 MLD depth was significantly fitted by a hyperbolic function in the Atlantic Ocean ($r^2 = 0.31$, $p < 0.05$, n= 18, Agawin et al. 2013).
532 *T. thiebautii* and *T. tenue* were the predominant species in the oceanic waters around New
533 Caledonia and in the open lagoon of the east coast, as previously stated in Rodier & Leborgne
534 (2010). By contrast and according to these authors, *T. erythraeum* dominate in the SW lagoon of
535 New Caledonia, which is a semi-closed and larger lagoon than the SE lagoon. Generally, *T.*
536 *thiebautii* and *T. tenue* are poorly represented in coastal regions (Relevante & Gilmartin 1982).
537 They dominate in the oligotrophic gyre of the Northern Pacific, Western Pacific (Marumo &
538 Nagasawa 1976) and eastern part of the China Sea (Marumo & Asaoka 1974) and are likely better
539 adapted to low-nutrient conditions than *T. erythraeum*. In our study, *T. tenue* was inversely
540 correlated with *T. thiebautii,* which suggests competition between the two species for an
541 apparently similar environment. According to Romans et al. (1994), *T. tenue* is a common species
542 often confused with *T. thiebautii* in optical microscopy, which can explain the lack of information
543 on the species' distribution and quantification. The predominance of *T. tenue* observed on

544 occasion during the Diapalis cruises is a relatively original finding. In this study around New
545 Caledonia, *T. contortum* and *T. hildebrandtii* occur in very low densities as in most oceans
546 (Bergman et al. 2013).

547 Information on *Katagnymene* species in the field is very scarce. They are mentioned in the
548 Southwestern Pacific (Lundgren et al. 2001, Dupouy et al. 2011, Giraud et al. 2016). Both
549 *Katagnymene* species, *K. pelagica and K. spiralis,* should now be considered as two
550 morphological forms of the same species (Lundgren et al. 2005). In our study, both forms are
551 frequently observed, but always in low abundance <284 trichomes $l^{-1}$. The strongest
552 concentrations were observed in the first 40 meters, although a deeper maximum (60 m) was
553 found in the Loyalty Channel in Feb 03. At this maximum, the K. s*piralis* form accounts for 81
554 % of the *Katagnymene* genus. Along the Revelle cruise track between New Caledonia and the Fiji
555 Islands (April 1998), Lundgren et al. (2001) found both *Katagnymene* forms at 34 of the 51
556 stations and counted up to 400 trichomes $l^{-1}$ of *Katagnymene* in the 0-25 m layer, with 89 % *K.*
557 *pelagica.* It should be noted that *Katagnymene* trichomes are generally long (1 cm) and extremely
558 fragile and can be broken up by handling before counting; hence the recommended use here of
559 surface and total volume for biomass estimates. The significant relationship of *K. spiralis* with all
560 nutrients, while *K. pelagica* was correlated only with phosphate, may indicate a different
561 ecological niche for these two species.

562 Like *Trichodesmium* and *Katagnymene*, the heterocystous *Richelia intracellularis* is
563 widespread in warm tropical and subtropical waters (Luo et al. 2012, Bonnet et al. 2015), whereas
564 *Calothrix rhizosolenia*, as epibionts of *Chaetoceros*, have been observed in the Indian and
565 Western Pacific Oceans (Sarma 2012, Giraud et al. 2016). Around New Caledonia in this study,
566 both *Richelia* and *Calothrix* species (*RC* groups) were sometimes present as free-living
567 organisms, but more frequently in endosymbiotic association with diatoms (mainly in
568 *Rhizosolenia* sp.). *RC* were present in all seasons with a maximum (>700 trichomes $l^{-1}$) in the
569 Loyalty Channel in summer (Feb 03), as was *Trichodesmium*. Based only on samples where *RC*
570 was detected, its biomass correlated with the number of diatoms in the >10 µm fraction (Spearman
571 rank correlation between 0.52 and 0.57, $p < 0.0001$; data not shown). In endosymbiosis, the host
572 cell provides a protective micro-environment and positive buoyancy to the symbiont, which
573 enables the cell to remain in the euphotic layer, as, unlike *Trichodesmium,* this species does not
574 have gas vacuoles (Janson et al. 1999). *Richelia* has a very uneven space distribution in the oceans,
575 like *Trichodesmium*, depending on whether the environment is conducive to their growth. Both

576 genera often inhabit the same types of ecosystems, as they have similar physiological properties,
577 like all diazotrophs (Bergman et al. 2013 and references therein). In the case of cyanobacteria-
578 diatom associations, environmental conditions must meet the needs of both organisms, and
579 particularly sufficient silicate concentration for the diatom host growth. Iron and silica were not
580 limiting factors in our area of study and are provided by leaching from terrestrial soils (Shiozaki
581 et al. 2014). In our study, *RC* was more correlated with *T. thiebautii* and *K. spiralis*, and its
582 positive dependence on the density structure of the water column (MLD) and ammonium needs
583 to be further explored. In the Northern Pacific Gyre, the greatest abundance in *Richelia*
584 *intracellularis* is also observed in summer, when the species can form blooms of about $10^4$
585 filaments per liter (Venrick 1974), values which are thus greater than ours (max 734 filaments $l^{-1}$
586 ). Around the Hawaiian Islands (Sohm et al. 2011), a high abundance of all three groups, i.e.
587 *Trichodesmium*, *Katagnymene* and *Richelia,* was reported in late boreal summer 2002 (Sep-Oct)
588 and summer 2003 (July-Aug), with *Richelia*-diatom associations more abundant to the south of
589 the islands. The high variability in *Richelia* size in our samples is another reason to underline the
590 inaccuracy of biomass measurements in terms of filament numbers rather than total filament
591 surface area or volume. The contribution of *RC* in terms of volume (or carbon) was minor (<1 %)
592 but must not mask the great impact of this species on carbon sequestration by DDA's as shown
593 in the North Tropical Pacific (Dore et al. 2008, Karl 2014; Karl & Church 2014).
594 In our study, large diatoms did not show significant correlations with any of the other groups
595 enumerated nor with any of the environmental variables. Dinoflagellates correlated positively
596 with total trichome abundance. Their positive correlation with the percentage of *T. erythraeum*
597 and their negative correlation with the percentage of *K. pelagica*, suggested affiliation to more
598 coastal than oceanic environments. The diversity of trophic status in dinoflagellates
599 (photosynthetic, mixotroph, heterotroph) was, nevertheless, not considered in the analysis. In the
600 Northern Tropical Pacific, the dinoflagellate group revealed no specific ecological pattern nor any
601 characteristic assemblages (Giraud et al. 2016). During the Diapalis cruises, the picoplanktonic
602 groups did not show any correlation with environmental parameters (Table 9), which was
603 surprising, as *Synechococcus* and *Prochlorococcu*s have distinct ecophysiology, *Synechococcus*
604 being better adapted to mesotrophic conditions and *Prochlorococcus* to oligotrophic conditions
605 (Partensky et al. 1996). This could be explained for *Prochlorococcus* by the fact that their
606 abundance varied little over the annual cycles. *Prochlorococcus* never dominated the
607 phytoplankton C biomass, but were often the second contributor to C biomass after filamentous

608 cyanobacteria in oceanic waters (Fig. 12). All phytoplankton share a requirement for light and
609 nutrients. A more specific mechanism underlying the covariation between *Synechococcus* and the
610 picoeukaryotes, as opposed to *Prochlorococcus*, is that they have a larger cell size than the latter,
611 and are able to use nitrate. This could explain they tend to respond positively to enhanced nutrient
612 supply as observed mostly in the lagoon station and at Santal Bay. The covariation between
613 *Synechococcus* and the picoeukaryotes has been described before in the Atlantic subtropical gyres
614 (Marañón et al. 2003) and in the Pacific Ocean (Worden et al. 2004). The significant positive
615 correlation between *Synechococcus* and picoeukaryotes may also occur due to similarities in
616 favorable light conditions (Gutiérrez-Rodríguez et al. 2016). *Synechococcus* and picoeukaryotes
617 dominated the C biomass in the lagoon, but may also be large contributors in oceanic waters, as
618 observed in winter (Fig. 12).
619
620 **2001-2003 Inter-annual variability**
621
622 The difference in large diazotroph abundance (and nitrogen fixation rates, Garcia et al.
623 2007; Fig. 12C) observed between the summers of 2001-2002 and 2002-2003 could be the result
624 of a relatively low *in situ* sampling frequency. Satellite imagery nevertheless confirmed the very
625 low chlorophyll values during summer 2001-2002 compared to summer 2003 (Dupouy et al.
626 2011). Moreover, satellite-classified *Trichodesmium* pixels in the region 5°S-25°S, 160°E-170°W
627 determined using the TRICHOSAT model were clearly lower in summer 2001-2002 (La Niña
628 years) than summer 2003 (an El Niño year). The spectral fluorescence signature of surface blooms
629 as measured by the MERIS sensor (Gower et al. 2014) was also high during El Niño summers
630 (2003, 2007, 2010) but low during La Niña summers. The difference in cumulative precipitation
631 between 2001-2002 and 2003 summers is striking, with 2003 being drier despite the occurrence
632 of two tropical storms, namely Beni in January 2003 and Erica in March 2003 (Neveux et al.
633 2009). Law et al. (2011) reported that nitrogen fixation increased owing to an iron supply from a
634 wet-dust deposition 8 days after Tropical Cyclone Wati in the Tasman Sea. Australian dust
635 peaking in 2001 and 2002 was cited as a potential factor in increasing occurrence in the seas to
636 the east of Australia (estimated by satellite) in 2001-2005 (Mackie et al. 2008). The regional
637 distribution of aerosols was probably also influenced by the continuous passive (non-explosive)
638 volcanic degassing of the Melanesian Volcanic Arc, as indicated by sulfur dioxide ($SO_2$), the
639 composition of which has been analyzed using satellite data and modelling (Lefèvre et al. 2015).

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 as against falling Sverdrup transport and strengthening northwesterly winds during La Niña (Kessler & Cravatte 2013; Cravatte et al. 2015). 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 cyanobacteria 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 practically 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), which were consistently present in low abundance. Microscopic examination also revealed the presence of unknown filamentous species formed by round-shape cells not yet described by molecular tools. Picoplanktonic cyanobacteria (*Prochlorococcus* and *Synechococcus*) were consistently present in abundance with less

672 pronounced seasonal variations than filamentous cyanobacteria. *Trichodesmium* dominated in
673 terms of carbon content in the Loyalty Channel and Santal Bay in summer, while *Synechococcus*
674 dominated at the Ouinne station and in winter at all the three stations. The comparison of the 2002
675 and 2003 summers showed major inter-annual variability. Environmental factors that could
676 explain this variability were not clearly identified. One hypothesis could be the effects of
677 alternation between La Niña and El Niño with maximum diazotrophs and *Trichodesmium*
678 abundance during a well-established El Niño. Some hypotheses relating to the El Niño/La Niña
679 cycle need verifying, i.e. dust inputs and links with precipitations, winds, vertical movements and
680 horizontal circulation within the upper 200-meter layer, gyre formation in relation to wind
681 strength and direction and increased mesoscale circulation.
682
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693
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916 TABLES

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918 **Table 1:** Dates of the 9 Diapalis cruises
919

| Diapalis cruises | Dates | Austral 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 |

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922 **Table 2:** Morphometric characteristics of filamentous cyanobacteria, in the >10 µm size fraction,
923 encountered during the Diapalis cruises. NDO: not distinctly observed.
924

| Species | Cell diameter (µm) | Cell length (µm) | Cell shape |
|---|---|---|---|
| *T. erythraeum* | 8 to 13.6 | 4 to 7 | as wide as or up to twice as wide as long |
| *T. thiebautii* | 6 to 14 | 7 to 19 | as long as or up to twice as long as wide |
| *T. tenue* | 4 to 5 | 12 to 20 | 3 to 4 x longer than wide |
| *T. contortum* | 26 to 36 | 5 to 7 | 5 to 6 x wider than long |
| *T. hildebrandtii* | 18-20 | 5 to 6 | 2.7 to 3 x wider than long |
| Unidentified filaments | 2.7 to 3 | NDO | NDO |
| *K. spiralis* | 14 to 28 | 3 to 8 | 2.2 to 4.8 x wider than long |
| *K. pelagica* | 17 to 28 | 2.5 to 3.5 | 4 to 14 x wider than long |
| *R. intracellularis*/*C. rhizosoleniae* group | cell 2-7 Ø<br>heterocyst 3-10 Ø | - | - |
| Colonies of unidentified cyanobacteria (spherical cells) | type 1: 2.7-3 Ø<br>type 2: 6 Ø | - | - |

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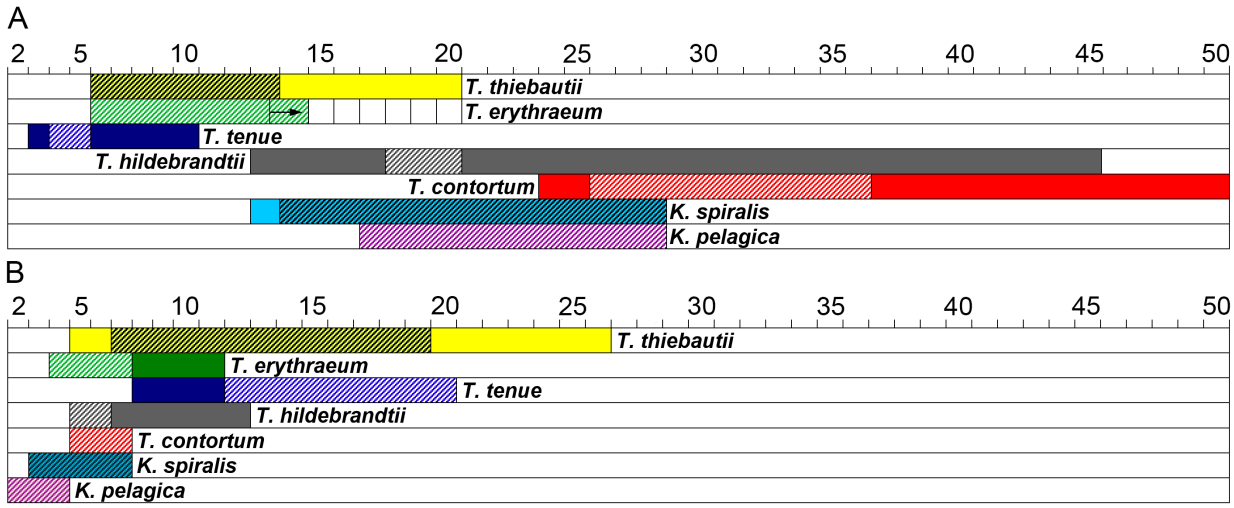
927 **Table 3:** A) Cell diameter (µm) and B) cell length (µm) of *Trichodesmium* and *Katagnymene*
928 species. The colored areas represent size ranges published in the literature and shaded areas part

929 of the size range observed during the Diapalis cruises in the New Caledonian Lagoon. A black
930 arrow indicates observation of a few *T. erythraeum* trichomes with a slightly wider diameter
931 than previously published.

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947 **Table 4.** Statistical data for the different groups enumerated by inverted microscopy in the
948 >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for
949 identified filamentous cyanobacteria and cell number for all other groups and the colonies
950 of unidentified cyanobacteria with spherical cells), at ST1, the Santal Bay station, during the
951 9 Diapalis cruises (2001-2003). Concentrations taken at 4-6 depths down to 60 m ($l^{-1}$) and
952 integrated values ($m^{-2}$) in the 0-60 m layer. Trichomes: sum of *Trichodesmium* and
953 *Katagnymene* trichomes.
954

| ST1 Diapalis 01-09 | min | max | average | stdev | 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$^3$ Trichome $m^{-2}$ | 0.16 | 1.15 | 0.52 | 0.37 | 7 |
| *R. intracellularis/C. 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 (cell $l^{-1}$) | 0 | 20 | 1 | 5 | 25 |
| Dinoflagellates $l^{-1}$ | 22 | 1927 | 322 | 405 | 31 |
| Diatoms $l^{-1}$ | 14 | 4516 | 640 | 1096 | 31 |
| *T. 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 |
| *K. 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 |

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966 **Table 5.** Statistical data for the different groups enumerated by inverted microscopy in the
967 >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for
968 identified filamentous cyanobacteria and cell number for all other groups and the colonies
969 of unidentified cyanobacteria with spherical cells), at the ST2, Loyalty Channel station,
970 during the 9 Diapalis cruises (2001-2003). Concentrations taken at 4-6 depths down to 60 m
971 ($l^{-1}$) and integrated values ($m^{-2}$) in the 0-60 m layer. Trichomes: sum of *Trichodesmium* and
972 *Katagnymene* trichomes
973

| ST2 Diapalis 01-09 | min | max | average | stdev | n |
|---|---|---|---|---|---|
| Trichomes $l^{-1}$ | 4 | 4578 | 676 | 1089 | 45 |
| $10^6$ Trichomes $m^{-2}$ | 0.46 | 235 | 44 | 66 | 11 |
| $10^6$ µm$^3$ Trichome $l^{-1}$ | 0.03 | 62.22 | 9.53 | 15.29 | 36 |
| $10^9$ µm$^3$ Trichome $m^{-2}$ | 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 (cell $l^{-1}$) | 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 |

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985 **Table 6**. Statistical data for the different groups enumerated by inverted microscopy in the
986 >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for
987 identified filamentous cyanobacteria and cell number for all other groups and the colonies
988 of unidentified cyanobacteria with spherical cells), at ST3, the Ouinne station, Eastern
989 Lagoon, during the 9 Diapalis cruises (2001-2003). Concentrations taken at 4-6 depths down
990 to 30 m ($l^{-1}$) and integrated values ($m^{-2}$) in the 0-30 m layer. Trichomes: sum of
991 *Trichodesmium* and *Katagnymene* trichomes.
992

| ST3 Diapalis 01-09 | min | max | average | stdev | 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$^3$ Trichome $m^{-2}$ | 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 (cell $l^{-1}$) | 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 |

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995 **Table 7:** Cell number per trichome of each *Trichodesmium* and *Katagnymene* species during the
996 Diapalis cruises.
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| | *T. tenue* | *T. thiebautii* | *T. erythraeum* | *T. hildebrandtii* | *T. contortum* | *K. spiralis* | *K. pelagica* |
|---|---|---|---|---|---|---|---|
| min | 3 | 3 | 4 | 16 | 19 | 2 | 10 |
| max | 206 | 209 | 235 | 132 | 62 | 140 | 334 |
| Mean | 54 | 55 | 64 | 62 | 40 | 42 | 57 |
| STD | 36 | 38 | 45 | 40 | 22 | 34 | 77 |
| N | 584 | 469 | 322 | 9 | 3 | 49 | 18 |

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**Table 8:** Morphometric characteristics of *Trichodesmium and Katagnymene* species at the *Trichodesmium* peak abundance in February 03 (Diapalis 7). Their chlorophyll *a* (Chla), phycoerythrin (PE), carbon (C) content per trichome and volume of trichome. 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 | STD | Number |
|---|---|---|---|
| **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. hidelbrandtii* trichome diameter (µm) | 16.00 | 2.49 | 9 |
| *T. hidelbrandtii* 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 |

**Table 9**. Spearman's correlation coefficient for the relationships between integrated abundance (cell or trichomes $m^{-2}$ over 60 m for ST1 and ST2 and 30 m for ST3) of autotrophic groups, the percentage of the different species of filamentous cyanobacteria and environmental parameters (n = 28). Data from the Diapalis cruises (2001-2003). *p < 0.01, **p < 0.05, 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** | 0.63** | ns | ns | ns | ns | ns | 0.50** | ns | ns | -0.57** | 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* | ns | ns | ns | ns | ns | ns | ns | ns | 0.62* | ns | ns | 0.51** | -0.48** |
| *Rich* | | | | 1 | ns | ns | ns | ns | ns | 0.61* | 0.69* | ns | 0.60** | ns | ns | ns | 0.85* | ns |
| *Proc* | | | | | 1 | -0.42** | ns | ns | ns | -0.50** | ns | ns | ns | ns | ns | ns | ns | ns |
| *Syn* | | | | | | 1 | 0.52** | 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* | -0.59** | ns | ns | 0.49** | ns | ns | -0.57** | 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** | 0.55** | 0.59** |
| %*K. p* | | | | | | | | | | | | 1 | ns | ns | ns | ns | ns | 0.63** |
| MLD | | | | | | | | | | | | | 1 | ns | ns | -0.46** | ns | ns |
| T(°C) | | | | | | | | | | | | | | 1 | -0.63* | ns | ns | -0.55* |
| Sal | | | | | | | | | | | | | | | 1 | ns | ns | 0.52** |
| $NO_x$ | | | | | | | | | | | | | | | | 1 | ns | 0.48** |
| $NH_4$ | | | | | | | | | | | | | | | | | 1 | ns |
| $PO_4$ | | | | | | | | | | | | | | | | | | 1 |

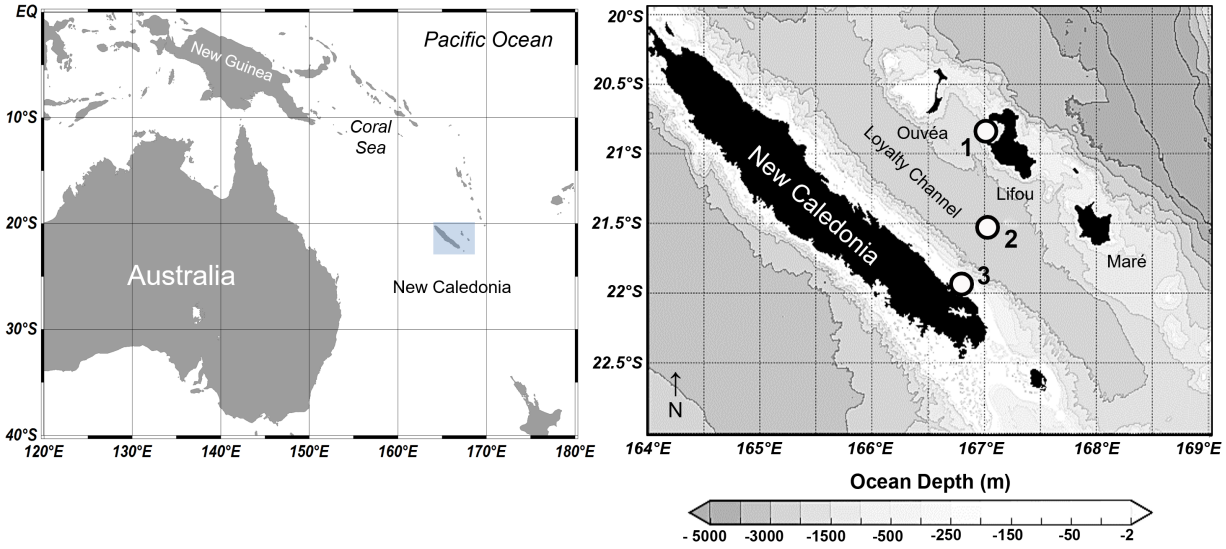


**Figure 1.** Location of the 3 main stations sampled during the nine Diapalis cruises in the New Caledonian waters: ST1 (Santal Bay) on Lifou Island (167°04'E 20°51'S, 1050 m depth), ST2 (Loyalty Channel, 167°E 21°30'S, 2100 m depth) and ST3 (Ouinne station) in the East Coast lagoon (166°45'E 21°57'S, 35 m depth). Figure Courtesy J. Lefèvre.

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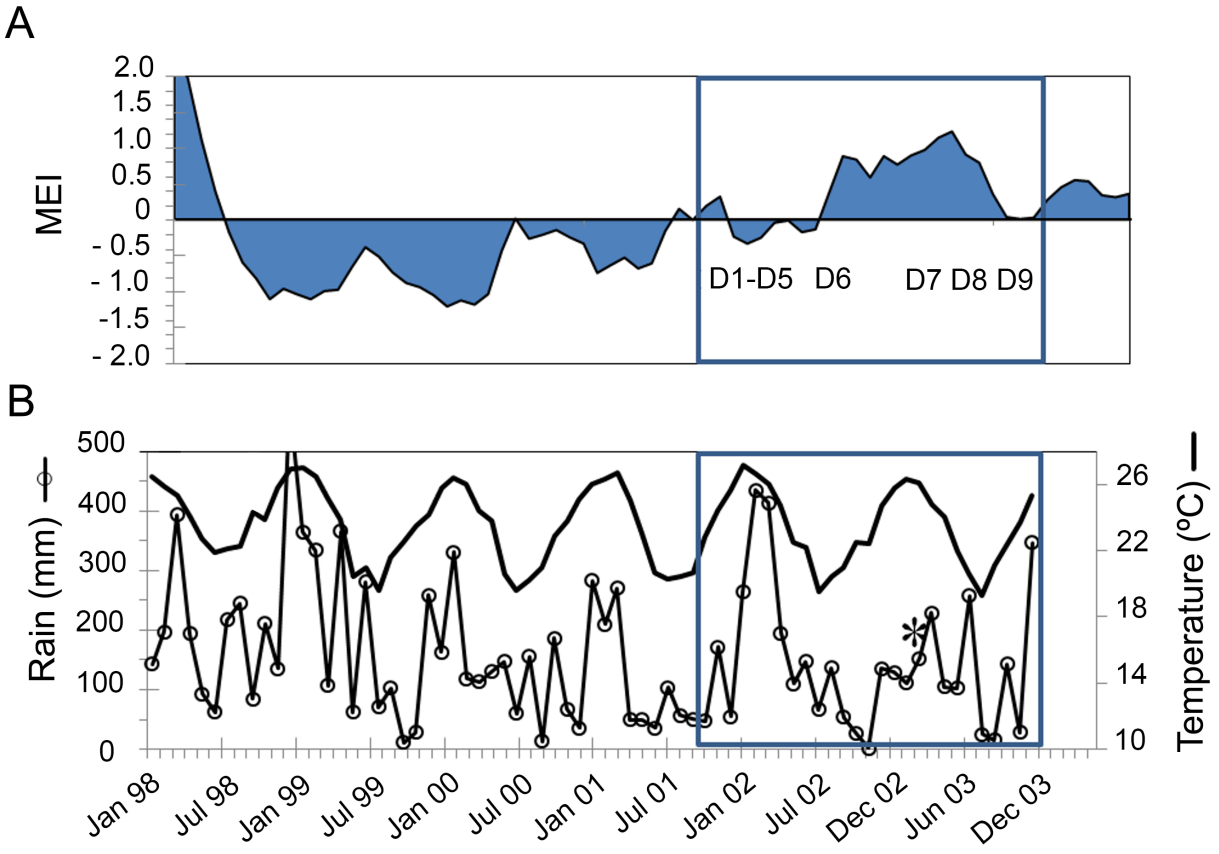


1030
1031
1032 **Figure 2**. (A) Multivariate El Nino Index, (B) air temperature and (T °C) and monthly
1033 cumulative precipitation (mm) for 1998-2003 on New Caledonia's east coast (Thio station). The
1034 framed area represents the Diapalis sampling period. Tropical Storm Beni (29 Jan - 5 Feb 2003)
1035 is indicated as (*). See Table 1 for the dates of the cruise.
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1037

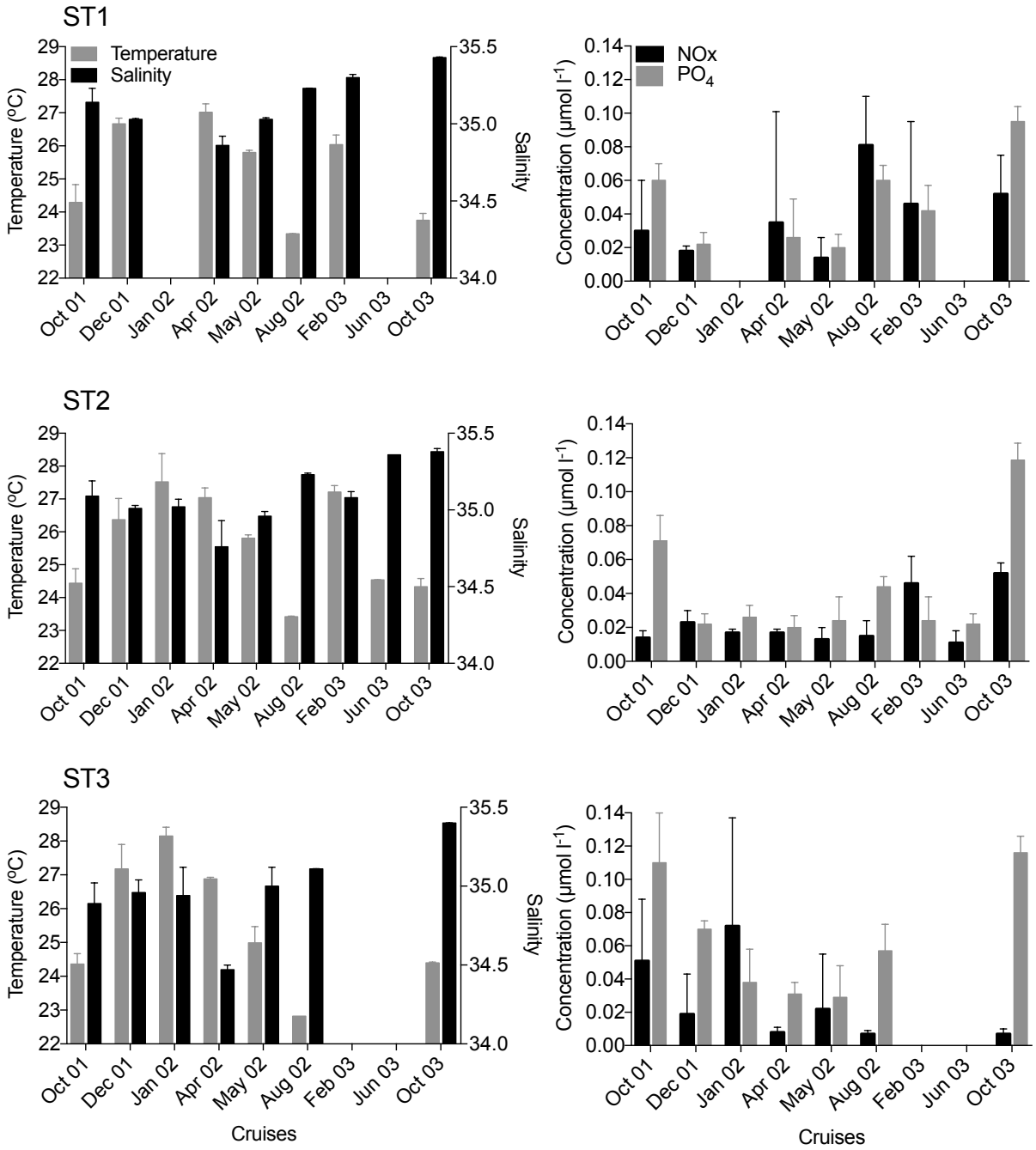


**Figure 3.** Mean temperature, salinity (left panel) and nutrients ($NO_x$, $PO_4^-$) (right panel) during the Diapalis cruises. Values are averaged from 0-60 m at ST1 and ST2 and 0-30 m at ST3. ST3 was not sampled in Feb 03 and Jun 03.

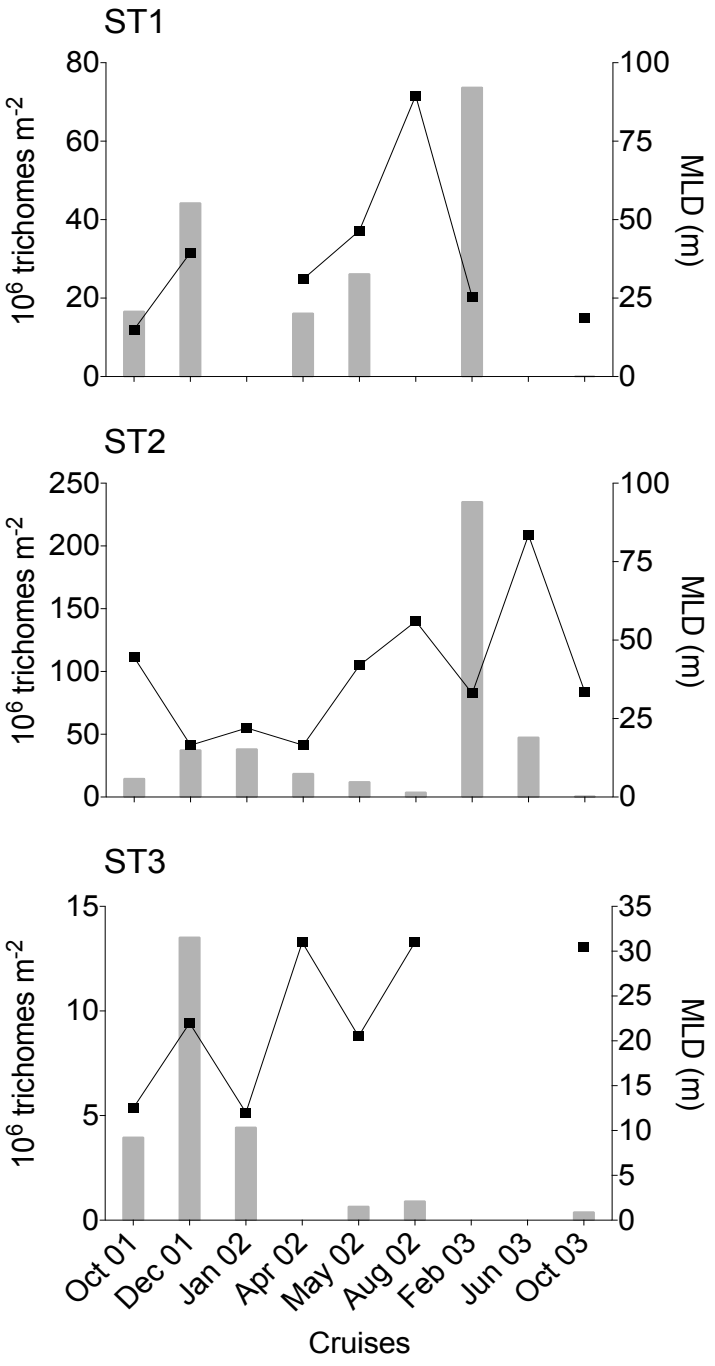


**Figure 4.** Integrated concentrations of the total filamentous cyanobacteria (trichomes $m^{-2}$) in the >10 µm fraction (*R. intracellularis* and *C. rhizosoleniae* not included) (bars) and the mixed layer depth (MLD) (lines) during the Diapalis cruises. Integration depths: 0-60 m at ST1 and ST2 and 30 m at ST3.

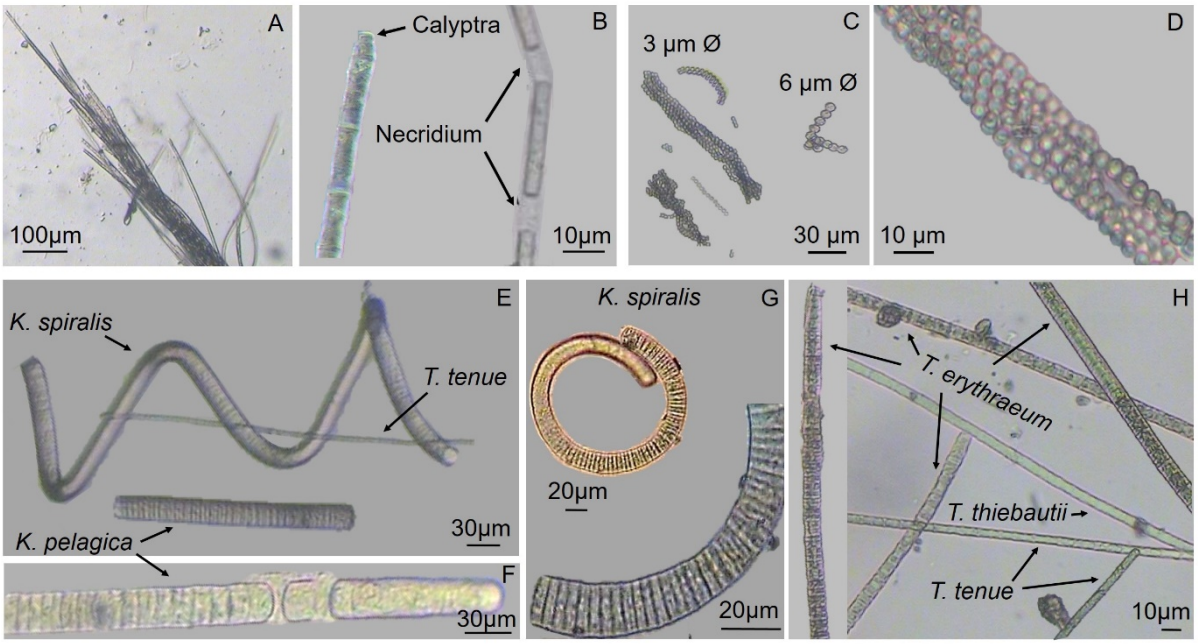


**Figure 5**. Photomicrographs of filamentous cyanobacteria obtained from the Diapalis cruises. A) *Trichodesmium tenue*, B) Details of *T. tenue* trichomes: note that cell length is about 3 times longer than wide; the calyptra (formation at the tip of the trichome) and the necridium (structure formed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

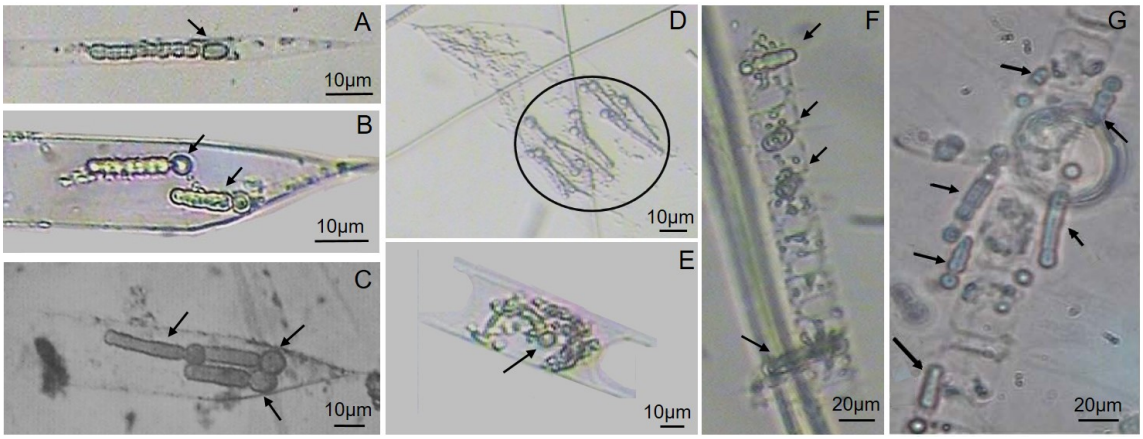


**Figure 6. A-D)** *Richelia intracellularis* symbiotic with *Rhizosolenia* spp. and **E)** *Hemiaulus* sp.; **F-G)** *Calothrix rhizosoleniae* epiphyte on *Chaetoceros* sp. Size and number of *Richelia* vary based on the size of the host. Photomicrographs obtained from the Diapalis cruises.

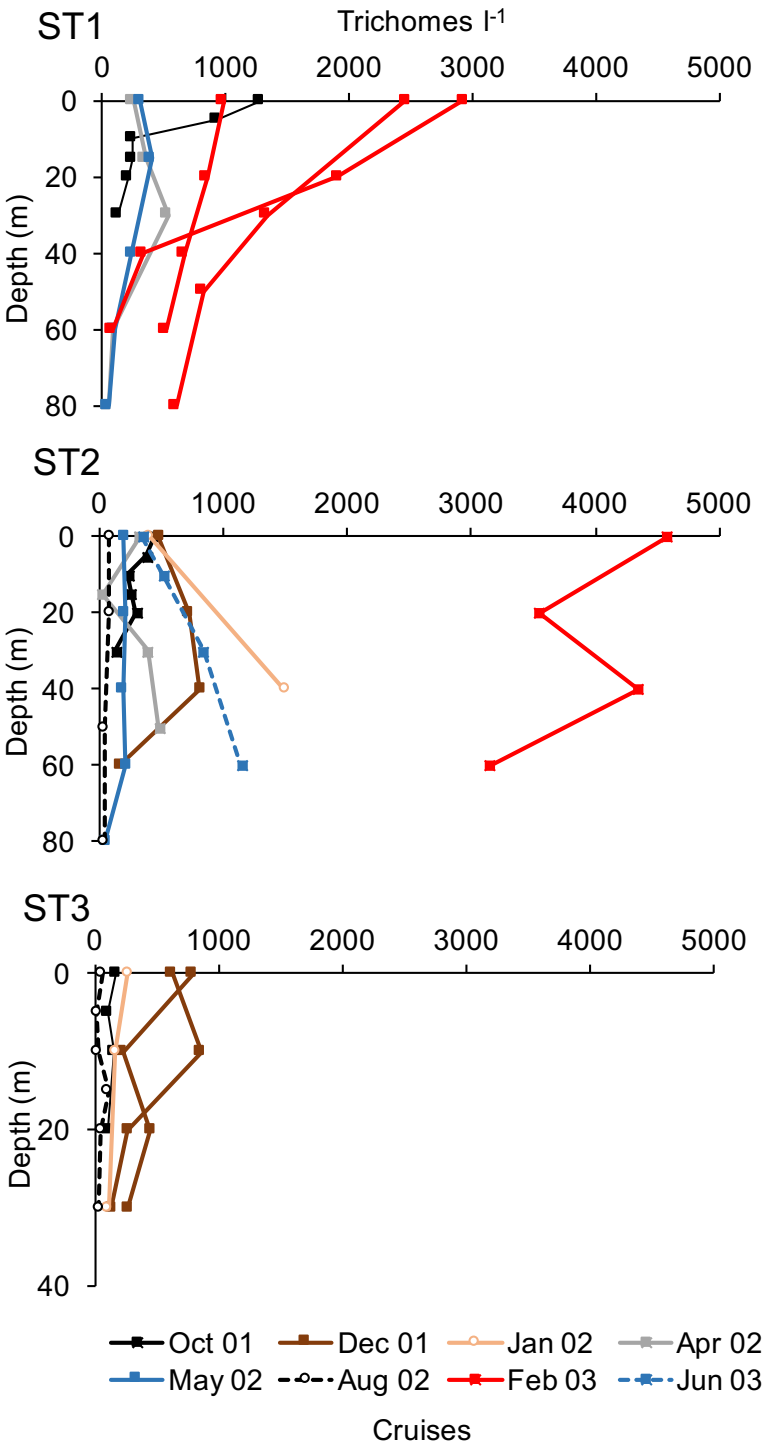


**Figure 7.** Vertical profiles for filamentous cyanobacteria abundance (Trichomes $l^{-1}$) (except *R. intracellularis* and *C. rhizosoleniae*) during the Diapalis cruise stations. ST2 (open ocean, Loyalty Channel) is well representative of the seasonal variations (some cruises are missing at ST1 and ST3). Note that at ST1 in February 2003, 3 profiles were performed with intervals of approximately 24h.

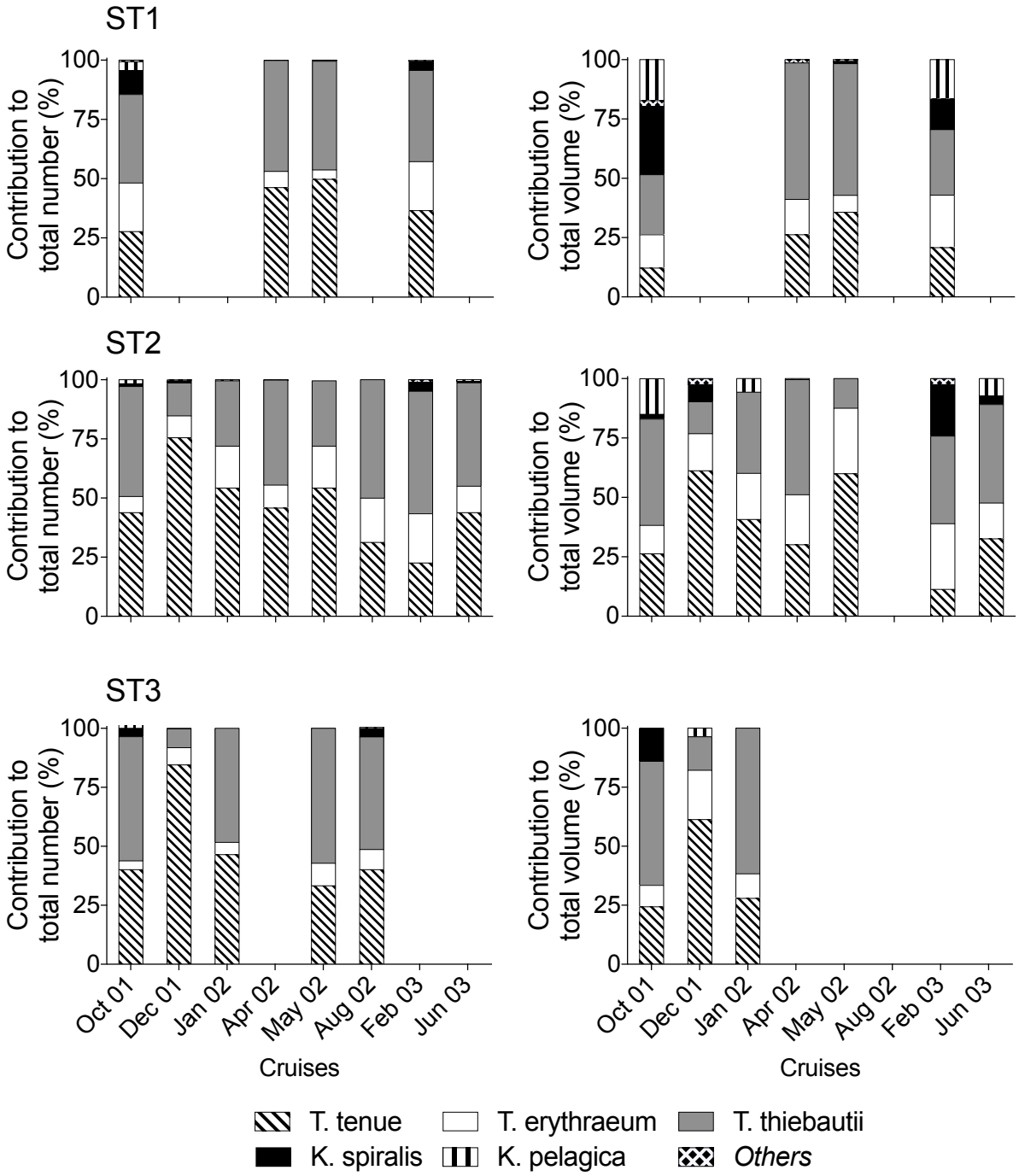


**Figure 8:** Contributions of the various species (%), in terms of trichome number (left panels) and trichome volume (right panel) to the total community of filamentous cyanobacteria (*R. intracellularis* and *C. rhizosoleniae* not included) at the three stations sampled during the Diapalis cruises. Integration depths: 0-60 m at ST1 and ST2; 0-30 m at ST3. At ST1 "Others" = *T. hildebrandtii* (October 2001, May 2002 and February 2003) or *T. contortum* (October 2001); At ST2 "Others" = *T. hildebrandtii.* At ST2, species contributions were determined at each cruise, except in August 2002 for the *Trichodesmium* volume contribution. Some cruises are missing at ST1 and ST3.

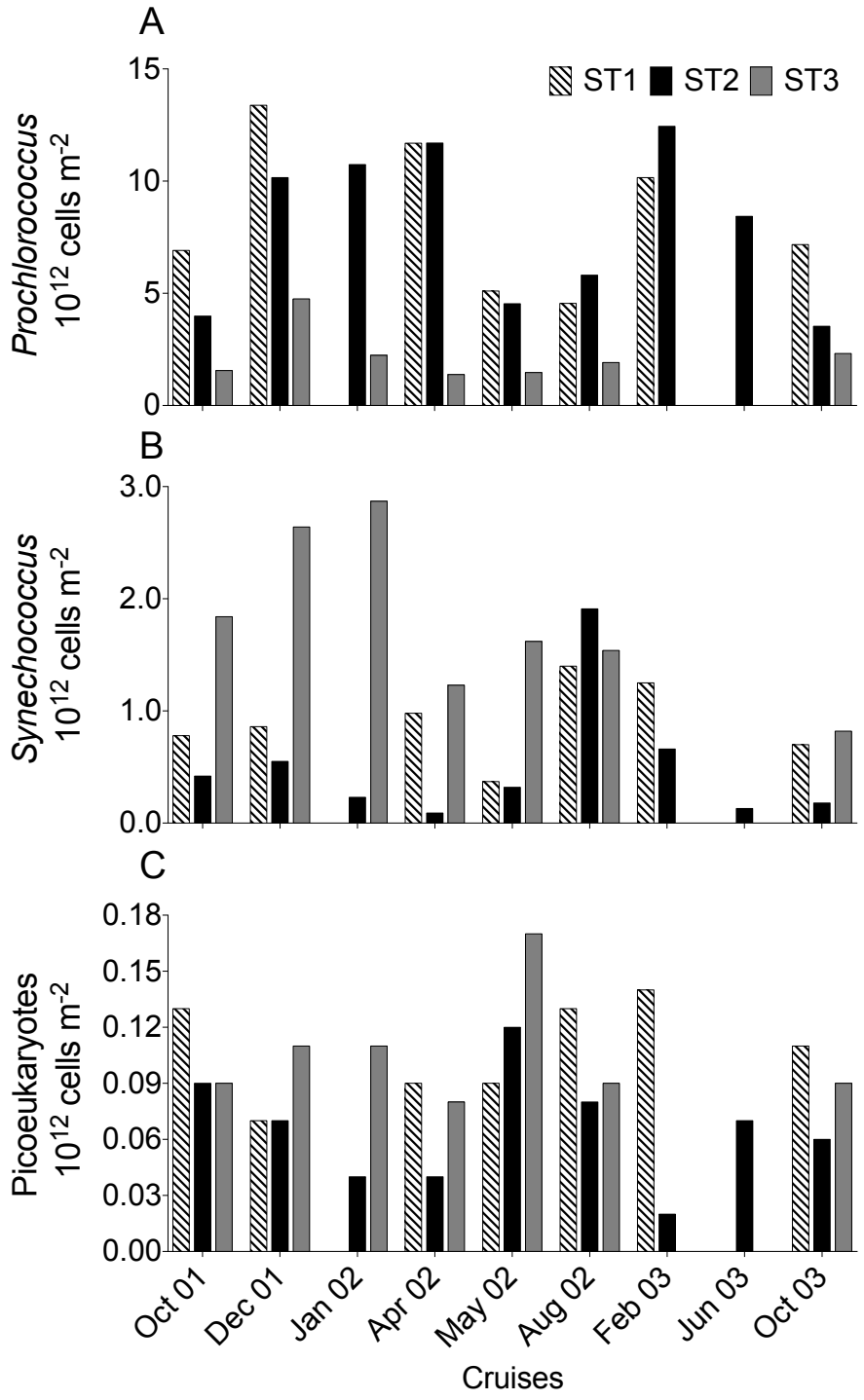


**Figure 9.** Integrated concentrations of (A) *Prochlorococcus,* (B) *Synechococcus*, and (C) picoeukaryotes at the three stations sampled during the Diapalis cruises. Integration depths: 0-60 m at ST1, ST2; 0-30 m at ST3.

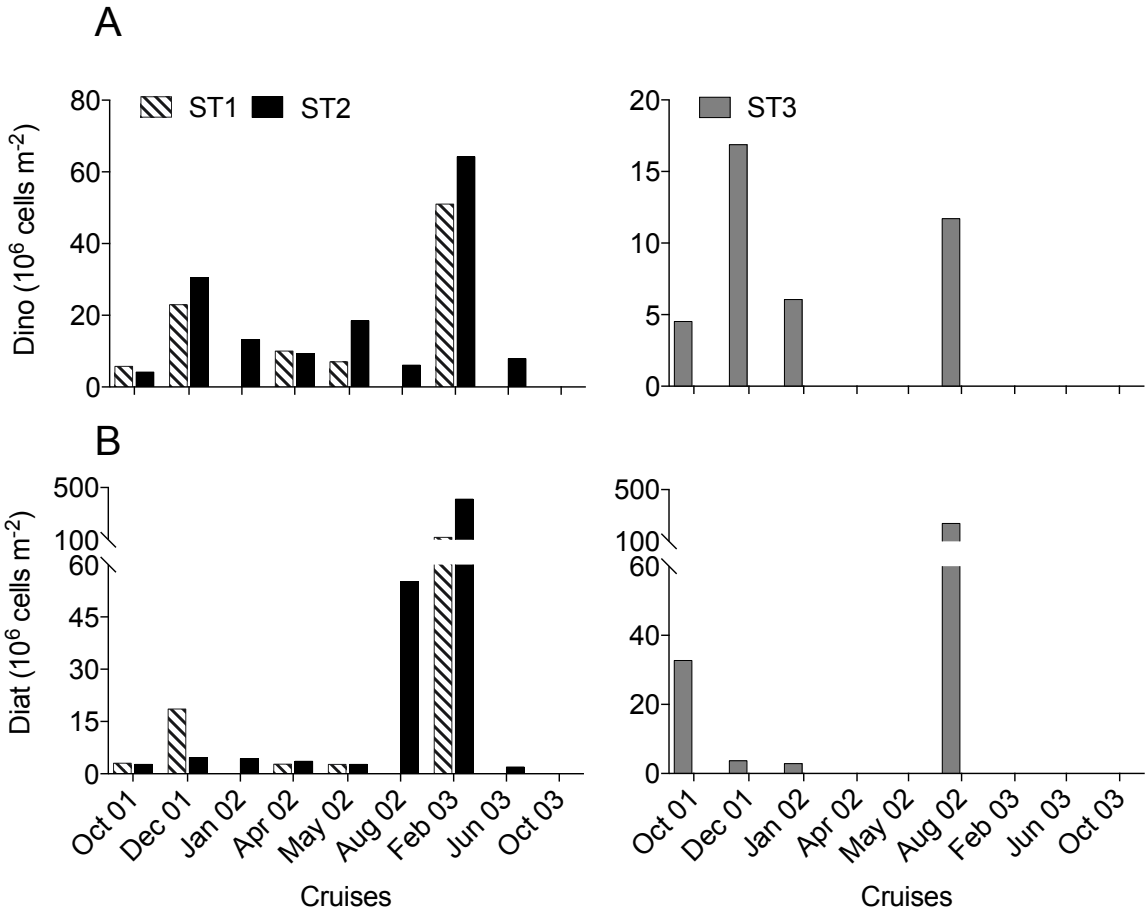


**Figure 10.** Integrated concentrations of (A) dinoflagellates (Dino) and (B) diatoms (Diat) in the >10 μm fraction. Enumeration was carried out by inverted microscopy at the three stations sampled during Diapalis cruises. Integration depths: 0-60 m at ST1, ST2; 0-30 m at ST3. At ST2, integrated concentrations were determined at each cruise, except in August 2002. October 2003 abundance was too low for enumeration. Some cruises are missing at ST1 and ST3.

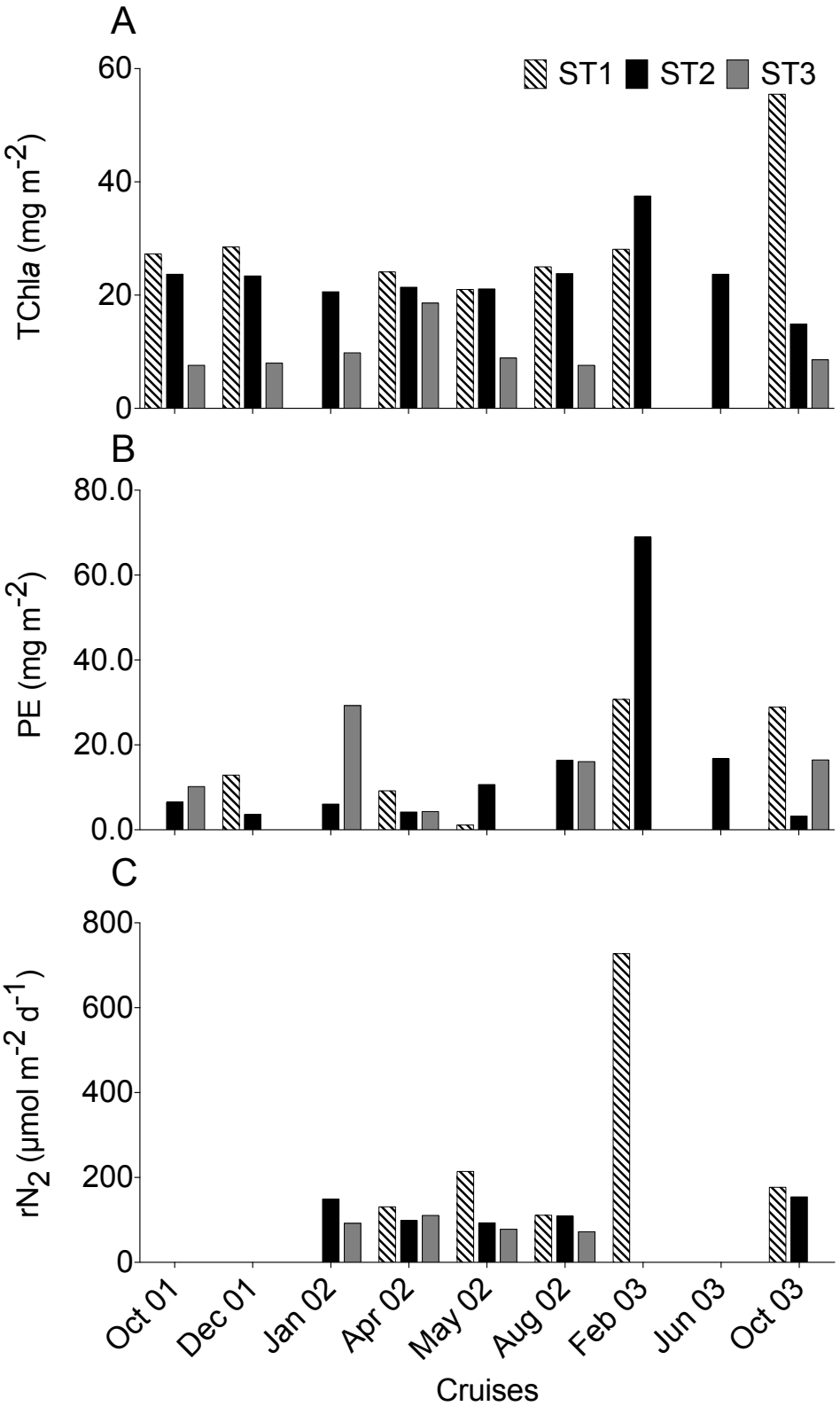


**Figure 11.** Integrated Tchla (A) and phycoerythrin (B) concentrations and (C) $N_2$ fixation rates (Garcia et al. 2007) at the three stations sampled during the Diapalis cruises: Integration depths: 0-60 m at ST1 and ST2, and 0-30 m at ST3.

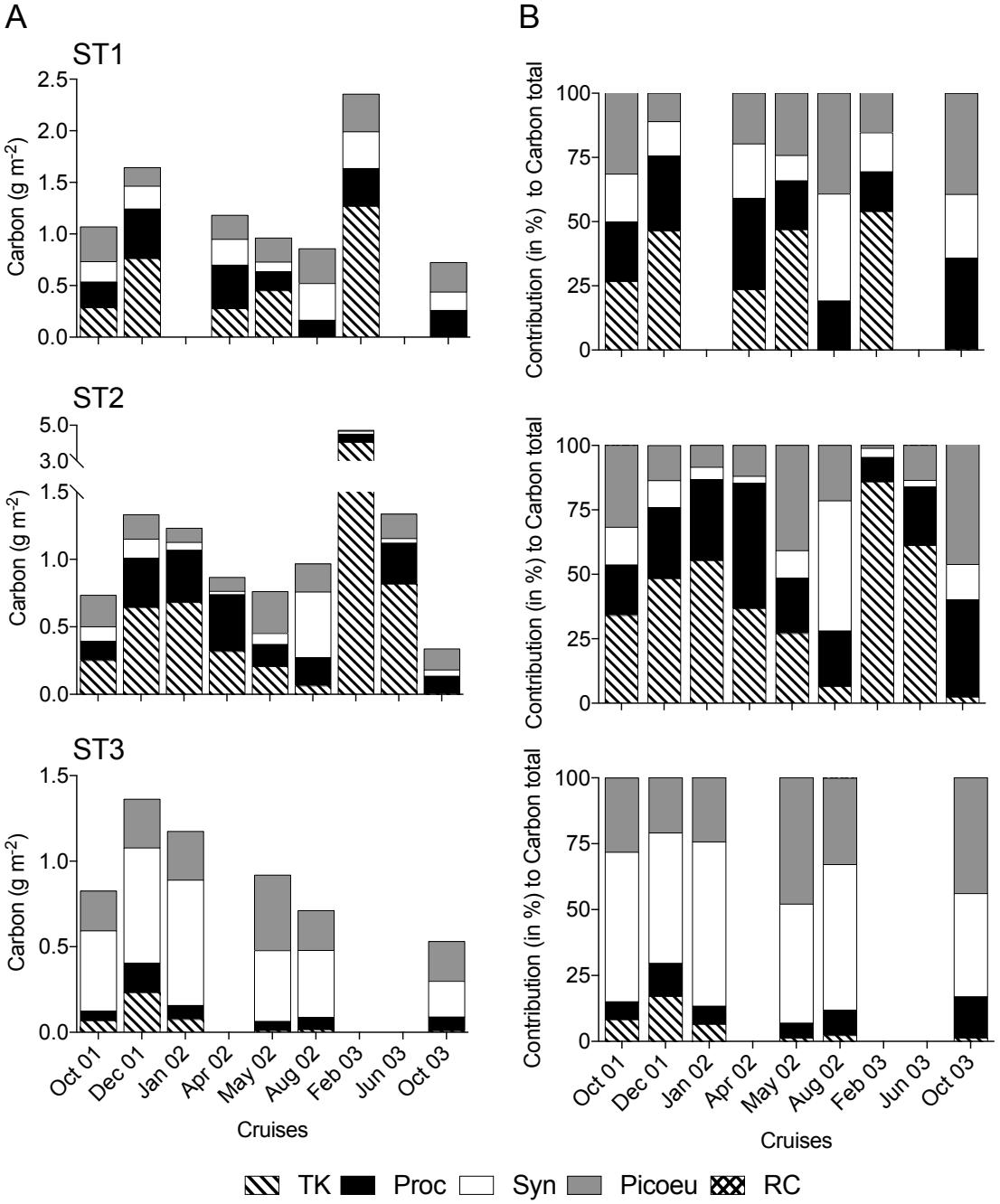


**Figure 12**. A) Carbon biomass of picoplankton and filamentous cyanobacteria at the three stations sampled during the Diapalis cruises. The conversion factors were (fgC cell$^{-1}$): *Prochlorococcus* – Proc (36), *Synechococcus* - Syn (255), picoeukaryotes - Picoeu (2590), *Richelia* - *RC* (10), sum of *Trichodesmium* and *Katagnymene* – TK (17.22 $10^6$ fgC trich$^{-1}$). B) Contribution of each group to the total carbon shown in A).