CONTRIBUTION OF NITROGEN FIXATION TO PLANKTONIC FOOD WEBS NORTH OF AUSTRALIA

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CONTRIBUTION OF NITROGEN FIXATION TO PLANKTONIC FOOD WEBS NORTH OF AUSTRALIA

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SUMMARY

Nitrogen fixation is no longer considered to be a minor factor of the nitrogen cycle in oceanic ecosystems. Recent geochemical and biological efforts have led to a significant increase in the estimated input of nitrogen to marine ecosystems by biological fixation, while molecular studies have increased our knowledge of the number and diversity of nitrogen fixers known to be active in the ocean. Although *Trichodesmium spp.* have long been viewed as the primary marine nitrogen fixers, recent efforts have shown that various members of the picoplankton community are also actively involved in nitrogen fixation. The relative abundance of different nitrogen fixers is an important ecosystem parameter since nitrogen fixers may differ significantly in their physiology, life history and ecology. Here we combine rate measurements and stable isotope natural abundance measurements to constrain the impact of N_2 fixation in the waters north of Australia. Samples were collected in the Coral, Arafura, and East Timor Seas, thus spanning three distinct hydrographic regions. Our data show that *Trichodesmium* has a significant influence on the stable nitrogen isotope ratios of particulate and zooplankton biomass and suggest that *Trichodesmium* is a significant source of nitrogen for the pelagic ecosystem. Based on stable carbon isotope ratios, it is also likely that the pathways are indirect and nitrogen fixed by *Trichodesmium* enters the higher trophic levels via decomposition as dissolved organic and inorganic nitrogen. Picocyanobacteria showed high diazotrophic activity at some stations, but unlike *Trichodesmium*, their N_2 fixation rate was not reflected in the stable N isotope ratios of particulate and zooplankton biomass. Our results suggest an important N contribution to biomass by diazotrophs in the Coral Sea, Arafura Sea and East Timor Sea.

1. INTRODUCTION

Nitrogen is a critical component of proteins, nucleic acids and many secondary metabolites and often limits primary production in oligotrophic waters. The most important nitrogen source for primary production in aquatic ecosystems is nitrate, which is most abundant in deep waters and in surface waters where coastal and equatorial upwelling occurs. Denitrification removes large amounts of biologically available nitrate from the ocean, and to maintain primary production, this deficit needs to be filled. In general, nitrogen fixation is the most important process that can add to the biologically active nitrogen pool of oligotrophic marine ecosystems. Geochemical budgets show a large mismatch between the inputs of nitrogen to the ocean by biological fixation (>135 Tg N per year) and the losses due to denitrification (>400 Tg N per year, Codispoti 2001 and 2007). But other opinions (Deutsch et al. 2007) suggest that the dimension of this mismatch between the losses and input of nitrogen is smaller. Other studies revealed only a mismatch between the inputs of new N by *Trichodesmium* and total N_2 fixation necessary to make up for the N loss, leaving the question about other possible sources open (Gruber and Sarmiento 1997, Karl et al. 1997). Conditions in many tropical and subtropical marine waters, such as the ocean gyres and some coastal waters like those along northern Australia, are oligotrophic, nitrogen-limited, and cover relatively large areas on the global seas. This makes N_2 fixation a critical factor in the global N cycle.

Generally, there are two possibilities to reconcile the apparent imbalance in the marine nitrogen budget. The first is that the nitrogen fluxes are not balanced and that the loss of nitrogen from the ocean is greater than the inputs. The other possibility is that

global nitrogen inputs have been underestimated and some biological sources of nitrogen, including new diazotrophic groups or new locations of significant nitrogen fixation, have not been adequately considered. The latter of these two assumptions has gained more weight with the discovery of new diazotrophic groups in the past few years (see below). Furthermore, a most recent study has challenged the idea that the Atlantic is the most important site of nitrogen fixation based on the finding that the amounts of total nitrogen fixation in the Pacific have been severely underestimated (Deutsch et al. 2007).

The abundant and cosmopolitan cyanobacteria from the genus *Trichodesmium* have long been regarded as the main contributors of biological nitrogen fixation in the pelagic water column (Capone et al. 1997, Capone 2001). Diazotroph-diatom associations (DDAs) comprised of symbiotic cyanobacteria living in association with diatoms are also important in some oligotrophic waters, particularly those influenced by riverine runoff (Carpenter et al. 1999, Foster et al. 2007). Other diazotrophs may also make significant contributions to the N budget, particularly small (< 10 μm diameter) unicellular picocyanobacteria from various genera (Zehr et al. 2000, 2001). More recent studies have strengthened this idea; significant nitrogen fixation rates by picocyanobacteria have now been measured in the central Pacific Ocean and in the tropical waters off Northern Australia (Zehr et al. 2001, Montoya et al 2004, Zehr et al. 2007). Similar findings have been reported for the North Atlantic by Falcón et al. 2004, though their use of concentrated cell suspensions instead of bulk water samples makes a direct comparison with the results of other studies difficult.

Currently, research focuses on picocyanobacteria (<10μm) that occur in the mixed layer of tropical and subtropical waters of the open ocean and shelf areas, which have

shown high rates of nitrogen fixation. Their detailed classification is still in progress, so we do not have as much knowledge of their ecology, physiology, distribution and phylogeny as we do for *Trichodesmium*. But we know about their potential biogeochemical significance, due to N_2 fixation rate measurements of selectively filtered water samples (Montoya et al. 2004, Zehr et al. 2007). And with molecular biological methods, a beginning has been made in classification based on the sequence analysis of the nitrogenase (*nifH)* by Zehr et al. (2007, 2001), Church et al. (2005) and Falcón et al. (2004). Currently, most of the unicellular diazotrophic cyanobacteria that are abundant in the open ocean fall into two groups, named Groups A and B (the latter includes the well known *Crocosphaera* spp.), based on *nifH* sequence analysis.

Overall, we have an increased number of diazotrophs that need to be considered. Yet, the relative importance of each group in supplying nitrogen for the higher trophic levels of an ecosystem remains an open question, since different diazotrophs can have different ecological and biogeochemical impacts due to environmental and biological factors. Specific grazers of the copepod genera *Macrosetella*, *Miracia* and *Oculosetella* are known to graze on *Trichodesmium* (O'Neil & Roman 1994, O'Neil 1998). But certain *Trichodesmium* species such as *T. thiebautii* are known to produce toxins as metabolic products, which reduces grazing pressure by other organisms significantly (Hawser et al. 1992). Thus, populations of toxic *Trichodesmium* species can be expected to pass on their N_2 fixation products into the food web indirectly rather than by grazing (Capone et al. 1994, Letelier & Karl 1996, Glibert & O'Neil 1999). Picocyanobacteria of the *Synechococcus* genus were found to be strongly exposed to grazing pressure (Tsai et al. 2005, Worden & Binder 2003, Landry & Kirchman 2002). At this point, we lack

critical information about unicellular diazotrophs. Therefore, we cannot predict at this point whether new nitrogen fixed within that size class goes go through similar pathways as new nitrogen of *Trichodesmium*.

Trichodesmium cells have relatively long doubling times and tend to form colonies (Mulholland & Capone 1999, LaRoche & Breitbart 2005), therefore populations are likely to respond to environmental changes differently and their blooms are expected to last longer than for small unicellular cyanobacteria.

A variety of biological and geochemical approaches can help in resolving the contributions of different groups of N_2 fixers to the overall flux of nitrogen into the ocean. Here, we report on a suite of biomass measurements for *Trichodesmium* and picoplankton groups, rate experiments, and stable isotope abundance measurements from the waters north of Australia, an oligotrophic, N-limited marine environment. Our findings suggest important contributions from diazotrophs to the overall nitrogen pool of the biomass. Furthermore, our data suggest an important role for *Trichodesmium*, bud do not allow us to resolve the importance of unicellular diazotrophs in supplying N to these waters.

2. MATERIALS AND METHODS

2.1. Sample Collection

Samples were collected during cruise EW9912 aboard the R/V *Maurice Ewing* (Figure 1). The first leg of the cruise (28 Oct 1999 - 11 Nov 1999) began in Townsville, Queensland, Australia, then passed through the Torres Strait westward through the Arafura Sea, the East Timor Sea and on to Broome, Western Australia. The second leg of the cruise (11 - 28 Nov 1999) extended eastward from Broome to Darwin, Northern Territory, and on to Townsville.

Standard hydrographic measurements were made with a SeaBird CTD (conductivity-temperature-depth) system equipped with standard conductivity, temperature, pressure, and fluorescence sensors and mounted on a standard rosette equipped with 10 L sampling bottles. Water samples from different depths were collected from selected depths with the CTD-rosette system.

Bulk suspended particles were collected by gentle vacuum filtration onto precombusted GF/F filters as described in Montoya et al. (2002), dried at 60˚C and stored over desiccant for later analysis ashore.

Trichodesmium colonies were collected with small, nets (30 cm diameter, 64 μm mesh size) deployed by hand while the ship drifted. When abundant at the surface, *Trichodesmium* was also collected by bucket. *Trichodesmium* was quantified by microscopic trichome counts in water subsamples from the CTD rosette. Picocyanobacteria were also quantified in subsamples taken from the CTD rosette with a BD FacScan flow cytometer operated with CellQuest software. In water sample

analyses, different groups of picocyanobacteria were identified based on relative side scatter of light, red fluorescence (chlorophyll) and orange fluorescence (phycoerythrin).

Zooplankton were collected using either meter nets to carry out oblique tows (OT) through the upper water column or a Multiple Opening/Closing Net and Environmental Sampling System (MOCNESS) to collect depth-stratified samples in 50m intervals. Once aboard ship, zooplankton were size-fractionated using nitex sieves, producing samples in the size ranges 250-500µm, 500-1000µm, 2000-4000µm and > 4000µm. All zooplankton were dried at 60ºC and stored over desiccant for analysis ashore.

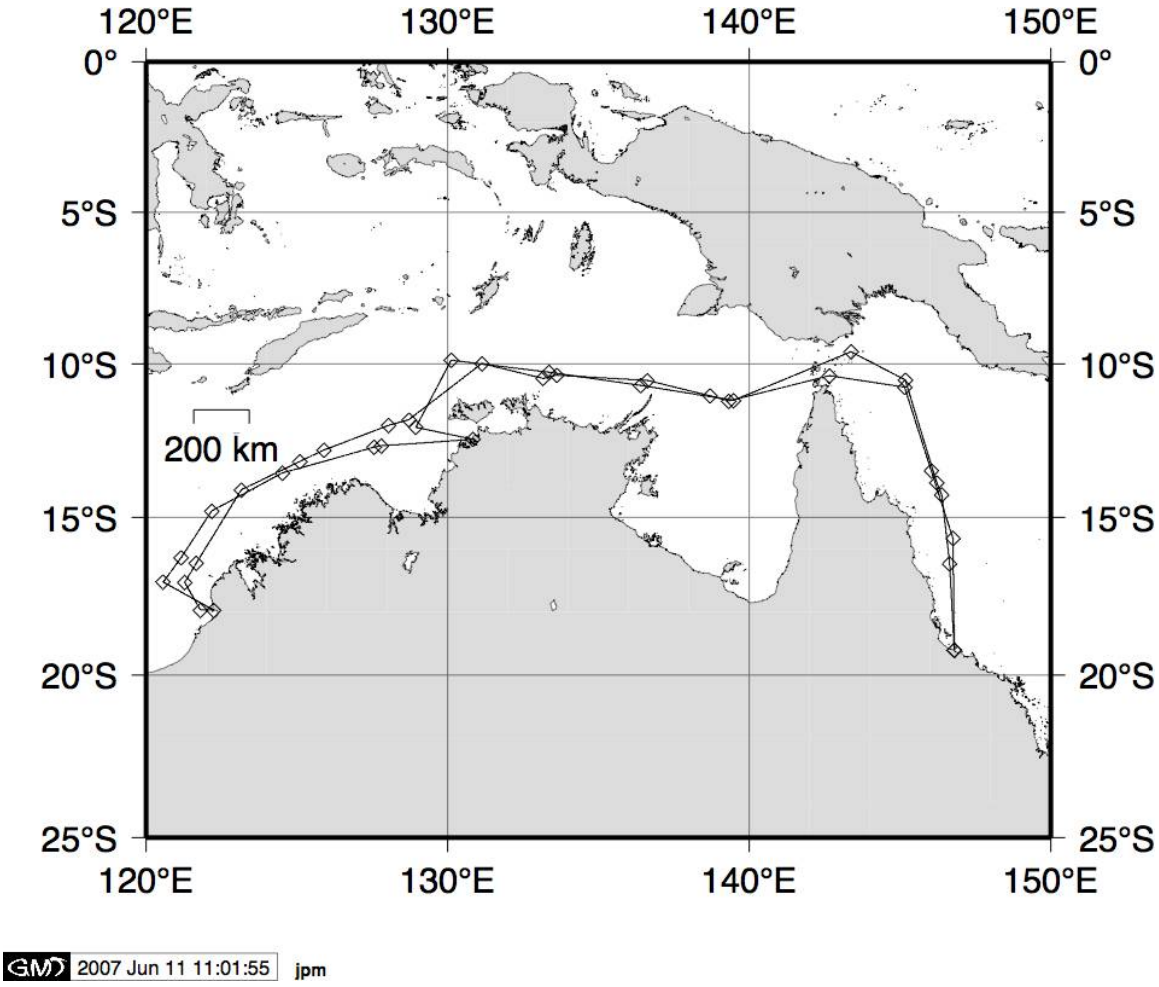

Figure 1: Track of Cruise EW9912 along the Northern Australian Coast, leading from Townsville to Broome (Leg 1) and back (Leg 2).

2.2. Isotopic Analysis

2.2.1 Analytic Procedures

We measured stable nitrogen and carbon isotope ratios by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Carlo Erba NA2500 elemental analyzer interfaced to a Micromass Optima mass spectrometer. Filters containing particle samples were trimmed, sectioned, then loaded into tin capsules and palletized for isotopic analysis. Zooplankton samples were ground to a fine powder, then weighed into tin capsules and pelletized for analysis. For stable carbon isotope ratio analysis, the zooplankton material was acidified with 10% HCl before loading the material into the tin capsules to remove inorganic carbon from the homogenate. When sufficient material was available, we carried out duplicate analyses of zooplankton and averaged the results. The stable N and C isotope ratios measured for each sample were corrected against the values obtained from standards with defined nitrogen and carbon element and isotopic compositions (methionine and peptone, respectively) by mass balance. Isotope abundances are expressed using the delta convention:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} \right) \times 1000 \quad (1)$$

where X is either ^{15}N or ^{13}C and R represents the isotope ratio ^{15}N:^{14}N or ^{13}C:^{12}C. $\delta^{15}N$ values are expressed relative to atmospheric N_2, and $\delta^{13}C$ values are expressed relative to PDB.

Stable isotope values of particulate organic matter (POM) were obtained from various depths in the water column at each station. For each station, the mean upper water

column $\delta^{15}N$ and $\delta^{13}C$ were calculated as the mass- and depth-weighted averages as described by Montoya et al. (2002).

2.2.2. Diazotroph Contributions to Biomass.

We used the mass-balance approach of Montoya et al. (2002) to estimate the contribution of diazotroph N to suspended particles (PN) and zooplankton. For particles, we used subsurface nitrate and the $\delta^{15}N$ of diazotrophs as end members:

$$\%\ \text{Diazotroph N} = 100 \times \left(\frac{\delta^{15}N_{PN} - \delta^{15}N_{nitrate}}{\delta^{15}N_{diazotrophs} - \delta^{15}N_{nitrate}} \right) \quad (2)$$

We used a value for $\delta^{15}N_{nitrate}$ based on literature estimates of the global average for deep water NO_3^- (4.5‰; Liu & Kaplan 1989, Sigman 2000) which fuels primary production by vertical flux to the euphotic zone. The $\delta^{15}N$ value for diazotrophs used for the calculations was -0.88‰, which is the mean value for $\delta^{15}N$ of *Trichodesmium* colonies picked from samples at various stations during the EW 9912 cruise (see Table 1).

For zooplankton (Zpl) in the size ranges of 250-500µm, 500-1000µm and 1000-2000µm , respectively, we used the highest $\delta^{15}N$ found for each size class on the cruise as a reference value (see Table 2):

$$\%\ \text{Diazotroph N} = 100 \times \left(\frac{\delta^{15}N_{Zpl} - \delta^{15}N_{RefZpl}}{\delta^{15}N_{diazotroph} - \delta^{15}N_{RefZpl}} \right) \quad (3)$$

In this case, the reference values represent zooplankton with minimal inputs of nitrogen from diazotrophs. This approach provides a conservative estimate of the importance of diazotrophic N in supporting zooplankton biomass.

Table 1: reference values for $\delta^{15}N$ of diazotrophs, obtained from picked *Trichodesmium* colonies of samples from the EW 9912 cruise.

Station	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
002	-0.8	-17
003	-0.1	-18.1
005	-0.7	-16.6
021	-0.3	-17.9
025	-1.6	-18
026	-1.2	-20.1
031	-1.7	-18.9
031	-1.1	-23
032	-0.2	-19.8
032	-1.1	-19.2
Mean (SD)	-0.88 (0.55)	-18.86 (1.84)

Table 2: reference values for $\delta^{15}N$ of zooplankton samples from each size class used for the mass balance calculations

Size class	Reference Value ($\delta^{15}N$)	Station	Depth
250-500µm	7.2	010	Mixed layer
500-1000µm	8.12	017	Mixed layer
1000-2000µm	8.34	017	Mixed layer

2.3. Nitrogen Fixation Experiments

The Acetylene Reduction Assay (ARA; Capone 1993, Capone et al. 2005) was used to measure rates of N_2 fixation by *Trichodesmium* collected at the surface using buckets and hand-nets, or in gentle net tows through the upper 25 m of the water column. Picoplankton N_2 fixation activity was measured using the ^{15}N-N_2 assay (Montoya et al. 1996) in water samples collected from the pigment maximum at the base of the mixed layer. These water samples were filtered through 100-µm Nitex mesh before incubation to exclude *Trichodesmium* and other large organisms. Tracer incubations were carried out under simulated in situ conditions in deck incubators and were terminated by gentle vacuum filtration through precombusted GF/F filters. These filters were dried at 60˚C and stored over desiccant for isotopic analysis ashore. Rates were calculated using the approach of Montoya et al. (1996).

2.4. Statistical Analysis

Statistical analyses were performed with the JMP software package (SAS Institute). Since we have a limited amount of data, we used simple univariate correlation analyses to test variables of interest for correlation coefficient and significance. Data from each leg of the cruise were analyzed separately. Pairwise correlations and the number of data pairs used for each test are shown in the result sections below.

3. RESULTS

3.1. Plankton Abundance

3.1.1. *Trichodesmium*

Trichodesmium was frequently present at high abundance near the surface and in the mixed layer (Fig. 2). On the first leg of the cruise, peak concentrations were seen in the Arafura Sea at stations 7 and 11. On Leg 2, a high peak was found at station 025 and a smaller peak at station 031. No significant correlations were found between *Trichodesmium* abundance and the abundance of picocyanobacteria (see Table 3).

3.1.2. Unicellular Picoplankton

Pigment maxima in deeper layers of the euphotic zone reflected high abundances of various picoplankton including *Synechococcus*. *Prochlorococcus* generally occurred at much higher densities than *Synechococcus* on both legs of the cruise though *Synechococcus* dominated at Stations 011, 013 and 018 on Leg 1 and at station 029 on Leg 2. *Prochlorococcus* and *Synechococcus* tended to be negatively correlated, though none of the correlations among cyanobacterial groups were significant (see Table 3).

Table 3: Correlation matrix for *Trichodesmium* and picoplankton abundance on the two legs of the cruise. Each cell shows the correlation coefficient (r), the associated p-value and the number (n) of data pairs. Correlations from Leg 1 (8 stations) are shown to the upper right of the main diagonal and correlations from Leg 2 (10 stations) are shown to the lower left of the main diagonal.

	Trichodesmium	*Synechococcus*	*Prochlorococcus*
Trichodesmium		Leg 1 r = 0.47 p =0.24 n = 8	Leg 1 r = -0.71 p =0.05 n = 8
Synechococcus	Leg 2 r = 0.56 p =0.09 n = 10		Leg 1 r = -0.62 p = 0.01 n = 8
Prochlorococcus	Leg 2 r = 0.02 p =0.95 n = 10	Leg 2 r = -0.54 p =0.11 n = 10	

Table 4: Correlations (r) between cyanobacteria abundances and other parameters. Correlations of Leg 1 are shown on the upper right of the main diagonal, of Leg 2 on the lower left. Significant correlations are marked with an asterisk. Below the correlations, numbers (n) of data pairs tested for correlation are shown.

	Tricho	SYN	PRO	[PN]	N_2 Fix (Tricho)	$\delta^{15}N$ POM
Tricho	•	0.47 n = 8	-0.71 n = 8	0.26 n = 9	0.87* n = 9	-0.71* n = 9
SYN	0.56 n = 10	•	-0.62 n = 8	0.51 n = 8	ND	<0.01 n = 8
PRO	0.02 n = 10	-0.54 n = 10	•	-0.13 n = 8	ND	0.9* n = 8
[PN]	0.09 n = 9	0.76* n = 13	0.52 n = 13	•	-0.15 n = 9	0.32 n = 9
N_2 Fix (Tricho)	0.03 n = 9	ND	ND	-0.16 n = 9	•	-0.52 n = 9
$\delta^{15}N$ POM	-0.66 n = 9	-0.41 n = 13	0.07 n = 13	-0.32 n = 9	0.25 n = 9	•

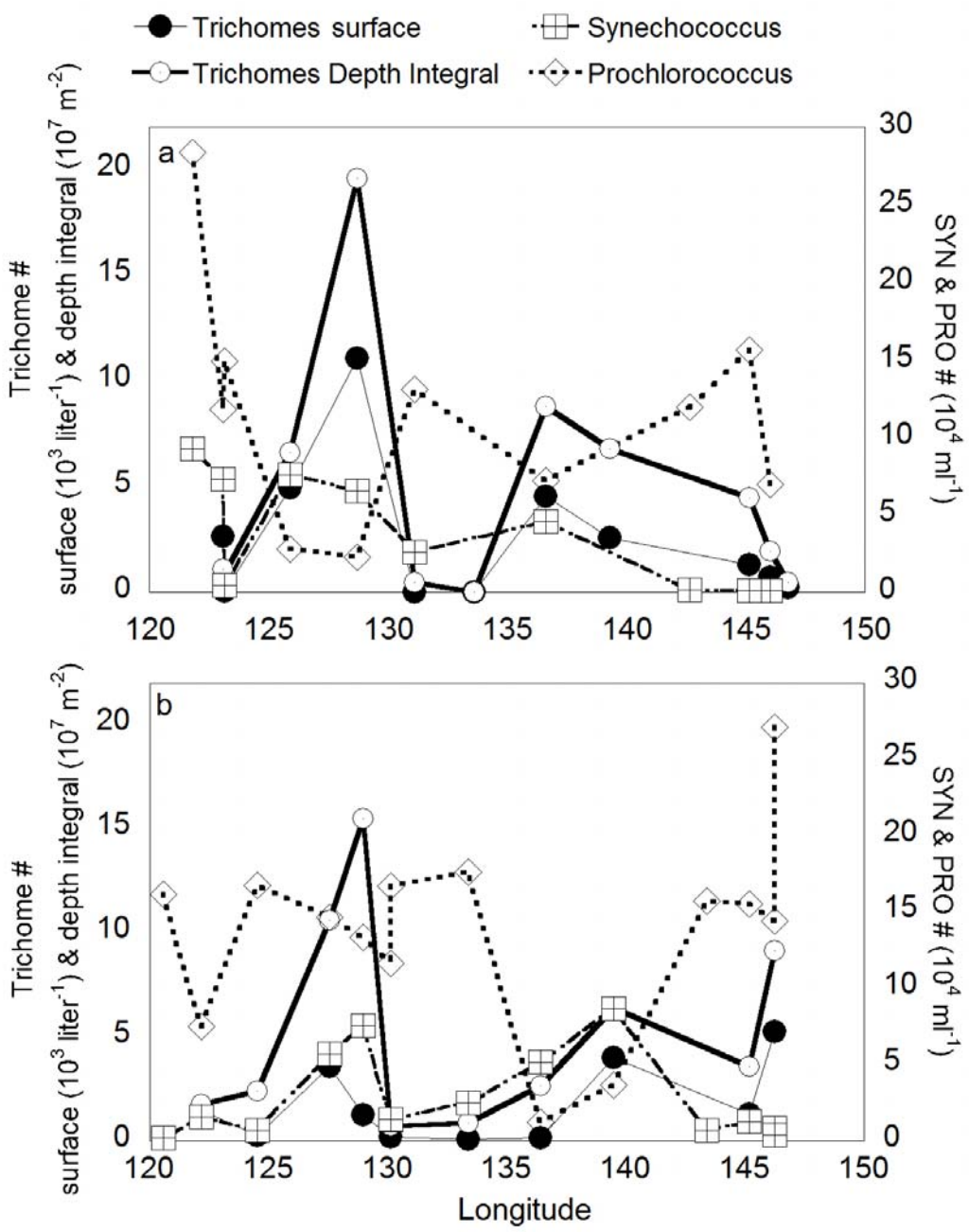

Figure 2: Densities of trichomes and unicellular cyanobacteria in water samples from cruise Leg 1 (a) and Leg 2 (b). Trichome abundances are given for the surface and mixed layer, picoplankton abundances are given for the pigment maximum layer.

3.2. Particulate Nitrogen Concentrations [PN]

There were similar longitudinal trends in [PN] at the surface and in the mixed layer. In general, concentrations were highest in the East Timor Sea and the Arafura Sea on both legs (see Fig. 3 a, b). PN concentration did not show significant correlations with the $\delta^{15}N$ of PN, *Trichodesmium* abundance, picoplankton abundance, or nitrogen fixation rates. (see Tables 4, 5).

For *Synechococcus* we have found a significant correlation with [PN] on Leg 2 (see Tables 4, 5). No significant correlations were found for the *Prochlorococcus* group.

Table 5: Cyanobacteria abundances and their correlations with [PN]

	[PN] mixed layer (μmol liter^{-1}) Leg 1	[PN] mixed layer (μmol liter^{-1}) Leg 2
Trichomes (10^7 * m^{-2})	r = 0.26 p = 0.5 n = 9	r = 0.09 p = 0.82 n = 9
Synechococcus (10^4 * ml^{-1})	r = 0.51 p = 0.2 n = 8	r = 0.76 p < 0.02 n = 13
Prochlorococcus (10^4 * ml^{-1})	r = - 0.13 p = 0.76 n = 8	r = 0.52 p = 0.07 n = 13
δ^{15}N particles (mixed layer)	r = 0.21 p = 0.79 n = 9	r = 0.32 p = 0.40 n = 9

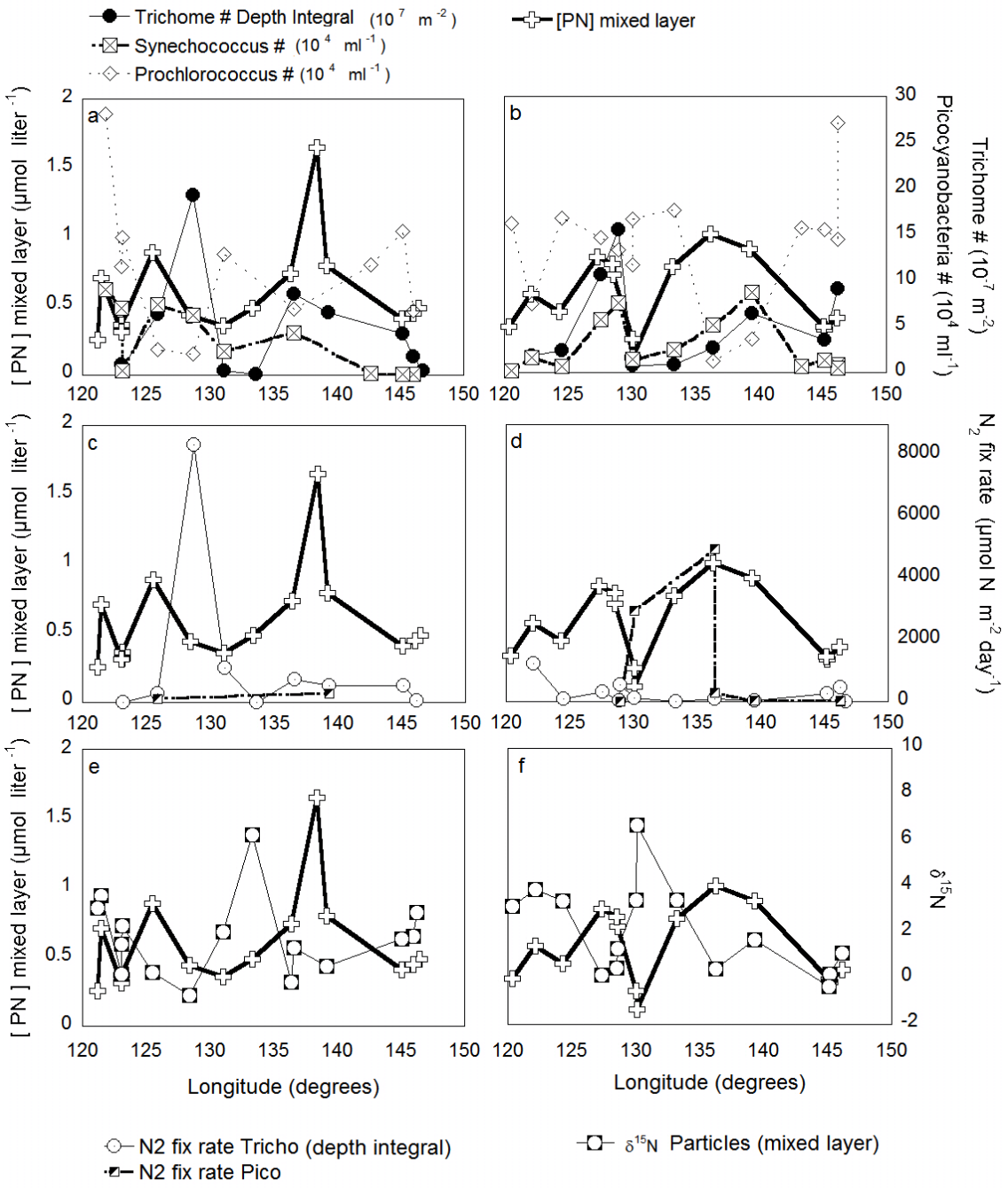

Figure 3: PN concentrations plotted with cyanobacteria abundances, N_2 fixation and $\delta^{15}N$ of particles as a function of longitude on Leg 1 (a, c, e) and Leg 2 (b, d, f).

3.3. Nitrogen Fixation Rates

Nitrogen fixation rates showed different spatial patterns on the two legs of the cruise. For *Trichodesmium*, fixation rates under non-bloom conditions were up to 136 µmol N m^{-2} day^{-1}. For picoplankton, we have no information about the abundances of unicellular diazotrophs, therefore we cannot make statements about rates under different bloom conditions.

Generally, high N_2 fixation rates for *Trichodesmium* during Leg 1 of the cruise (550 µmol N m^{-2} day^{-1} at station 003 in the Coral Sea, stations 005, 007, 011 with 543, 738 and 8361 µmol N m^{-2} day^{-1}, respectively, in the Arafura Sea) coincided with high abundances of trichomes (Fig. 4 a). The correlation between N_2 fixation rates and Trichome abundances was highly significant (Table 6). In contrast to the high rates measured for *Trichodesmium*, the N_2 fixation rates measured for picoplankton in pigment maximum areas within the deeper euphotic zone were negligible except from two stations with considerable rates (station 5 with 106 and station 13 with 281 µmol N m^{-2} day^{-1}) in the western and eastern Arafura Sea, respectively.

On Leg 2, peaks in *Trichodesmium* N_2 fixation rates were lower than on Leg 1. Yet, rates were relatively high at stations in the East Timor Sea (1247.1 µmol N m^{-2} day^{-1} at station 021), Arafura Sea (stations 023 and 025 with 323 and 567 µmol N m^{-2} day^{-1}, respectively) and Coral Sea (station 031 and 032 with 258 and 481 µmol N m^{-2} day^{-1}, respectively). There were no significant correlations between *Trichodesmium* N_2 fixation rates and any parameter on that leg.

Picoplankton N_2 fixation measurements on the second leg of the cruise revealed high rates of nitrogen fixation at two stations in the Arafura Sea (026 and 027 with 2961 and 4950 μmol N m^{-2} day^{-1}, respectively) where fixation rates and abundances of *Trichodesmium* were rather low (Fig. 4 b, d). These high picocyanobacterial fixation rates coincided with moderate abundances of *Synechococcus* (Fig. 4 b). Picocyanobacterial fixation rates showed no correlation with [PN] or the $\delta^{15}N$ of particles (Fig. 4). The stations with high picocyanobacterial nitrogen fixation activity coincided both with very high (6.7‰) and rather low (0.4‰), respectively, $\delta^{15}N$ values for suspended particles (Fig. 4 d).

Table 6: Correlation analysis between *Trichodesmium* nitrogen fixation rates and Trichome abundances, stable N isotope ratios of particles and [PN], respectively.

	Tricho N_2 fixation rate Leg 1	Tricho N_2 fixation rate Leg 2
$\delta^{15}N$ of particles	r = -0.52 p = 0.16 n = 9	r = 0.25 p = 0.52 n = 9
Trichome abundance	r = 0.87 p < 0.01 n = 9	r = 0.03 p = 0.94 n = 9
[PN]	r = -0.15 p = 0.70 n = 9	r = -0.16 p = 0.92 n = 9

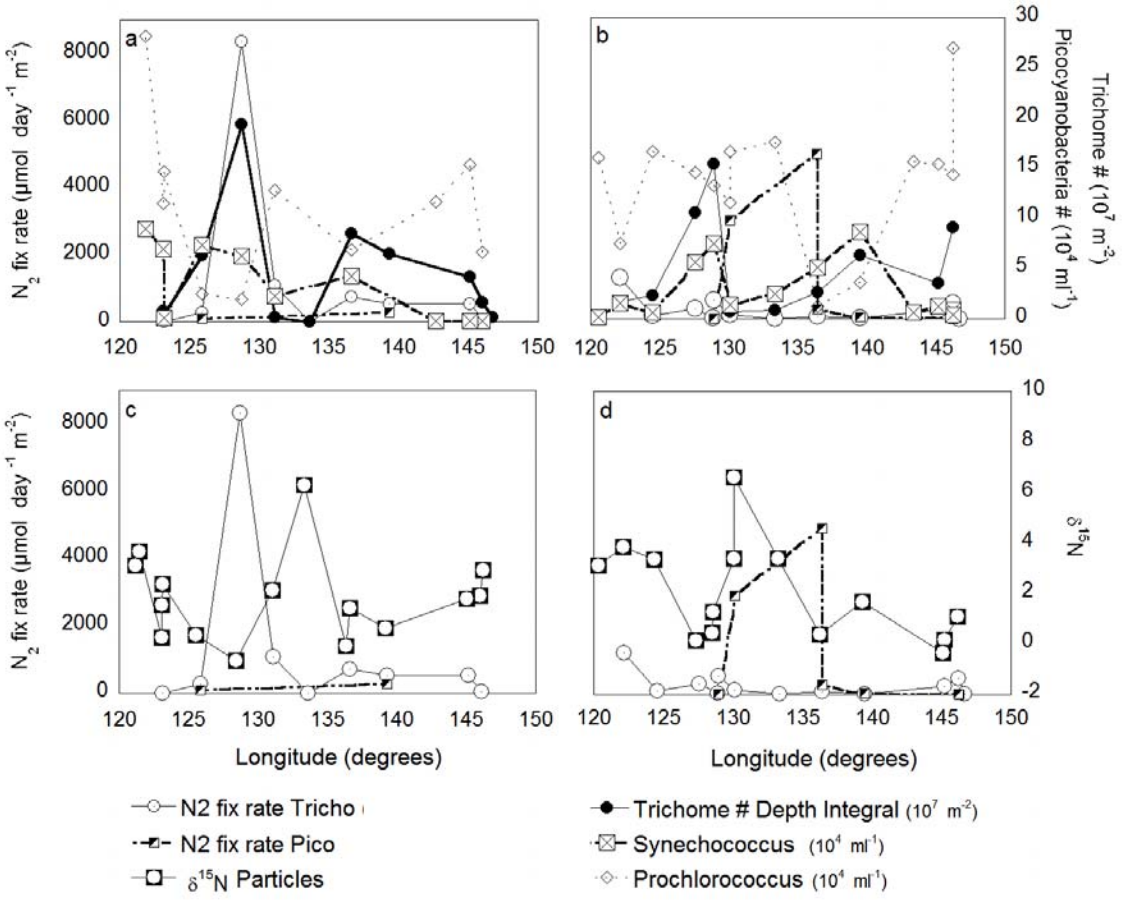

Figure 4: N_2 fixation rates plotted with the abundance of cyanobacteria (a for Leg 1, b for Leg 2) and $\delta^{15}N$ for particles (c for Leg 1, d for Leg 2) as a function of longitude on each leg. Correlations of Tricho N_2 fixation with concentration and $\delta^{15}N$ of particulate N are shown in e (Leg 1) and f (Leg 2).

3.4. Nitrogen stable isotope abundance

3.4.1. $\delta^{15}N$ of PN in The Mixed Layer

For both legs of the cruise, the zonal distributions of minimal and maximal $\delta^{15}N$ values were similar. For Leg 1, a comparison of the $\delta^{15}N$ of PN and *Trichodesmium* abundance revealed a significant negative correlation (Table 7, Fig. 5a). A significant positive correlation was found between *Prochlorococcus* abundances and $\delta^{15}N$ of particle samples on Leg 1.

On Leg 2, no significant correlations were found among the $\delta^{15}N$ of particles, particle concentration, and cyanobacteria abundances (Table 7). Yet, the tendencies between $\delta^{15}N$ of PN and *Trichodesmium* abundance were similar as on Leg 1 (Fig. 5 b, d).

Table 7: Correlations between $\delta^{15}N$ of particulate organic matter and [PN] concentrations and cyanobacteria abundances, respectively, on both cruise legs.

	$\delta^{15}N$ of POM Leg 1	$\delta^{15}N$ of POM Leg 2
Trichodesmium	r = -0.71 p = 0.03 n = 9	r = -0.66 p = 0.05 n = 9
Synechococcus	r < 0.01 p = 0.99 n = 8	r = -0.41 p = 0.16 n = 13
Prochlorococcus	r = 0.9 p < 0.01 n = 8	r = 0.07 p = 0.82 n = 13
[PN]	r = -0.21 p = 0.50 n = 9	r = -0.4 p = 0.16 n = 9

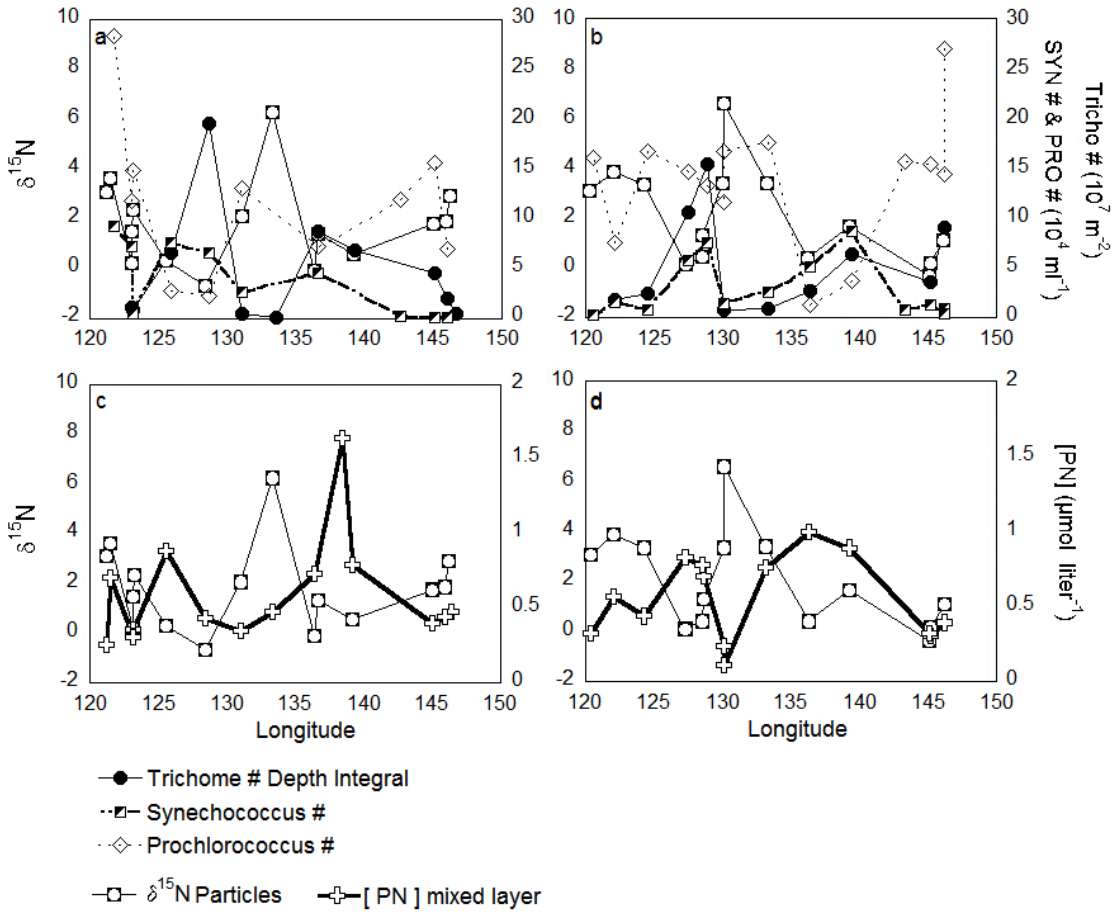

Figure 5: $\delta^{15}N$ of particles plotted with cyanobacterial abundance on Leg 1 (a), Leg 2 (b) and with [PN] on Leg 1 (c) and Leg 2 (d) as a function of longitude.

3.4.2. $\delta^{15}N$ of Zooplankton throughout the water column

In general, we found an increase in zooplankton $\delta^{15}N$ with increasing depth. At some stations, the differences between the depth range of 100-50m and the one immediately below were relatively large. In the Arafura Sea at station 026, the five different size classes of zooplankton showed on average a difference of 2.3 ‰ (SD 0.86) between $\delta^{15}N$ of MOCNESS samples from the upper 100m (Fig. 7b, e) and those taken from greater depths (Fig. 7h, k, n). Maximal differences between zooplankton of these water layers were associated with the steepest water temperature gradients. In the Eastern and Western areas of the cruise track, the differences between the $\delta^{15}N$ of the upper 100m and deeper waters were smaller. In the Coral Sea, the average increase from one 50m-depth interval to the next deeper one was less than 1‰ (see Fig. 7c, f, i, l, o). In the East Timor Sea (Fig. 6 and 7a, d, g, j, m), average increases for a size classes with depth ranged between 0.36 (SD 0.2) and 1.1 (SD 0.6).

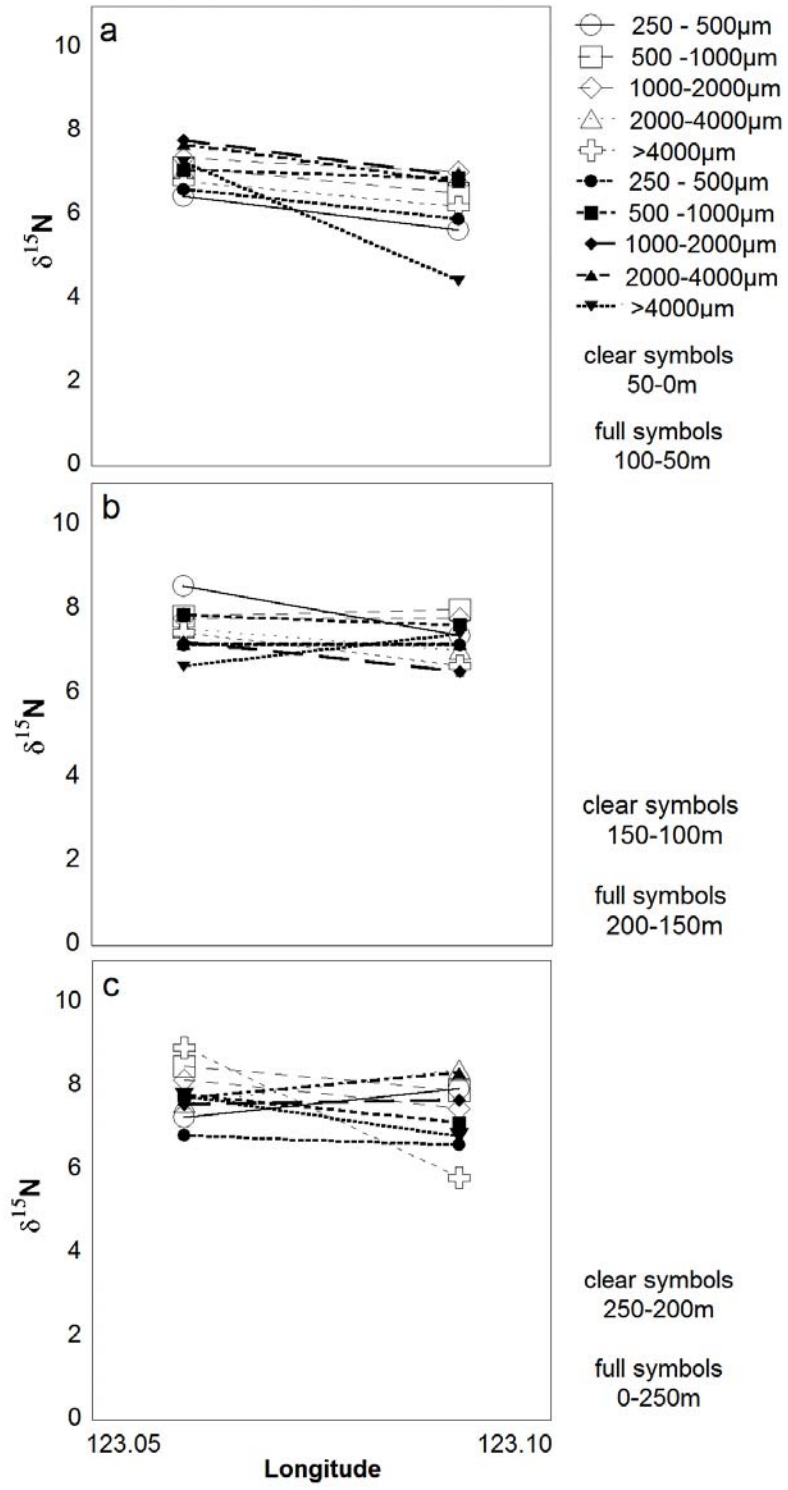

Figure 6: MOCNESS samples of mesozooplankton collected in the East Timor Sea at station 015 during the first leg of the cruise. Graph a) shows upward tows from 100 - 50 and 50 - 0m; b) upward tows from 200-150m and 150-100m; c) upward tow from 250-200m and downward tow from the surface to 250m.

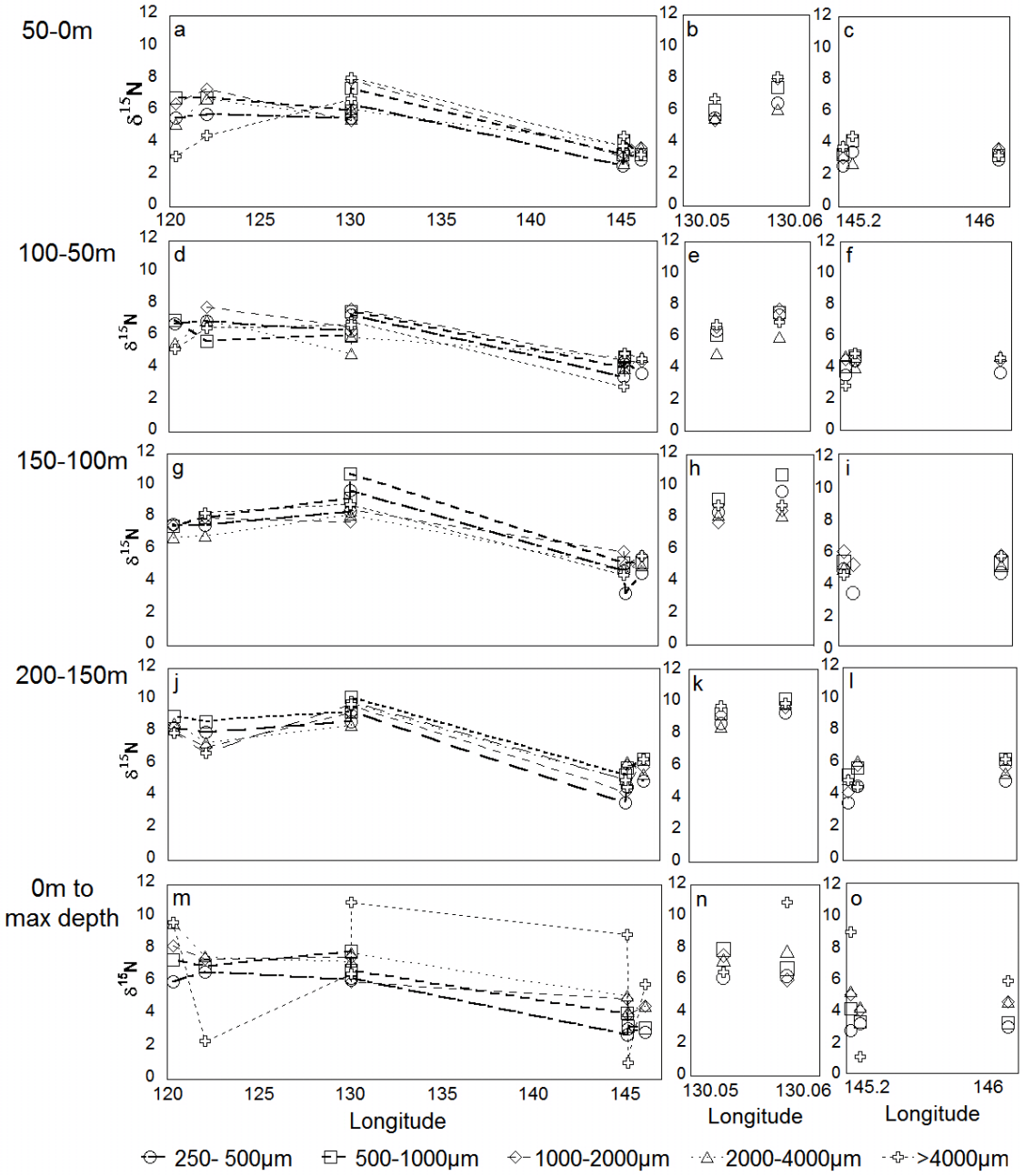

Figure 7: Zooplankton MOCNESS samples collected during the second leg of the cruise by upward tows in the upper 200m of the water column and the entire water column in downward tows to the bottom of the sampling range. Each row shows a plot with the total longitudinal sampling range (a, d, g, j, m) and one detailed plot of the Arafura Sea (b, e, h, k, n) and Coral Sea (c, f, i, l, o).

3.4.3. N Isotopes in Mixed Layer Zooplankton

The $\delta^{15}N$ of mixed layer mesozooplankton showed east-west patterns that were in general consistent with those of trichome abundances and the $\delta^{15}N$ of particles (see Fig. 8 and 9). On Leg 1, $\delta^{15}N$ values of zooplankton tracked those for PN (see Fig. 8), yet there were some spatial differences between them, particularly in the Arafura Sea. No significant correlation of zooplankton $\delta^{15}N$ isotope ratios with $\delta^{15}N$ of particles was found on Leg 1, even though distributions of maxima and minima were similar for both data groups. On Leg 2 the correlation between the values of the smallest zooplankton size fraction (250-500μm) and particles was highly significant ($r=0.80$; $p<0.001$; $n = 13$). On both cruise legs, oblique tow and MOCNESS samples showed minimal $\delta^{15}N$ values in the eastern and western Arafura Sea and a peak of $\delta^{15}N$ in the central Arafura Sea (see Fig. 8 and 9).

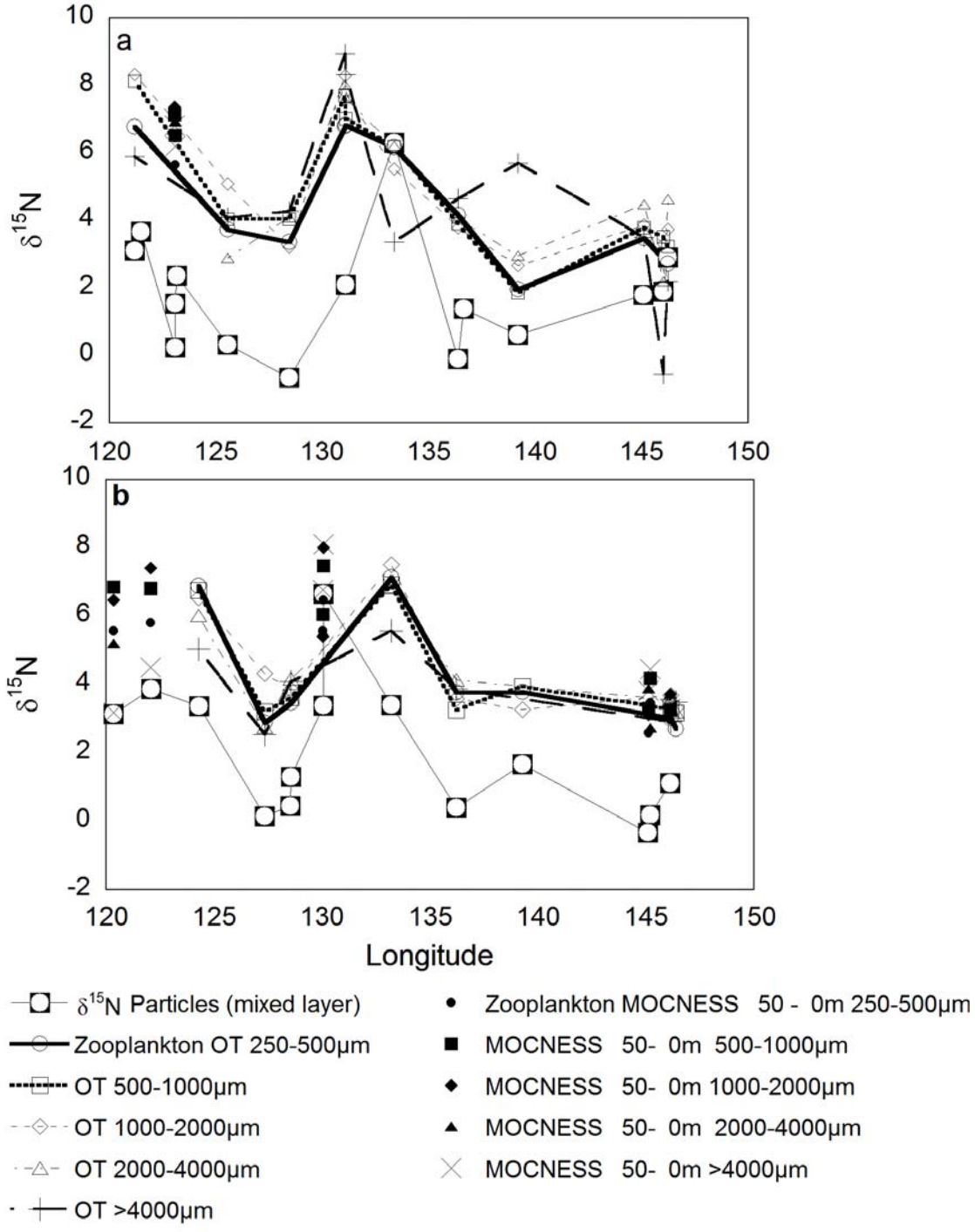

Figure 8: $\delta^{15}N$ of particles and zooplankton from the mixed water layer collected by Oblique Tow (OT) and MOCNESS for Leg 1 (a) and Leg 2 (b).

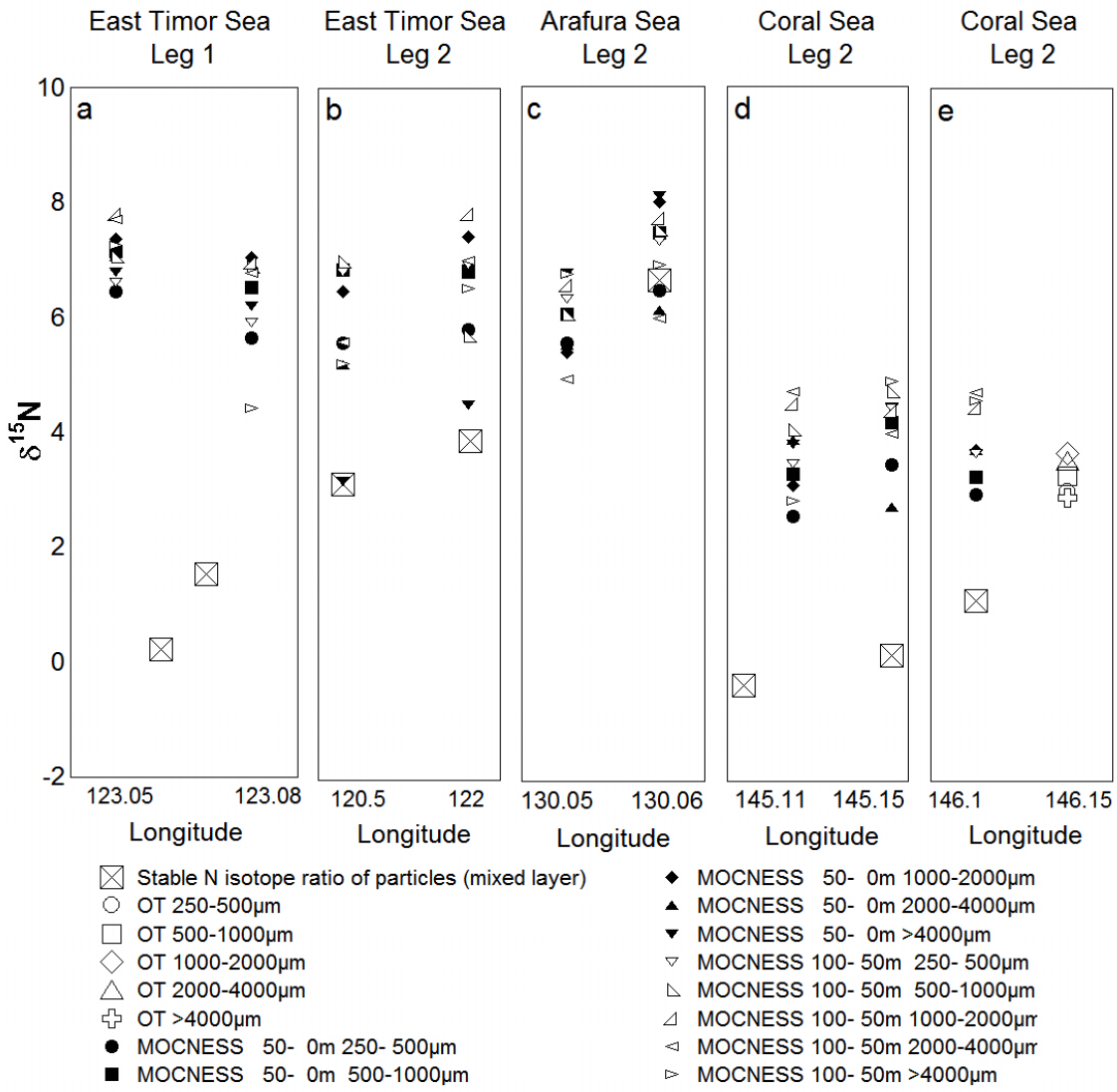

Figure 9: detailed plots with focus on longitudinal areas of each MOCNESS sampling area in showing the $\delta^{15}N$ of zooplankton collected in the upper 100m of the water column in detail. Graph a shows $\delta^{15}N$ of MOCNESS samples in the East Timor Sea on Leg 1 and particle samples sampled nearby. Graphs b (East Timor Sea), c (Arafura Sea), d and e (Coral Sea) show particle and zooplankton samples collected on Leg 2.

3.5. C and N Stable isotope abundances

3.5.1. Water Column

From the East Timor Sea, we have MOCNESS data available from both cruise legs. On Leg 1, $\delta^{15}N$ of POM varied between 0.4 and 7‰, increasing with depth. The $\delta^{13}C$ of particles ranged from -17 to -23.7‰. We found no regular pattern in $\delta^{13}C$ variations with depth or station, nor was there a significant correlation between $\delta^{15}N$ and $\delta^{13}C$ at any depth or between any size classes of zooplankton and particles.

For Leg 2, we have MOCNESS data from stations in all three hydrographic regions of the cruise track. In the East Timor Sea (stations 019 and 021, Fig. 14 and 15, respectively), we found $\delta^{15}N$ for POM samples ranging from 2.3 to 4.5‰ throughout the water column, whereas $\delta^{13}C$ for these samples was broadly scattered between -18 and -23‰. No correlations could be observed between $\delta^{13}C$ and any other data. In the Arafura Sea at station 26, where high unicellular N_2 fixation was measured in the pigment maximum layer around 50m, the $\delta^{15}N$ of particles was lowest in the upper 50m at around 2‰, increasing with depth and close to 8‰ in the deepest sampling range. $\delta^{13}C$ for particles was -20‰ with a variation of about 2‰. In the Coral Sea, we found a low $\delta^{15}N$ at all depths for POM. $\delta^{15}N$ values did not exceed 2‰ even at depth.

Zooplankton on Leg 1 increased in $\delta^{15}N$ by 1-2‰, depending on size class, from the top (50-0m) to the bottom depth interval (250-200m) sampled (Fig.12a – e). Zooplankton $\delta^{13}C$ varied widely among zooplankton size classes in the upper 50m (Fig. 12 a), with less variation in deeper samples (Fig. 12b-d). We found similar $\delta^{13}C$ values among zooplankton size classes within stations but no significant correlations with depth.

For zooplankton on Leg 2, $\delta^{13}C$ showed no regular changes with depth or longitude. The $\delta^{13}C$ values were much higher for zooplankton than for particles (differences larger than 5‰ between a zooplankton sample and its respective particle sample from a depth layer). We found no significant correlation between the C isotopic composition of particles and zooplankton, nor was there a continuous trend with changing depths or size classes. Generally, we found smaller variations in $\delta^{13}C$ between zooplankton size classes within a single station (Fig.11 to Fig. 17).

Overall, zooplankton in all three hydrographic regions showed an increase of $\delta^{15}N$ with depth and size. Zooplankton in the Arafura Sea at station 026 (Fig. 14) followed similar trends, but the difference between particles and zooplankton were smallest in this region throughout the water column. There were no correlations between $\delta^{13}C$ and $\delta^{15}N$.

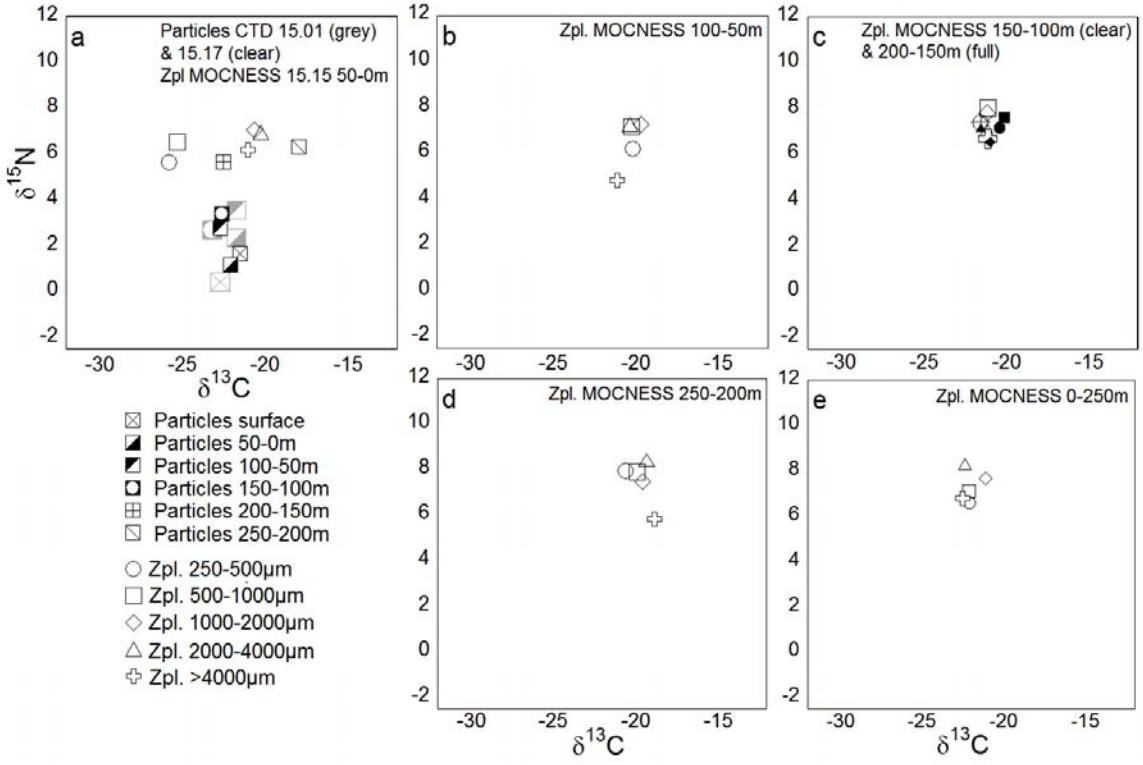

Figure 10: Isotopic cross plots for particles from station 015, particle samples from CTD 15.01 and 15.17 and depth-stratified zooplankton samples from MOCNESS tow 15.15 in the East Timor Sea.

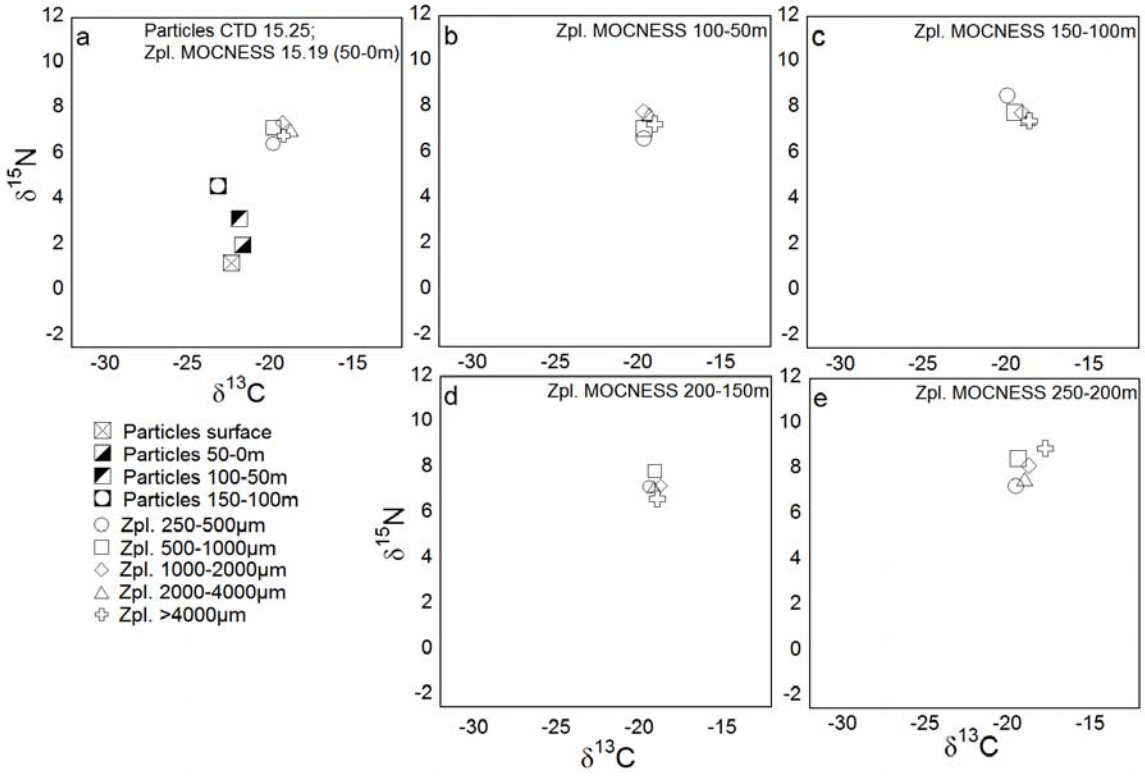

Figure 11: Isotopic cross plots for particles from station 015, CTD 15.25 and depth-stratified zooplankton from MOCNESS tow 15.19 in the East Timor Sea.

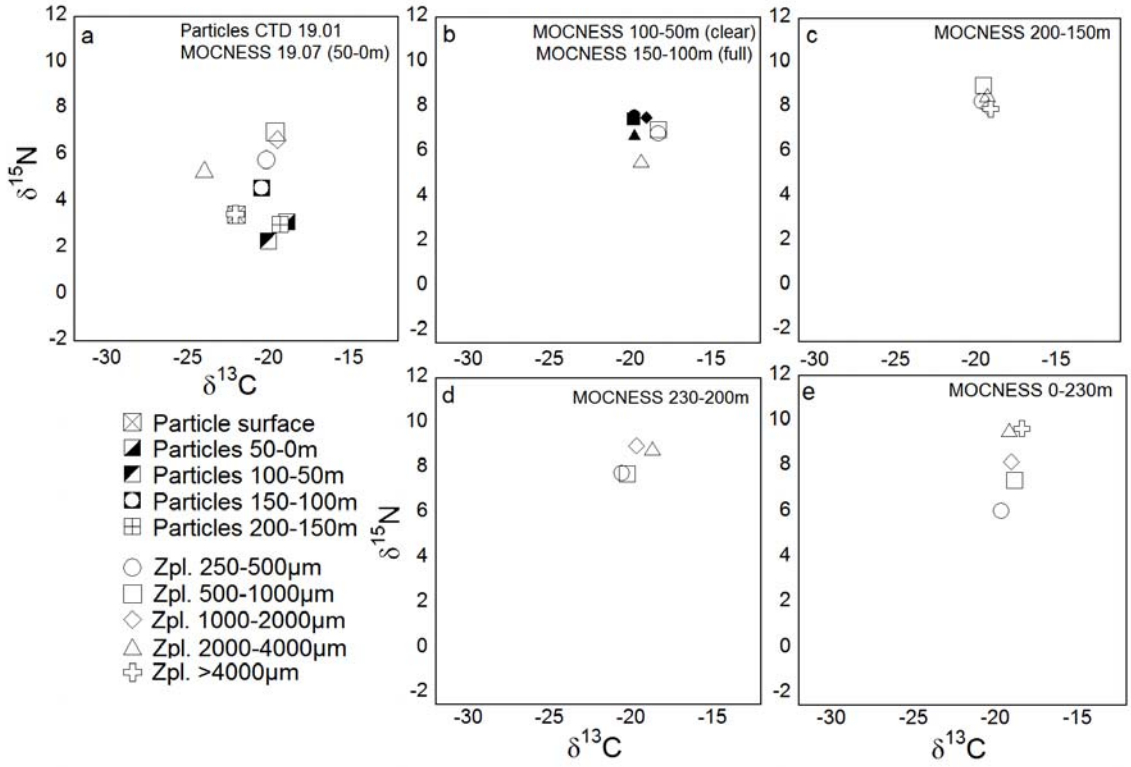

Figure 12: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values from station 019; particles from CTD 19.01 and zooplankton of each depth range from MOCNESS tow 19.07 in the East Timor Sea.

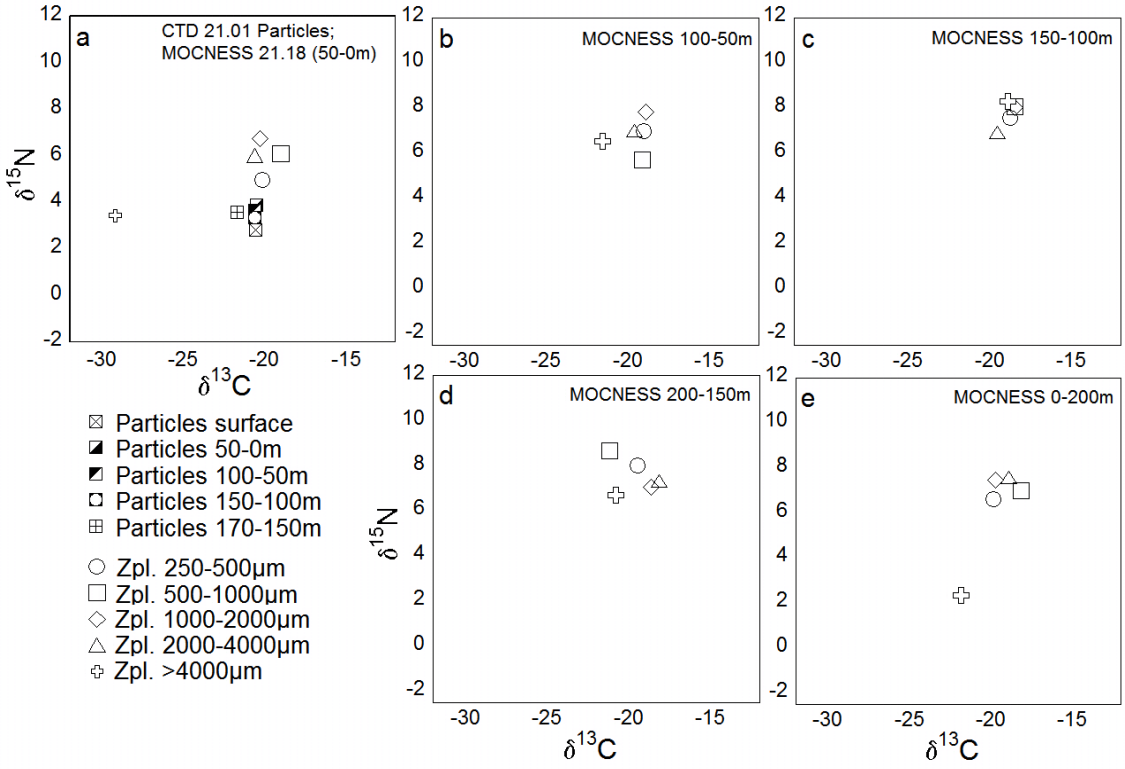

Figure 13: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values from station 021; particle samples from CTD 21.01 and zooplankton of each depth range from MOCNESS tow 21.18 in the East Timor Sea.

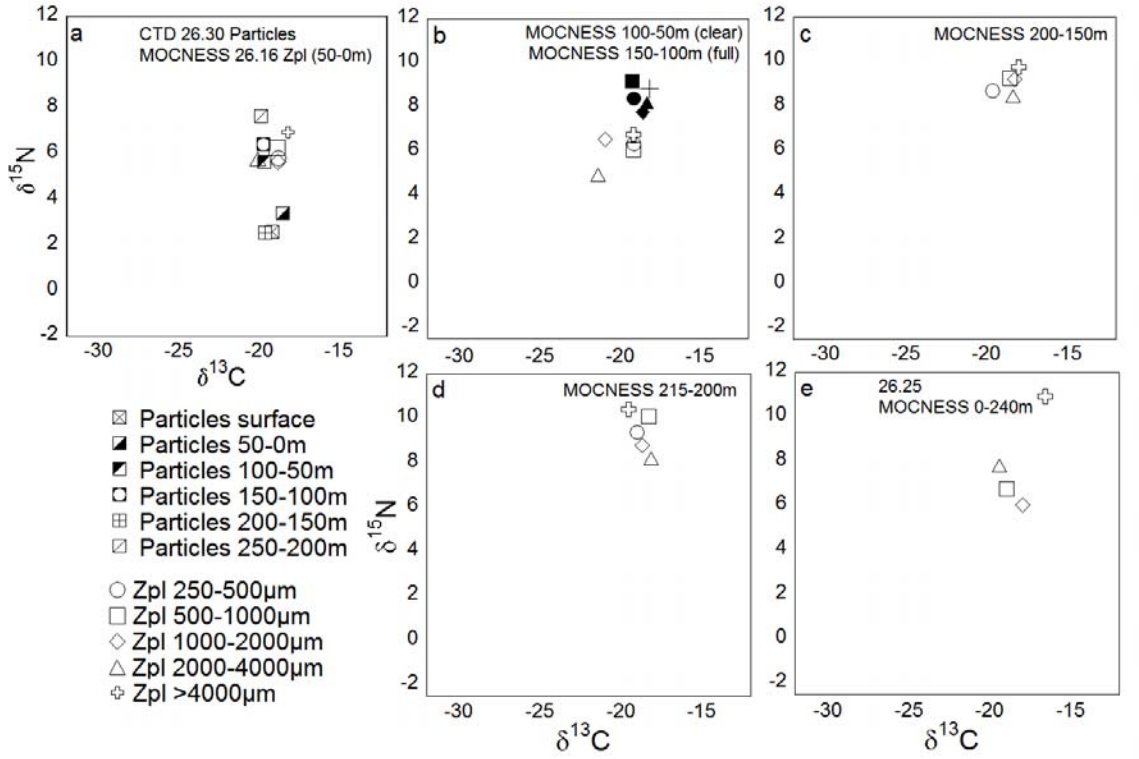

Figure 14: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values from station 026; particles from the CTD 26.30 and zooplankton of each depth range from MOCNESS tow 26.16 and a downtown sample of MOCNESS tow 26.25 in the Arafura Sea.

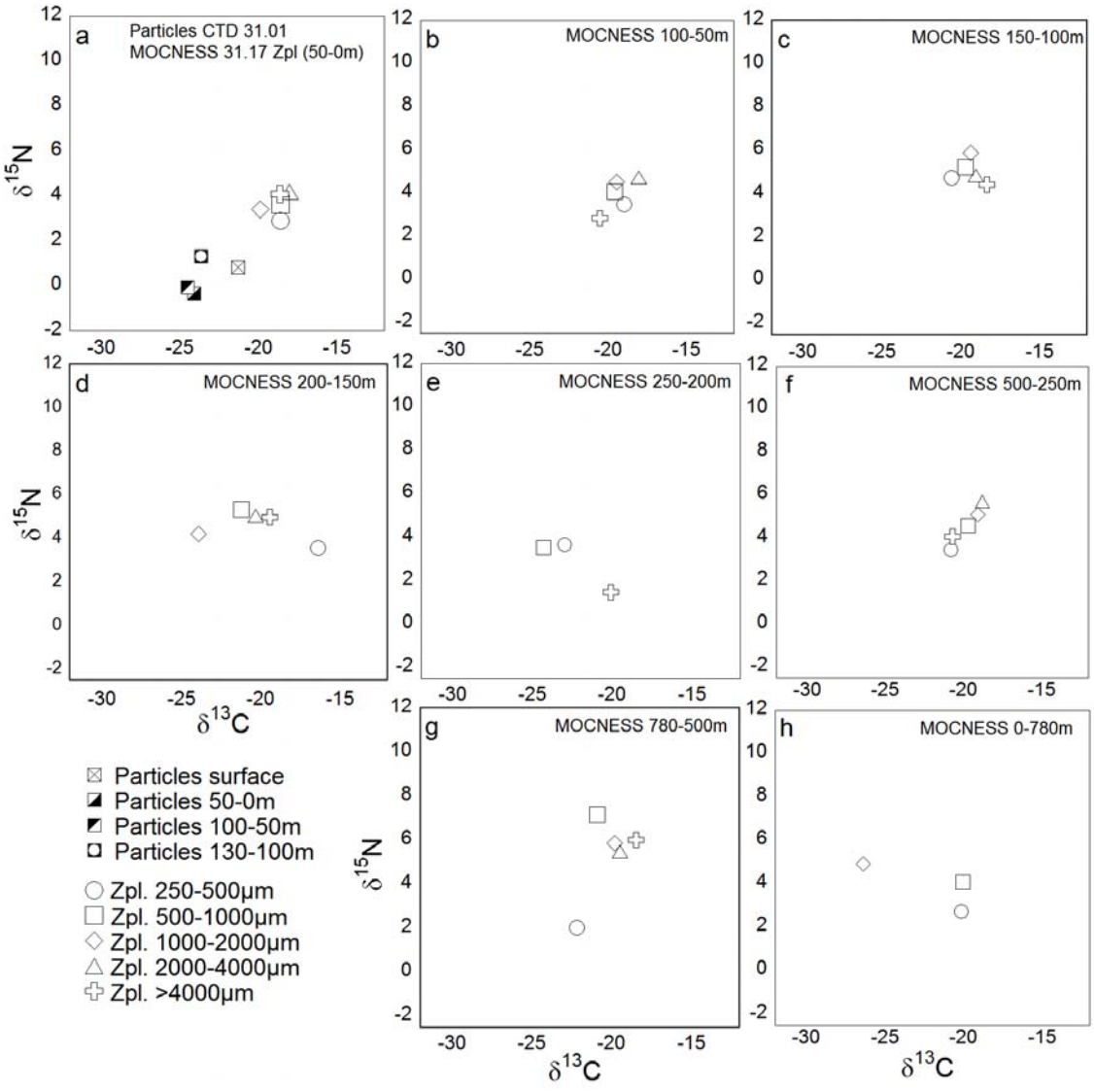

Figure 15: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values from station 031; particles from the CTD 31.01 and zooplankton of each depth range from the MOCNESS tow 31.17 in the Coral Sea.

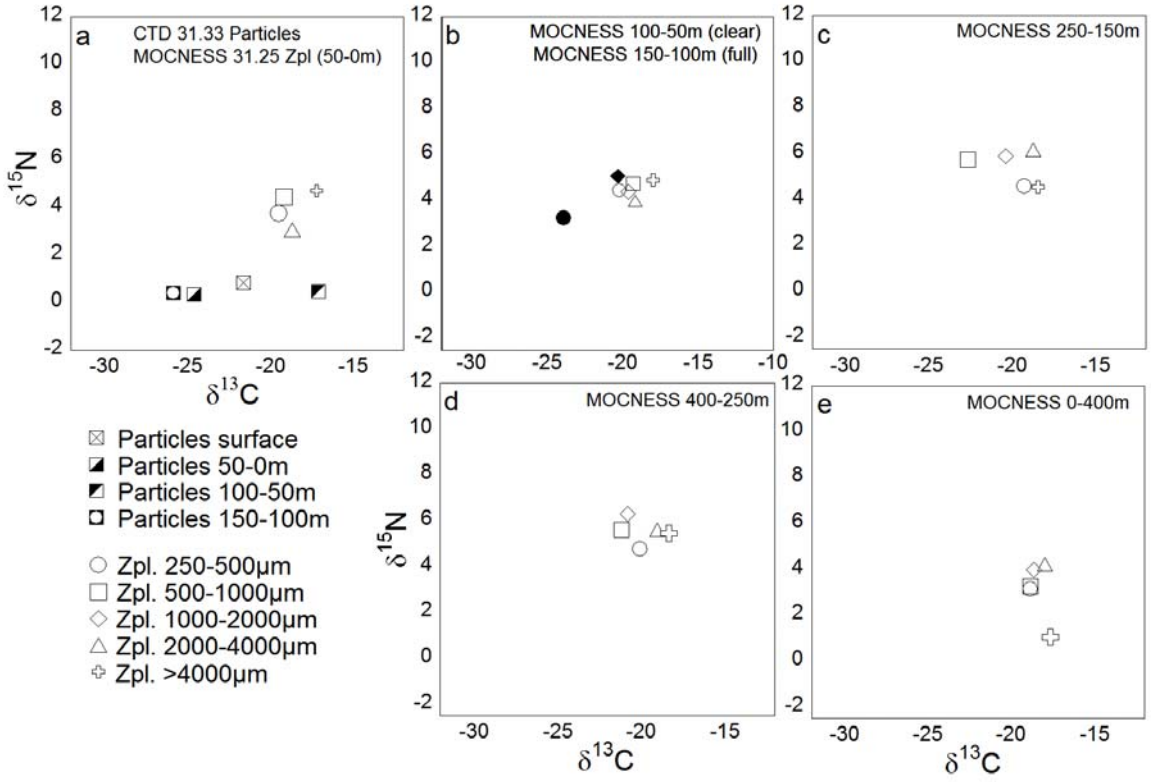

Figure 16: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values from station 031; particles from CTD 31.33 and zooplankton of each depth range from MOCNESS tow 31.25 in the Coral Sea.

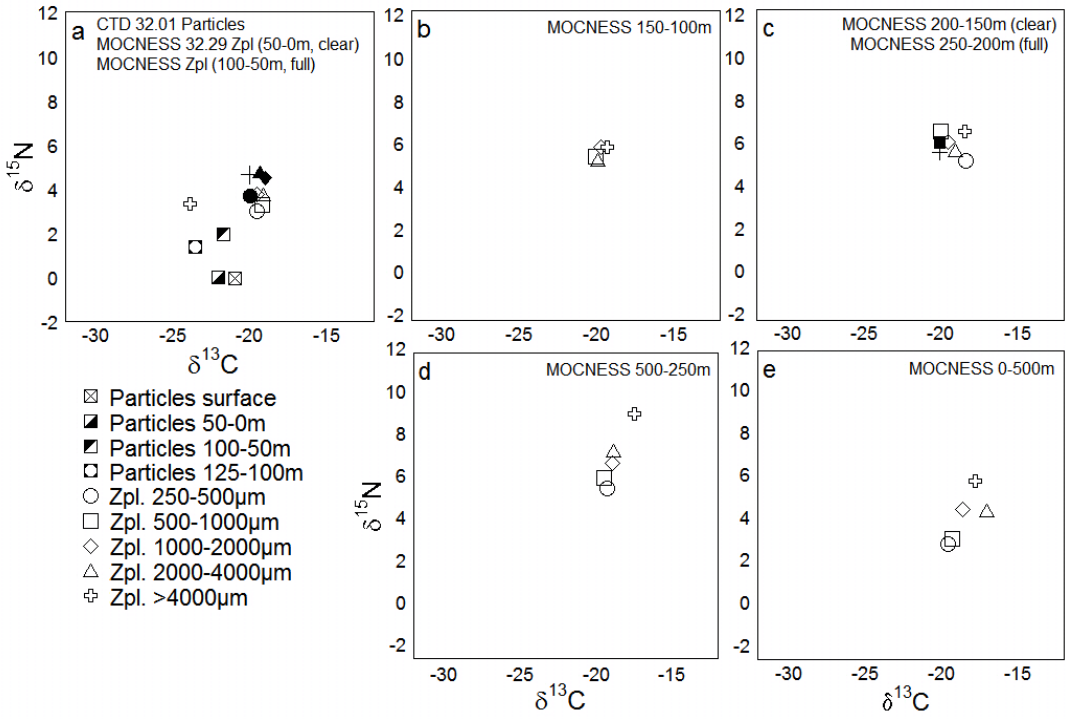

Figure 17: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values from station 032; particle samples from CTD 32.01 and zooplankton samples from MOCNESS tow 32.29 in the Coral Sea.

3.3.2. Mixed Layer

Isotope ratios of C and N showed different patterns of change with depth and location. The $\delta^{15}N$ of zooplankton generally increased from smaller to larger size classes of plankton. At most stations, we found a difference of 2-3‰ between the $\delta^{15}N$ of particles and mesozooplankton. We found no regular pattern in $\delta^{13}C$ of PN and zooplankton, and particle samples with the lowest $\delta^{15}N$ values showed some variation in their $\delta^{13}C$ (integrated values for $\delta^{13}C$ in the mixed layer ranged from -15.89‰ to -24.61‰).

On Leg 1, $\delta^{13}C$ ranged from -23‰ at stations 005 (Fig. 18 d) and 007 (Fig. 18 e) in the eastern Arafura Sea to -19.5‰ at station 13 in the western Arafura Sea (Fig. 18 i). Low $\delta^{15}N$ (0.25‰ and 0.21‰, respectively) values were also found in the Coral Sea in particle samples from the surface (Fig. 18 b, c). The $\delta^{13}C$ of these surface samples were -24‰ and -22.8‰. On Leg 2, $\delta^{13}C$ of particles with low nitrogen isotope ratios were between -20 and -21 ‰ in the western Arafura Sea at stations 023 (Fig. 19 d) and 025 Fig. 19 e). In the Coral Sea, the range in $\delta^{13}C$ was broad, between -21 and -25‰.

We found a difference of up to 5 ‰ between the $\delta^{13}C$ of particles and zooplankton (see Fig. 18 and 19), but the differences tended to be smaller in the Arafura Sea (see Fig. 18 g, h, 11 e, f, g, h). We found no regular variations with stations or size classes in the $\delta^{13}C$ values of zooplankton.

Overall, we have found similar patterns for $\delta^{15}N$ of particles and zooplankton in each longitudinal area on both cruise legs. Maxima in the East Timor Sea and central Arafura Sea were found on both legs as well as the minima in the Arafura Sea and Coral Sea.

Results for $\delta^{13}C$ have shown no consistent longitudinal pattern, and we found no significant spatial correlation between $\delta^{13}C$ and $\delta^{15}N$.

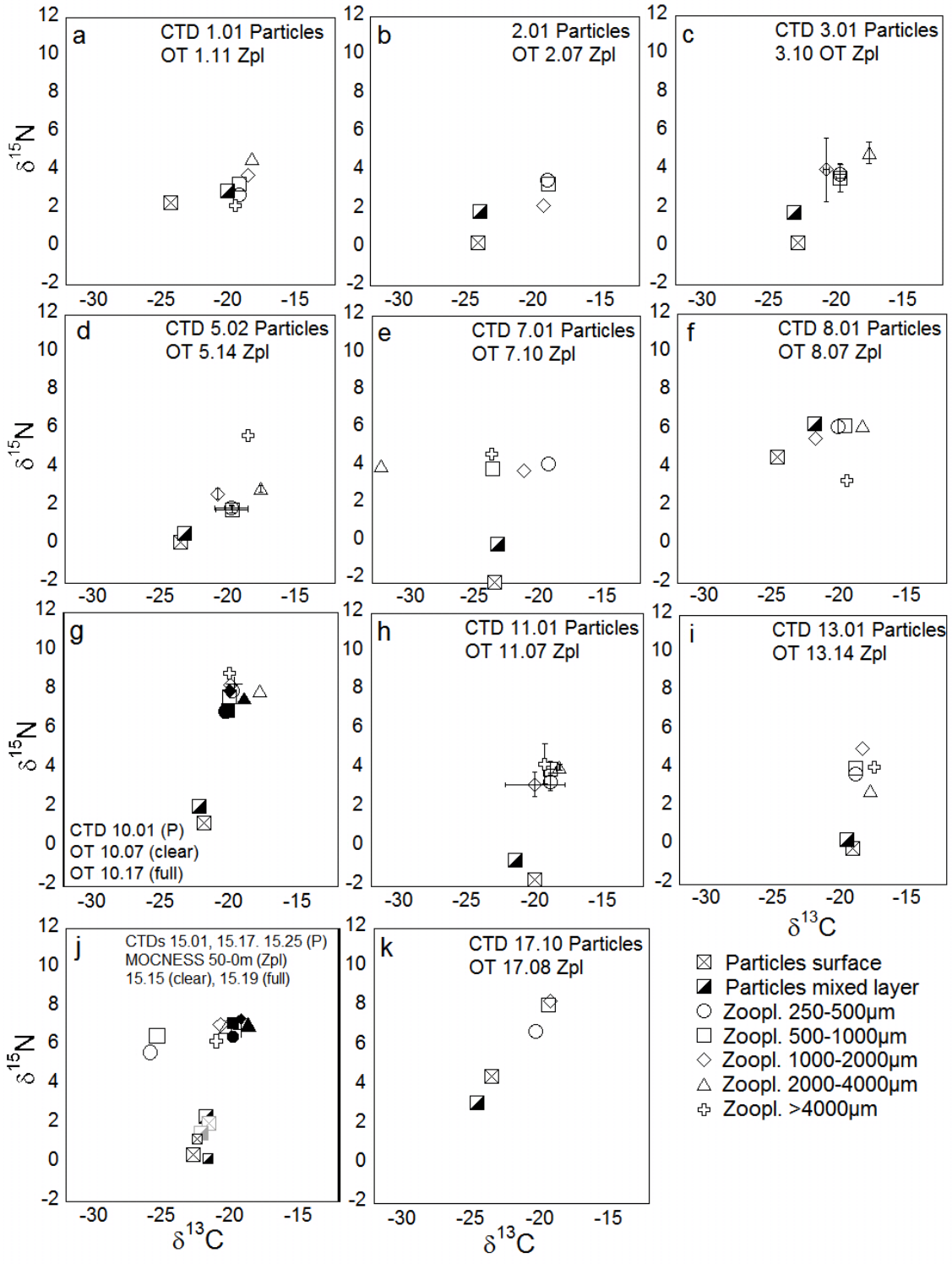

Figure 18: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values of particle and zooplankton samples from the mixed layer of each station on Leg 1.

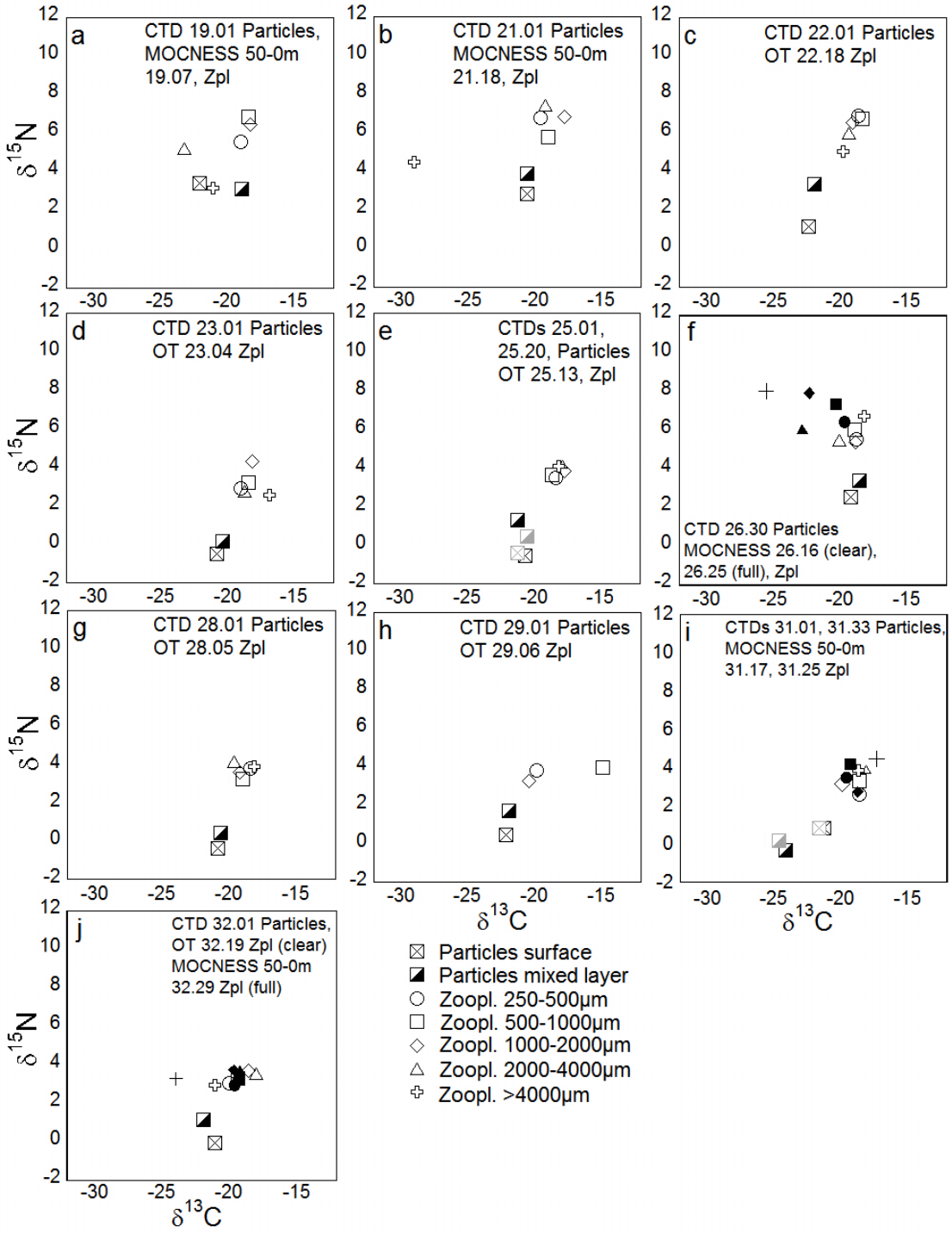

Figure 19: Isotopic cross plots with the $\delta^{15}N$ and $\delta^{13}C$ values of particle (P) and oblique tow (OT) zooplankton samples from the mixed layer of each station on Leg 2

3.4. Diazotroph Contribution to Particles and Zooplankton

Our mass balance calculations showed relatively high contributions of diazotroph nitrogen to particles and zooplankton throughout the cruise range, with the exception of some stations in the central Arafura Sea and the East Timor Sea (Tables 8, 9). On Leg 1 (Fig. 20 a, Table 8), we found an average diazotroph contribution of 51.9% for particles in the mixed layer. The diazotroph contribution to particles ranged between 29.6 and 50.4% in the Coral Sea, whereas local maxima reached 85.5 and 96.1% in the Arafura Sea. These two maxima were separated by a minimum of 0% (i.e., no measurable diazotroph contribution). In the East Timor Sea, diazotroph nitrogen comprised between 15.6 and 79.4% of PN with a decline towards the west.

The different zooplankton size classes were generally similar in their diazotrophic N percentages; the average diazotroph contribution was 28.8% (250-500µm), 32.9 % (500-1000µm) and 32% (1000-2000µm). In the Coral and Arafura Seas, many stations showed diazotroph contributions greater than 50 or even 60% for all three size classes investigated. Geographical distributions of maxima and minima in N content derived from diazotrophs were similar to what we observed for particulate nitrogen. For both particles and zooplankton, we found similar longitudinal patterns between diazotrophic N content in biomass and [PN], but correlations were not significant. We found a similar match between areas of high diazotroph contribution to biomass and trichome abundance, except from stations in the East Timor Sea, where high percentages for particle biomass co-occurred with low trichome abundances.

On Leg 2 (Fig. 20 b, Table 9), the overall patterns were similar to those on Leg 1. Estimated N contributions by diazotrophs to PN were around 48.2% on average. Values

ranged between 11.5 and 25.5% in the East Timor Sea and local maxima of 76% and 80.8% occurred in the Arafura Sea. As on Leg 1, these peaks the percentages were separated by a region with very low diazotroph contribution to biomass. In the Coral Sea, diazotrophs accounted for most of the biomass, with contributions ranging between 62.8 and 89.8%.

As on Leg 1, zooplankton and particles showed very similar spatial patterns in diazotrophic nitrogen content. Average percentages for the size classes of 250-500µm, 500-1000µm and 1000-2000µm were 35.1, 36.7 and 36, respectively. As on Leg 1, the diazotrophic N content of biomass had similar distributions of its maxima as [PN] and trichome abundances (Fig. 20a vs. 20c and Fig. 20b vs. 20d), but there were no significant relationships. In the Arafura Sea and Coral Sea, there were also stations with high percentage contributions (>50%) but low trichome abundances (Fig. 20b, d).

Table 8: Contributions (%) of biological N_2 fixation to nitrogen in particulate and zooplankton biomass in the mixed layer on Leg 1. At station 010, two oblique tows (OT) and at station 015, two MOCNESS tows were made.

	PN	Zpl 250-500µm	Zpl 500-1000µm	Zpl 1000-2000µm
Reference $\delta^{15}N$ values (‰)	4.5	7.2	8.1	8.4
Stn 001 (OT)	29.6	55.5	54.4	50
Stn 002 (OT)	48.5	ND	51.2	54.6
Stn 003 (OT)	50.4	46.2	48.1	48.6
Stn 005 (OT)	72.5	64.8	69.7	61.5
Stn 006 (CTD)	57.8	ND	ND	ND
Stn 007 (OT)	85.5	37.8	46.8	49.5
Stn 008 (OT)	0	12.6	21.6	30.6
Stn 010 (OT)	44.7	0	4.7	0.43
Stn 010 (OT)	ND	4.6	12.2	5.8
Stn 011 (OT)	96.1	47.5	45.4	55.5
Stn 013 (OT)	77.7	43	45.4	35.2
Stn 015 (MOCNESS 50-0m)	39.8	19.3	17.7	14
Stn 015 (MOCNESS 50-0m)	79.4	9.3	10.8	10.6
Stn 017 (OT)	25.8	5.3	0	0
Stn 018 (CTD)	15.6	ND	ND	ND

Table 9: Contributions (%) of biological N_2 fixation to nitrogen in particulate and zooplankton biomass in the mixed layer on Leg 2. At stations 026 and 031 each, two MOCNESS tows were made.

	PN	Zpl 250-500µm	Zpl 500-1000µm	Zpl 1000-2000µm
Reference $\delta^{15}N$ values (‰)	4.5	7.2	8.12	8.4
Stn 019 (MOCNESS 50-0m)	25.5	20.	14.1	20.3
Stn 021 (MOCNESS 50-0m)	11.5	17	14.3	10.1
Stn 022 (OT)	20.8	13.7	15.1	19.5
Stn 023 (OT)	80.9	53.3	54.6	43.6
Stn 025 (OT)	59.3	46.2	49.9	48.6
Stn 026 (MOCNESS 50-0m)	0	20.2	22.9	31.8
Stn 026 (MOCNESS 50-0m)	20.4	8.9	7.2	3.6
Stn 027 (OT)	20.3	0.6	13.3	9
Stn 028 (OT)	76	42.5	54	51.5
Stn 029 (OT)	52.4	42.2	46.4	55
Stn 031 (MOCNESS 50-0m)	89.8	57.2	53.4	56.6
Stn 031 (MOCNESS 50-0m)	80.3	46.2	43.7	ND
Stn 032 (OT)	ND	51	53.6	50.5
Stn 032 (MOCNESS 50-0m)	62.8	52.4	53.8	50
Stn 034 (OT)	ND	55.6	54.9	54.3

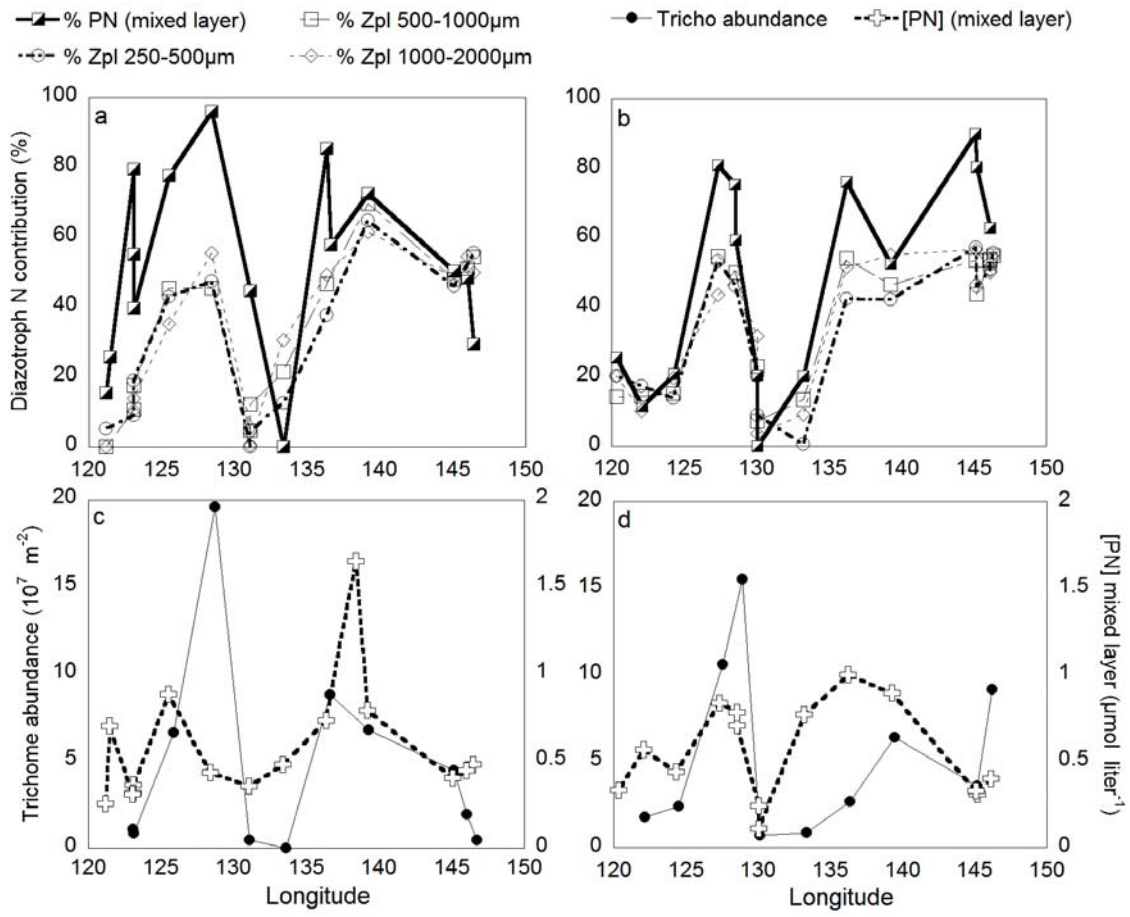

Figure 20: Estimated contributions of N by diazotrophs to particulate and zooplankton biomass on Leg 1 (a) and Leg 2 (b). Graphs c and d show *Trichodesmium* abundances and [PN] on Leg 1 and 2, respectively.

4. DISCUSSION

4.1. Background of this Study

This study focuses on a critical subject in the global marine environment. Nitrogen availability plays a central role in controlling oceanic production, yet we currently know little about the diversity and the biogeochemical and ecological importance of different diazotroph groups. The contribution of recently discovered diazotrophs to new production remains poorly known and will require a combination of molecular and biogeochemical techniques applied in the field. The methods used in this study included N_2 fixation rate measurements, stable isotope ratio analyses, measurements of particulate nitrogen concentrations [PN] and cyanobacterial abundances. We focused on nitrogen fixation rates and contributions of nitrogen to planktonic biomass by two different diazotrophic groups (*Trichodesmium* and picocyanobacteria) in waters north of Australia, where surface NO_x^- concentrations ranged between 0 and 2.2µm (cruise EW9912; Krauk et al. 2006).

Dense aggregations of *Trichodesmium* are commonly found at or near the surface of oligotrophic waters, due to gas vesicles in the cells that provide positive buoyancy (Van Baalen & Brown 1969). Yet, significant amounts of *Trichodesmium* can be found throughout the upper water column, due to wind driven mixing processes and probably also through active migration via buoyancy regulation (Carpenter 1983, Villareal & Carpenter 2003). For unicellular picoplankton, the focus in this study was on the N_2 fixing activity of small cells below 10µm in diameter without further discrimination among different diazotrophs within this size class. Abundance measurements were made

for the most ubiquitous picocyanobacteria genera of oligotrophic waters, *Synechococcus* and *Prochlorococcus*. In contrast to *Trichodesmium*, maximal densities of picocyanobacteria in this and other studies were not found in the upper but rather in the middle and lower range of the euphotic zone in the subsurface pigment maximum between depths of 25 and 50m, depending on the study locations (Montoya et al. 2004, Zehr et al. 2001, Falcón et al. 2004).

Geochemical indices, including the natural abundance of stable carbon and nitrogen isotopes in suspended particulate material and zooplankton, can also provide insight into the role of N_2 fixation in supplying nitrogen to oligotrophic waters. Generally, the $\delta^{15}N$ of primary producers is controlled by the N isotope ratios of the nitrogen source. The low ^{15}N content of atmospheric N_2 and the low isotopic fractionation associated with biological nitrogen fixation produces organic nitrogen with a ^{15}N content lower ($\delta^{15}N$ between 0 and -2‰) than typical marine nitrogen, which reflects the average $\delta^{15}N$ of 4-5‰ for deep water nitrate (Liu & Kaplan 1989, Sigman 2000). This in turn leads to an inverse relationship between high rates of biological nitrogen fixation and $^{15}N/^{14}N$ ratios in plankton biomass (Minagawa & Wada 1986, Saino & Hattori 1987, Carpenter et al 1997, Montoya et al. 2002, McClelland et al. 2003, Holl et al. 2007). Stable nitrogen isotopes also provide information on the trophic position of an organism in a food web since $\delta^{15}N$ increases on average about 3.4‰ from one trophic level to the next (DeNiro & Epstein 1981, Cabana & Rasmussen 1994, Montoya 2007, Montoya et al. 2007). Stable carbon isotope ratios on the other hand provide information on an organism's food source since the increase from one trophic level to another is only about 1‰ (DeNiro & Epstein 1978). As a result, organisms with a similar stable carbon isotope ratio are more likely to

be directly linked in a food chain or to share their main carbon source than would be the case for organisms with greater $^{13}C/^{12}C$ ratio differences. Thus, a combination of these methods can help resolve the relative importance of the various sources of N and C in an ecosystem.

The importance of nitrogen fixation to an ecosystem can be estimated both directly and indirectly. Measuring N_2 fixation rates for *Trichodesmium* or picoplankton in water samples via the $^{15}N_2$ tracer or acetylene reduction assays provide a direct measure. Stable N isotope abundances in organic matter are an in situ tracer of nitrogen fixation on longer time scales. In this case, N_2 fixation is addressed indirectly by estimating its relative importance from the $\delta^{15}N$ of organic matter.

As in any oceanographic study, we have to consider the temporal scales for each process or parameter we measured. Our rate measurements were carried out in experiments lasting hours and therefore provide a snapshot of the instantaneous rate of activity, which may change on a time scale of hours to days. On the other hand, [PN] and $\delta^{15}N$ measurements reflect processes acting over several days or even weeks. Another point to consider is that samples were not taken simultaneously at the various locations. Each cruise leg with its samples represents a series of snapshots taken from different locations at different times. Each area was sampled twice, once on each cruise leg, yet the time intervals between sampling were quite different in the three hydrographic regions of interest (3 to 4 weeks for stations in the Coral Sea versus 2 to 6 days for stations in the East Timor Sea). Apart from the different temporal scales of parameters, these differences in sampling interval have to be taken into account when interpreting the data in spatial terms. At this point, we will focus on correlations among variables rather

than the zonal distribution of properties. Regarding the correlation tests, our data sets were too small to allow robust tests for normality, but pairwise correlations with variables of interest were used as an indicator of general relationships among parameters. .

4.2. Cyanobacteria

Our measurements of cyanobacterial abundances are similar to the findings of a recent study in the SW Pacific Ocean (Campbell et al. 2005), in which *Trichodesmium* blooms were associated with higher *Synechococcus* abundances. The connection between *Trichodesmium* and *Synechococcus* is not entirely clear, but indirect pathways of nitrogen transfer such as extracellular release of amino acids and ammonia (Capone et al. 1994, Glibert & Bronk 1994, Glibert & O'Neil 1999), cell death (Berman-Frank et al. 2004) and probably viral lysis (Ohki 1999, Hewson et al. 2004) are all likely to be involved. These mechanisms will make nitrogen available to other primary producers and heterotrophic bacteria, rather than directly to grazing zooplankton. These extracellular release mechanisms could also help explain why the correlations between [PN] and *Synechococcus* abundances were much higher than between [PN] and *Trichodesmium* abundances. For *Prochlorococcus* abundances, we have found non-significant negative correlations to *Trichodesmium* abundance on Leg 1 and to *Synechococcus* abundances on both legs. The latter observation is well known from earlier studies (Partensky et al. 1999, Letelier et al. 1993). Unlike *Synechococcus*, *Prochlorococcus* abundances were negatively correlated to [PN] on both legs. This suggests a stronger connection between [PN] and the *Synechococcus* abundances, both of which are positively influenced by *Trichodesmium* blooms. The reason for the apparently stronger link between *Trichodesmium* and *Synechococcus* could be due to the

slightly higher growth rates of *Synechococcus* compared to *Prochlorococcus* (André et al. 1999, Crosbie & Furnas 2001). This is of advantage when increasing general productivity causes an increasing grazing pressure.

We have only limited information on the diversity of diazotrophic organisms in our study area, though our measurements provide good constraints on the abundance and activity of *Trichodesmium*. We also know that unicellular N_2 fixers were highly active, at least at some stations sampled on this cruise, though we lack information about their abundance and phylogeny. Marine unicellular diazotrophs are subdivided by *nif*h-sequences into two major groups (Groups A & B; Zehr et al. 2007). Group A N_2 fixers include various unclassified strains but also *Cyanothece* strains. Group B is represented mainly by *Crocosphaera* spp. and strains of *Synechococcus* and *Cyanothece* (Zehr et al. 2007). In our study, high unicellular fixation rates appeared to occur independently of daylight, but we have no information about the identity of the unicellular diazotrophs involved. We only have information about abundances of *Synechococcus* strains without detailed classification, which limits our interpretation. Nevertheless, *Synechococcus* strains have been found in the diazotrophic group B of unicellular cyanobacteria. Since high fixation rates were observed at night and during daylight (as observed for group B in the study of Zehr et al. 2007), it is possible that diazotrophic *Synechococcus* strains from Group B were involved.

4.3. Nitrogen Fixation – Rates, Limitation and Influence on $\delta^{15}N$

For an interpretation of N_2 fixation data, we have to consider the differences between *Trichodesmium* and picoplanktonic diazotrophs. Temporal and spatial patterns of diazotroph activity and abundance are influenced by different environmental factors such

as light, temperature, water mixing, salinity and grazing pressure, but these influences are likely to act differently on each of these two size classes. Unicellular picocyanobacteria are smaller in cell size (which means that the surface/volume ratio is smaller) than *Trichodesmium* and do not form colonies, therefore they are expected to respond more quickly to environmental changes in general. Furthermore, *Trichodesmium* and picocyanobacteria are expected to be linked to the rest of the food web in different ways. There are two ways of biomass flux from primary producers to the rest of the food web. The first way would be via the macroscopic food chain, in which primary producers get consumed by primary consumers; the latter are food for the larger secondary consumers, and this pattern is maintained up to the highest trophic level. The second way of nutrient transfer is via the microbial loop (Pomeroy 1974, Azam et al. 1983), in which primary production of autotrophic organisms and secondary production of heterotrophic microorganisms are connected. The latter utilize dissolved organic matter and inorganic nutrients released by decomposition and metabolism from primary producers, but also from higher trophic levels. Those secondary microproducers are food for microzooplankton grazers as well, so they pass on their biomass via the food chain as well as via the microbial loop.

The palatability of a primary producer is a critical factor for the way of its biomass flux. A toxic organism is more likely to pass on its biomass via the microbial loop than we would expect for a more palatable organism. Thus, a toxic primary producer is not grazed as much but releases more nutrients into its environment in the form of metabolic products and by decay of its dead cells. This is of benefit for other primary producers and for secondary producers. A palatable primary producer also releases nutrients and

metabolic products, but a larger fraction of its biomass gets utilized directly by grazers. These factors demonstrate how physiological traits such as toxin production can be important in development of populations of different group of diazotrophs. For example, *Trichodesmium thiebautii* is toxic to most grazers, but known unicellular cyanobacteria may be more exposed to grazing pressure, based on the high grazing rates reported for known *Synechococcus* species (Tsai et al. 2005, Worden & Binder 2003, Landry & Kirchman 2002; Caron et al. 1991). Then we would expect N fixed by *Trichodesmium* and picocyanobacteria to follow different pathways through the food web. The two diazotroph types are also likely then to show different population responses to grazing pressure. The much smaller size of picocyanobacteria is also critical because of the different possibilities for nutrient storage and nutrient uptake. A more efficient nutrient uptake due to a larger surface/volume ratio allows a quicker response by growth to increasing nutrient levels. Furthermore, fast growing populations of small organisms can deplete nutrients locally, making growth for larger organisms with lower division rates more difficult.

Another factor causing different population cycles is the growth rate of each group. *Trichodesmium* is known for rather low rates of 0.12 to 0.16 day^{-1} under optimal growth conditions (high temperature and salinity, sufficient Fe and P supply for growth, stratified water column with little water movement, see Mulholland & Capone 1999, LaRoche & Breitbarth 2005), whereas growth rates can be a magnitude higher for *Synechococcus* spp. (Crosbie & Furnas, 2001) and about two fold higher for *Crocosphaera* spp. (Tuit et al. 2004, JGI web database). For this reason and the different grazing pressures noted above, blooms of *Trichodesmium* can be expected to grow more slowly but to last longer

than those of unicellular cyanobacteria. For that reason, the isotopically light N fixed by *Trichodesmium* will be transferred into the food web over a much longer time span than would be the case for the shorter blooms of unicellular cyanobacteria. Due to the longer bloom period, the overall contribution of biologically fixed new nitrogen may also be much larger. This may explain why variables that integrate over longer time frames (such as [PN] or $\delta^{15}N$) are more strongly correlated to *Trichodesmium* than to picoplankton abundances in our results.

The N_2 fixation rates of *Trichodesmium* showed similar longitudinal distributions of maxima and minima on each leg of the cruise. Correlations between N_2 fixation rates of *Trichodesmium* and trichome abundances were found on both legs, particularly high and significant on Leg 1. On the other hand, the East-West pattern of maximal N_2 fixation rates in our study does not show significant correlations to the distribution of minimal $\delta^{15}N$ and maximal [PN]. This may reflect the different time scales that these variables integrate. Nitrogen fixation rates can alter within hours, but due to the release mechanisms and limits in uptake and assimilation efficiency of other primary producers and associated bacteria, it may take days until changes in N_2 fixation rate are mirrored in the isotope ratios of particulate and zooplankton biomass.

The indirect (geochemical) approach of estimating the importance of N_2 fixation by $\delta^{15}N$ of biomass has brought conclusive results in many oligotrophic waters. Earlier studies in the tropical North Atlantic have proven the correlations between high *Trichodesmium* abundances and low ^{15}N contents of particulate organic nitrogen (Altabet 1988, Carpenter et al. 1997, Dore et al. 2001) and both particulate organic nitrogen and zooplankton (Montoya et al 2002), respectively. Most studies on these relationships

were made in open ocean systems with a stratified water column and conditions more predictable and homogenous than in the waters of the EW 9912 cruise. In these waters between Indonesia and New Guinea in the north and Australia in the South water mixing in the euphotic layer is supposed to be different, due to shallow depths and variable water-motion creating forces (wind, water currents) from different directions. Due to these different mixing conditions, patchiness in the water column is also likely to be more important than in the ocean gyres.

Upon consideration of the conditions explained above, the correlations (even though they are low) between N_2 fixation, trichome abundance and [PN] we found can be seen as additional evidence for the influence of N_2 fixation by *Trichodesmium*. Still, the geographical overlap and inverse correlation between high trichome abundances and the $\delta^{15}N$ values of PN and zooplankton provide the best evidence for a significant N contribution by *Trichodesmium* in the North Australian waters. Regarding picoplanktonic N_2 fixation, we have found considerable rates at two stations on Leg 1. On Leg 2, the highest rates occurred at two stations in the central Arafura Sea, moderate and low rates were found in adjacent stations. The first station in the Arafura Sea with high picoplanktonic nitrogen fixation activity (station 026 at 130.10 E) was near MOCNESS tows 26.16 and 26.25 at 130˚06' E, and the high N_2 fixation rates measured co-occurred with high $\delta^{15}N$ of particles and zooplankton samples in CTD/rosette casts 26.16, 26.25 and 26.30 (>6‰) at all depths. Yet, at station 028 (136^{O} E) even higher fixation rates were geographically associated with low $\delta^{15}N$ around 0‰ for particles and 3-4‰ for the different size classes of zooplankton from an oblique tow. Since we lack abundance data for unicellular diazotrophs, we cannot say anything about a possible

bloom that may have started around the sampling time. For these unexpectedly high N isotope ratios in areas of high N_2 fixation at station 026, denitrification might also play a role. This process causes high $\delta^{15}N$ values in nitrate, which can increase $\delta^{15}N$ of organisms in the food web significantly. Denitrification might occur near the bottom and in the sediments of shallow shelf waters with high input of sinking organic matter, where oxygen concentrations can become low enough to promote NO_3^- reduction (Nishio et al. 1982). Denitrification also depletes the N pool in the water column and therefore increases the need of new nitrogen by biological N_2 fixation.

Among the general environmental factors discussed so far, the activity of picoplankton is likely to be sensitive to a different set of environmental factors than the activity of *Trichodesmium*. Furthermore, oxygen and therefore photosynthetic activity is known to interfere with nitrogenase activity (e.g. Fay 1992), and diazotrophs have various mechanisms to overcome this obstacle, one of which is a temporal separation between nitrogen fixation and photosynthesis (e.g. Mitsui et al, 1986). This is a strategy also used by Group B unicellular N_2 fixers. Group A cyanobacteria, on the other hand, are particularly active during day. It is not clear at this time how small unicellular organisms can maintain photosynthesis and N_2 fixation without any apparent spatial or temporal separation of the processes (Zehr et al. 2007). Another factor that might alter activity, particularly for diazotrophs well below the surface, is a changing supply of sunlight. Surface light irradiance and hence the light supply for primary producers can vary with water movements (Banse 1987). Therefore, if light-regulated (and not diurnal cycling) separation of the two processes were a mechanism for a significant group of

unicellular diazotrophs, temporal variations in sunlight irradiance might alter photosynthesis and N_2 fixation rates.

Nitrogen fixation is one possible nitrogen source for diazotrophs, but switching to an energetically less expensive method to meet nitrogen demand is possible. The relative energetic costs of nitrate uptake/assimilation and N_2 fixation remain unclear (Karl et al. 2002), but *Trichodesmium* does reduce its N_2 fixation rate and take up nitrate when given the opportunity (Holl & Montoya, 2005).

Usually, different ecotypes exist within a genus, and each has adaptations to a certain niche of light or nutrient exploitation (e.g. Moore et al. 2002, Partensky 1999). Different ecotypes within genera or groups of unicellular diazotrophs may be involved in the changing patterns of various diazotroph populations and activities we observed. Interspecific interactions such as competition may be important as well. In fact, N_2 fixation rates of picoplankton were particularly high where N_2 fixation rates and abundances of *Trichodesmium* were low, which may reflect the same sort of niche separation reported by Montoya et al. in the North Atlantic (Montoya et al. 2007). For a better understanding of such observations, it will be an important task for the future to find out more about the interactions between *Trichodesmium* and unicellular diazotrophs.

4.4. Information from $\delta^{13}C$

Carbon enters the biosphere via photosynthesis. In this process fractionation of carbon isotopes occurs, mainly because $^{13}CO_2$ is for energetic reasons less readily used by enzymes during the carbon assimilation process. This is why primary producers generally have a lower ^{13}C content than inorganic C. Atmospheric CO_2 has generally a $\delta^{13}C$ between -8.2 and -7.4‰ (Carbon Dioxide Information Analysis Center,

http://cdiac.ornl.gov) yet primary producers have $\delta^{13}C$ values between, -25 and -9‰ (Schidlowski 1983), depending on carbon fixation pathway, carbon supply and other environmental parameters. Growth rate and cell size (in case of phytoplankton) are important in determining the extent to which inherent isotopic fractionation effects are expressed. In several studies, high growth rates have led to increased ^{13}C contents in primary producers due to a smaller isotope fractionation during rate-limiting steps of the carbon assimilation (Popp 2005, Gu & Schelske 1996, Fry 1991, Fry & Wainwright 1996).

When primary producers are consumed and enter the food web, the consumer takes up carbon by feeding and loses carbon by respiration. These processes are accompanied by little isotopic fractionation, resulting in a small enrichment of ^{13}C isotopes within the organism. This enrichment causes a general increase in $\delta^{13}C$ of up to 1‰ between an organism and its food source. Thus, consumer and food source are in general similar in their $\delta^{13}C$ (DeNiro & Epstein 1978), making $\delta^{13}C$ values helpful in tracking down biomass of a certain organism group with a well-known C isotope ratio in the food web.

Stable isotope ratios for carbon showed no consistent spatial pattern in our study area, but are consistent with our current knowledge of nutrient flows of diazotrophs. Results from earlier studies revealed relatively high $\delta^{13}C$ for *Trichodesmium* between -15 and -12‰ (Carpenter et al. 1997). This enrichment in ^{13}C appears to reflect diffusion limitation of primary production in the large colonies sampled, which reduces the overall discrimination against heavier isotopes (Korb et al. 1996) and increases the $\delta^{13}C$ of the organic matter produced. Single trichomes or smaller colonies collected on the EW 9912 cruise had ratios between -17 and -23‰ (primary data, see Table 2). In our water column

samples, particulate material with the low $\delta^{15}N$ values typical of N_2 fixers (between 0 and -2‰) generally has a much lower $\delta^{13}C$ than has been reported for large *Trichodesmium* colonies (see Table 10). Our values for particulate biomass range between -25 and -19‰ and have an average value of -22‰, which is still somewhat lower than the values we measured for small colonies and single trichomes. Normally, there is a rich microbial community associated with *Trichodesmium* colonies (O'Neil & Roman 1991, Nausch 1996), primary and secondary producers. They benefit directly from the release of dissolved inorganic and organic nitrogen by Trichodesmium, but indirectly, the entire ecosystem is supplied with nitrogen and carbon fixation is amplified by the process of nitrogen fixation. The fauna and flora associated with *Trichodesmium* may have the extracellular nitrogen release of *Trichodesmium* as a main nitrogen source but, apart from *Trichodesmium*, other organisms as significant carbon sources. We have to consider the large amount of primary producers that benefit from the nitrogen release by *Trichodesmium* colonies. During a study in the Arabian Sea (Capone et al 1998), about one quarter of carbon fixation in the water column could be accounted to the *Trichodesmium* bloom. Even though it is a large contribution from one single species, other primary producers are of critical importance, too.

We do not yet have any information on the $\delta^{13}C$ or $\delta^{15}N$ values of unicellular diazotrophs. At the stations with the highest rates of picoplanktonic N_2 fixation, we found $\delta^{13}C$ values between -20 and -19‰ at the surface and between -18‰ and -21‰ in the mixed layer. These values are close to the data mentioned above for small colonies and single trichomes of *Trichodesmium*, yet it is too early to use this information for an interpretation of picoplankton $\delta^{13}C$ values. Future research will bring progress in specific

unicellular diazotroph phylogeny and abundance measurements in sampling areas. Then stable carbon isotope ratios of picoplanktonic diazotrophs in culture and isolated from in-situ samples may give helpful information to track down the ecological and biogeochemical pathways of nitrogen fixed by picocyanobacteria.

Table 10: Carbon isotope ratios of small picked *Trichodesmium* colonies in comparison of data from stations with high abundances and N_2 fixation rates of *Trichodesmium*.

Station	$\delta^{13}C$ (‰) of picked colonies	Station	Integrated $\delta^{13}C$ (‰) of mixed layer particles
002	-17	003	-23.1
003	-18.1	005	-23.2
005	-16.6	007	-23.2
021	-17.9	011	-21.4
025	-18	013	-19.51
026	-20.1	023	-20.3
031	-18.9	025	-21.3
031	-23	029	-21.9
032	-19.8	031	-24.1
032	-19.2	032	-21.9
Mean (SD)	-18.9 (1.8)	Mean (SD)	-22 (1.4)

4.5. Contributions of Diazotrophs to the N Pool

Our estimates of the diazotrophic contribution of N to the food web are based on a mixing model that integrates the $\delta^{15}N$ of diazotrophs, the $\delta^{15}N$ of samples and the $\delta^{15}N$ of nitrate. Diazotrophs are represented by the usage of the lowest $\delta^{15}N$ measured. Another part of the integration is the average $\delta^{15}N$ for deep water nitrate. Thus, the gain of new nitrogen by upwelling nitrate and the gain by biological fixation are taken equally into account. It is a simplified model, and we did not consider water mixing processes. Metabolic processes that may influence $\delta^{15}N$ of nitrogen passing through an organism are not considered either. Yet, such simplifying calculations with realistic reference values are helpful in estimating the importance of different nitrogen sources. Our confidence interval is based on a typical analytical confidence interval of ± 0.2‰ for a $\delta^{15}N$ measurement. Our particle sample at station 006 had a $\delta^{15}N$ of 1.8‰, which corresponded to a diazotrophic N contribution to particulate nitrogen of 50.4% (see Table 9). Our analytical error of ± 0.2‰ yields a range between 46.7% and 54.1% for the estimated diazotrophic contribution to PN. We conservatively estimate that our calculations have an error of roughly ±3.5%.

Our reference $\delta^{15}N$ for deep water nitrate (4.5‰) is based on measurements from the Southern Ocean (Sigman et al. 2000), the East China Sea (Liu & Kaplan 1989) and in an upwelling area near the Vietnamese coast in the South China Sea (Loick et al. 2007). We recognize that denitrification (Saino & Hattori 1987) may create local variations in $\delta^{15}N$, but our approach is a conservative one in the context of this study. For example, if nitrate $\delta^{15}N$ values were increased by denitrification, our approach would underestimate the contribution of diazotrophs to PN. Specifically, if we replace the reference value of

4.5‰ for nitrate with a value of 6‰, our estimate of the diazotroph contribution to PN would increase from an average of 51.9 to 60.9% on Leg 1 and from 48.2% to 57.4% on Leg 2 of the cruise. Our results from the mixing model suggest that diazotrophic N accounts for roughly 50% of the total N in particulate organic nitrogen and between 28 and 37% of zooplankton biomass in the tropical waters north of Australia. The longitudinal overlap of high percentages of diazotrophic N with areas of high [PN] and Trichome abundances in our study area supports the importance of diazotrophic activity as a new nitrogen source to the entire food web in these waters. At this point our data suggest that among the two diazotroph groups of our study, *Trichodesmium* dominates the flux of new nitrogen to the pelagic biomass in the study area. For picocyanobacteria, we need to get more knowledge about their activity patterns and their typical isotopic ratios for carbon and nitrogen before we can start estimating their contributions to the food web.

Different diazotrophs are likely to pass their biomass (and therefore nitrogen) to the macroscopic food web through different pathways, due to the higher grazing pressure on unicellular picocyanobacteria relative to *Trichodesmium*. If a large amount of their biomass is grazed, isotopically light N from diazotrophs gets assimilated directly into microzooplankton biomass. It is obvious that we are dealing with a different pattern here, more diffuse than what we know for *Trichodesmium* and therefore harder to track isotopically.

We have found geographical patterns of diazotroph N percentages and trichome abundances that match in general, but not completely. There were stations with high diazotroph N percentages for particles but low trichome numbers in the East Timor Sea

on Leg 1. On Leg 2, high percentages for both particles and zooplankton biomass overlapped with low trichome abundances as well at stations in the Arafura and Coral Seas. At this point, two explanations are possible. The first would be that samples were taken at the end of a *Trichodesmium* bloom period, which would result in low $\delta^{15}N$ and high [PN] as a result of a longer period of high productivity and an input of ^{15}N-depleted nitrogen into the ecosystem. But due to the poor conditions for *Trichodesmium* cells at the end of the bloom, there would be low activity rates and abundances for *Trichodesmium*. Another explanation would be that in these areas, unicellular diazotrophs made a significant contribution to the local organic N pool.

4.6. Summary

We have found coherent patterns in N_2 fixation activity and stable isotope abundance that reflect the impact of different diazotrophic groups on the N cycle. Our results for picoplankton and *Trichodesmium* show that the influences of their nitrogen fixation activities cannot be tracked in the same way, due to critical biological differences. Furthermore, we have gained a better idea about the relative contributions of diazotrophs to the biologically active N pool in the oligotrophic waters between Indonesia and Australia. This study has proven the ecological and biogeochemical importance of diazotrophs in a region of the Indo-Pacific area. Future research can benefit from the information about the activities of different diazotrophic groups when it comes to define their distribution and imprint of their environment further.

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