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Full Length Research Paper

Distribution and abundance of the cyanobacterium *Richelia intracellularis* in the coastal waters of Tanzania

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The filamentous heterocystous cyanobacterium, *Richelia intracellularis* Schmidt have been suggested to be among the most important nitrogen fixing cyanobacteria in tropical and subtropical waters, but they are less studied in the tropical Western Indian Ocean waters. The spatial and temporal distribution of this cyanobacterium was studied in the coastal waters of Zanzibar and Dar es Salaam, Tanzania. The *Richelia* sp. was found as an endosymbiont within five species of diatom *Rhizosolenia* spp., two *Hemiaulus* species and rarely as epiphyte to *Chaetocerus* spp. or freely in waters. The morphology and sizes of *Richelia* sp. did not show big variations but the number of vegetative cells per filament ranged from 4 to 14 cells. Abundance of *Richelia* sp. ranged from zero in some samples to mean maximum of 428 ± 105 filaments l^{-1}. The diatom–diazotroph associations were found throughout the year peaking during southeast monsoon. Blooms (up to 1554 filament l^{-1}) of *Richelia* sp. were recorded in July to August 1993. High rates of nitrogen fixation occurred during northeast monsoon with maximum value (2.75 ± 0.03 nmol N $h^{-1}l^{-1}$) in February corresponding to high numbers of *Trichodemium* spp. Lower values were obtained during southeast monsoon with the lowest value (0.03 ± 0.005 nmol N $h^{-1}l^{-1}$) recorded in August when the number and type of cyanobacteria including *Richelia* sp. was very low. The results clearly indicate that *Richelia* sp. may contribute significantly to the productivity of the studied waters through its ability to fix nitrogen.

Key words: Cyanobacteria, *Richelia Intracellularis*, symbiosis, nitrogen fixation, Western Indian Ocean.

INTRODUCTION

In marine tropical and subtropical open oceans, the heterocystous cyanobacterium *Richelia intracellularis* together with trichomatous, bundle forming non-heterocystous cyanobacterium *Trichodesmium* spp. have been considered as the most important primary producers (Venrick, 1974; Capone et al., 1997; Lugomela et al., 2001; Capone et al., 2005; Foster et al., 2009). These two cyanobacteria genera have also been considered the dominant nitrogen fixing plankton in marine tropical oceans and contribute a major fraction of new nitrogen input to the oligotrophic surface waters (Carpenter and Romans, 1991; Gallon et al., 1996; Karl et al., 1997; Capone et al., 1997, 2005; Zeev et al., 2008).

The genus *Trichodesmium* is the most abundant and widely studied based on their morphology, genetic and physiology (Thajuddin and Subramanian, 2002). For instance, along the Western Indian Ocean (WIO) region, Lugomela et al. (2001) reported phytoplankton primary production to range from 204 - 4,142 mg C m^{-2} day^{-1} in Tanzania, with major fraction of it coming from cyanobacteria. The mean rate of carbon fixation by *Trichodesmium* spp. was estimated to the order of 1.15 ± 0.3 ng C $trichomes^{-1}$ h^{-1} in the upper 5 m depth (Lugomela et al., 2002). The *Trichodesmium* species alone contributed about 0.03% of the total CO_2 fixation during South East Monsoon period when their abundance is low, and up to 20% during North East Monsoon when its abundance is high. In addition, Lugomela et al. (2002) reported rates of nitrogen fixation by *Trichodesmium* species to be in the order of 1.8 ± 1.6 pmol N $trichome^{-1}$ h^{-1} giving an average nitrogen fixation of 42.7 mmol N

$m^{-3}y^{-1}$. These estimates were within the range of those reported elsewhere (e.g. Carpenter et al., 1987; Kromkamp et al., 1997). The fixed nitrogen has been shown to be subsequently transferred to bacteria, other phytoplankton as well as zooplankton (Bryceson and Fay, 1981; Glibert and Bronk, 1994; O'Neil et al., 1996). However, little is known about the occurrence, diversity and contribution of *Richelia* species to the primary productivity in the WIO region, though is the only known heterocyst-forming cyanobacterium that occurs as plankton in tropical or subtropical marine waters.

The genus *Richelia* comprise of only one species *R. intracellularis* Schmidt (see images in result section) which is a filamentous with a polar heterocyst and a filament of three to ten vegetative cells (Geitler, 1932). It has been reported as endophyte in several species of the diatom genus *Rhizosolenia*, and *Hemiaulus* or as an epiphyte (rarely endophyte) in species of *Chaetocerous* and *Bacteriastrum* as well as free filaments (Bryceson, 1977; Lugomela, 2002; Gomez et al., 2005). *R. intracellularis* is often referred to as a member of the family Rivulariaceae based on the polarity of its filament, with single terminal heterocyst. Also, Janson et al. (1995) showed presence of phycoerythrine pigment in their heterocyst which is another characteristic of members of the family Rivulariaceae. However, Janson et al. (1995) argued that since the heterocyst are much larger than the vegetative cells and due to the absence of a tapering trichome, *Richelia* is closely related to the genera *Anabaena* and *Nostoc* than to members of the Rivulariaceae. In addition these two genera are the most frequently observed in symbiosis with other plants.

The symbiotic relationship between diazotrophic cyanobacterium *R. intracellularis* and diatoms enhance survival through mutual benefits for both partners. Experiments with laboratory cultures of *Rhizosolenia clevei* - *Richelia* symbiont showed that the cyanobiont could support growth in nitrogen depleted media, while in nitrate stuffed media the host could grow without its cyanobacterial symbiont (Villareal, 1990). This kind of experiments showed clearly that the cyanobiont is fixing nitrogen and the fixed nitrogen is liberated to the host. The presence of nitrogenase exclusively in heterocyst enables the *Richelia* to fix nitrogen during day time concurrently with photosynthesis (Janson et al., 1999). The ability to utilize molecular nitrogen would seem to give nutritional advantage to the *Richelia* sp., perhaps also to the host diatom.

In the tropical North Atlantic, extensive Nitrogen fixation by blooms of diatoms and *Richelia* sp. were estimated to produce nearly 70% of total N demand in surface waters (Carpenter et al., 1999; Janson et al., 1999). Similarly, Zeev et al. (2008) estimated Nitrogen fixation rates by *Richelia* sp. averaging 1.0 nmol Nl^{-1} day^{-1} which comprises 50 to 70% of total N-fixation in Eastern Mediterranean Sea. Also, Mague et al. (1974) observed high abundance of *Richelia* in *Rhizosolenia* spp. and estimated a total nitrogen fixation rate of approximately 800 µg N m^{-2} day^{-1} in central Pacific Ocean. In general, these studies point out the role of the *Richelia* sp. that they contribute substantially to the primary productivity of marine oligotrophic tropical and subtropical waters.

In Western Indian Ocean waters along the Tanzanian coast previous observation indicated the presence of *R. intracellularis* in diatoms *Rhizosolenia castracenei*, *R. clevei*, *R. setigera* and *R. styliformis* and in *Hemiaulus* spp., also attached epiphytically to *Chaetoceros* spp. and in few occasion found as free living (Bryceson, 1977; Lugomela et al., 2001; Lugomela, 2002). However, their special and temporal dynamics of this species has not been reported. This study therefore focused on the temporal and spatial dynamics of the filamentous nitrogen-fixing cyanobacterium - *R. intracellularis* off the Zanzibar and Dar-es-Salaam coasts of Tanzania, Western Indian Ocean.

MATERIALS AND METHODS

Sampling sites

Samplings were done in coastal waters of Zanzibar Island and Dar es Salaam in 1993/4 and 2008/9, respectively. Data collection in Zanzibar was conducted from March 1993 to February 1994 at an offshore station located at approximately 6° 9' S and 39° 9' E, west of Zanzibar town (referred to as site Z) with a depth of about 30 m. In Dar es Salaam, samplings were done from September 2008 to August 2009 at an offshore station (with a depth of about 40 m) located at approximately 6° 40' S and 39° 17' E, here referred to as site D (Figure 1). The differences in sampling time and stations were mainly due to financial constraints. However, the two station experience similar climatic characteristics as reported in various studies (Lugomela et al., 2002) and therefore are expected to show similar characteristics. The climate of Tanzania is tropical; with air temperatures in the coastal regions rarely below 20°C. Seawater temperature fluctuates between 25 and 30°C. The air is humid with an annual average precipitation ranging from about 1100 to 1500 mm. The dominant factors determining the hydrography of the East African Indian Ocean are the monsoon trade winds. The northeast monsoon (NEM) persists from December to April and the southeast monsoon (SEM) from June to October. The months of April/May and October/November may be considered as inter-monsoon periods (Newel, 1959; Lugomela et al., 2002). The NEM is characterized by higher temperature, lower wind speed and calmer sea. Tides in this area are semi-diurnal with a spring tidal range of approximately 3 m.

Physical and chemical parameters

Temperature and salinity of the seawater were measured *in situ* at each sampling site during samplings using a mercury thermometer and a hand-held refractometer (Salt Refractometer 300011 SPER SCIENTIFIC - China), respectively. Nutrient concentration (nitrate, nitrite and phosphate) was measured from the seawater samples after filtration using a suction pump equipped with filter papers (0.45 µm - GF/C). The filtered samples kept in cool box with ice and upon return to the laboratory, they were briefly stored at -20°C before analysed for nutrients concentrations according to Parsons et al. (1989).

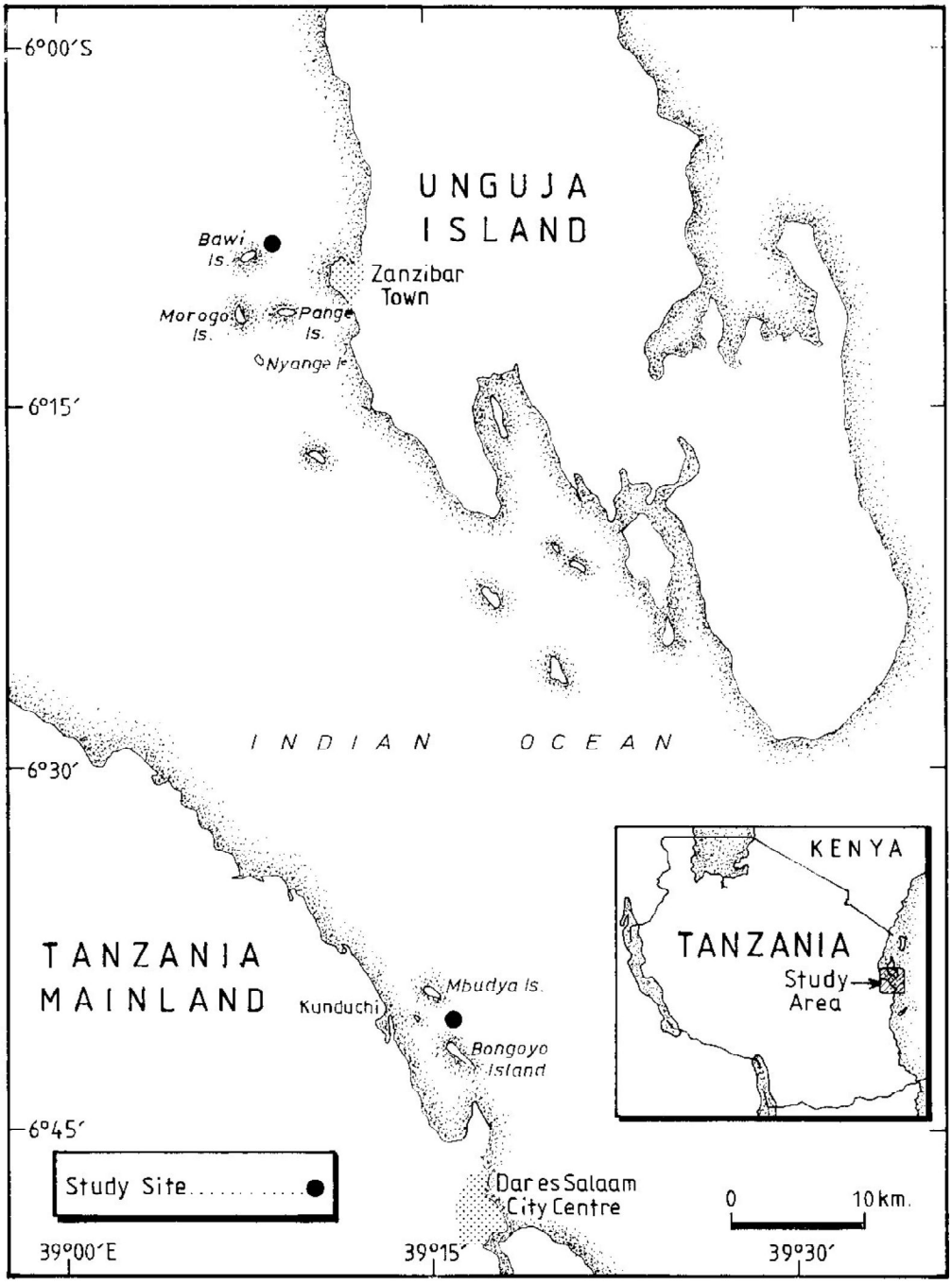


Figure 1. A map showing the study sites at Dar es Salaam (site D) and Zanzibar (site Z).

Distribution and abundance of *Richelia* sp.

In 1993/4, triplicate samples for seasonal phytoplankton abundance were collected at 1 to 2 week intervals by concentrating 10 to 20 L of seawater from the desired depths (that is 0, 5, 10, 15 and 20 m depth) with a 20 µm mesh size plankton net. In 2008/9 integrative net samples were collected by vertically hauling plankton net (30 cm in diameter, 50 µm mesh size) from a depth of 20 m. The concentrated samples were poured into 100 ml plastic vials and immediately preserved in 4% borax buffered (pH 8.0) formalin. Counting of filaments of *Richelia sp.* in *Rhizosolenia* spp. and *Hemiaulus* spp. was done under a light microscope (Olympus BH2, Japan) using a Sedgewick rafter cell. However, the *Richelia* sp. in *Hemiaulus* spp. could only be seen and counted using fluorescent light of the same microscope.

Nitrogen fixation

Nitrogen fixation rates were measured during 1993/4 samplings by

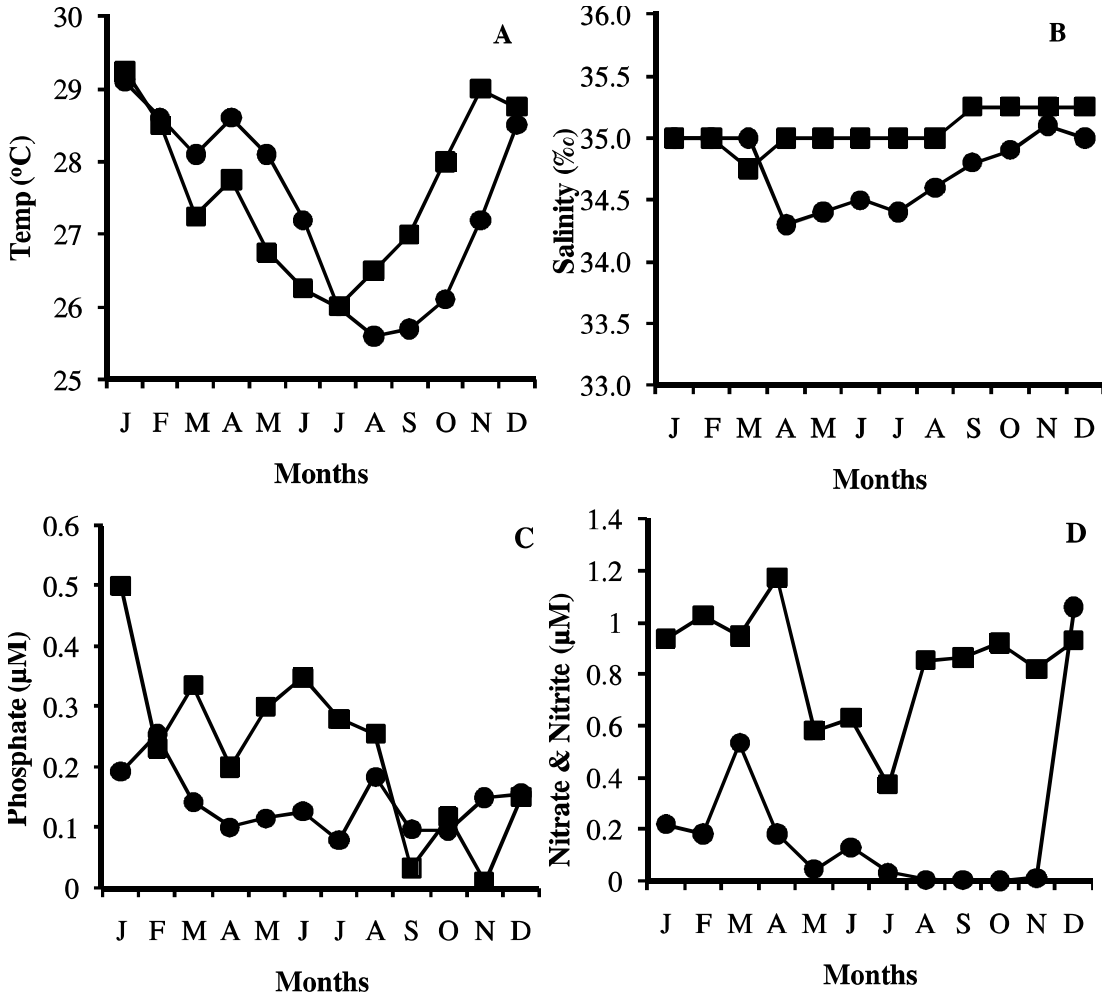


Figure 2. Seasonal variation of the measured physical chemical parameters in the sampling sites of Dar es Salaam (Squares) and Zanzibar (circles). (A) Temperature (B) Salinity (C) Phosphate concentration and (D) Nitrate and nitrite concentration.

the Acetylene Reduction Assay (ARA) as described by Capone (1993). To estimate rate by cyanobacteria, 10 l of surface seawater samples were taken and concentrated by plankton net (20 µm) to about 20 ml. The concentrates (4 ml) were then transferred to three 10 ml glass serum bottles, sealed by rubber stoppers and capped with aluminum caps. To estimate bacterial N_2-fixation, 500 ml of the filtrate was then filtered through 0.22 µm membrane filters, which retain bacteria. The filters, presumed to contain bacteria, were then suspended into 4 ml filtered seawater in 10 ml serum bottles as above. Ten percent of the air phase was withdrawn from the bottles and replaced with an identical volume of acetylene gas generated from calcium carbide. Samples were incubated *in situ* for ca. 2 h in a floating cage. Thereafter, 0.55 ml of gas was withdrawn from the gas phase by gas tight syringes, sealed in a rubber stopper and within 1 to 2 h injected into a Gas Chromatograph (GC). The GC was equipped with a Porapack N column with airflow of 50 to 75 cm^3 min^{-1}. The amount of ethylene produced was calculated as described by Capone (1993). Known concentrations of ethylene gas served as standard. In addition, each vial for cyanobacteria assay was opened and the concentrate was analyzed for cyanobacteria species and numbers using light microscope as described above.

Statistical analysis

Statistical tests were carried out using Graph Pad Instant t_m 1990 to 1993 software. Two-way analysis of variance (ANOVA) and *t*-test were used to analyse variables. Prior to the analysis, the data were subjected to normality and homogeneity of variance tests. Where assumptions for parametric test were not met, alternative non-parametric tests were used. P-values less than 0.05 were considered to represent significant differences.

RESULTS

Physical and chemical parameters

The monthly variations of physical and chemical parameter are shown in Figure 2. Seawater temperature ranged from an average of 29.3 ± 0.35°C in January at Dar es Salaam site (site D) to 25.6 ± 0.32°C in August at Zanzibar site (site Z), with values being consistently

higher during northeast monsoon (NEM) than in the southeast monsoon (SEM) (Figure 2A). Indeed, there were significant high temperature during NEM (December to April) than the SEM (June – October) ($p < 0.0001$; $t = 6.657$). There were small variations in salinity levels being lower during rainy months (March to June) and the values ranged from an average of 34.3 ± 0.66 in April (site Z) to 35.3 ± 0.35 ‰ in August through December at site D (Figure 2B). The seasonal variations were not significant but there was significant higher salinity at Site D than site Z ($p = 0.01$; $U = 117$). Phosphate concentrations was slightly higher during the NEM showing maximum value of 0.5 ± 0.17 µmol PO_4-P l^{-1} in January and minimum value of 0.01 ± 0.01 µmol PO_4-P l^{-1} in November (site D). The concentrations did not vary significantly with sites ($p = 0.057$; $t = 2.089$) or seasons ($p = 0.145$; $t = 1.639$). Nitrite and nitrate concentration in the seawater was significantly higher at site D than site Z (Figure 2D) ($p = 0.0002$; $U = 12$). The values obtained at site D ranged from 0.37 ± 0.02 in July to 1.17 ± 0.01 µmol NO_2/NO_3-N l^{-1} in April, while at site Z ranged from 0.002 ± 0.021 in October to 1.06 ± 0.98 µmol NO_2/NO_3-N l^{-1} in December. The nitrate/nitrate concentrations were significant higher during NEM than SEM at both sites D ($p = 0.040$; $t = 2.46$) and site Z ($p = 0.047$; $t = 2.34$).

R. intracellularis occurrence

R. intracellularis was commonly found as endosymbiont to diatoms of genus *Rhizosolenia* and *Hemiaulus*. On few occasions *Richelia* sp. were found freely in the samples. Figure 3 shows the most common species of *Rhizosolenia* and *Hemiaulus* that contained the endosymbiont *Richelia* sp. *R. styliformis* was the predominant species of *Rhizosolenia* that contained *Richelia* sp. which occurred in single or double at one or both ends of the host (Figure 3a-c). In most cases they were found in a pair at one end of the host with the heterocysts pointing towards the apex (tip). However, sometimes the trichomes of the *Richelia* sp. were found to have two heterocysts at both ends of the vegetative cells (Figure 3c). The morphology and sizes of *Richelia* sp. did not show big variations but the number of vegetative cells per filament ranged from 4 to 14 cells. Other common species of *Rhizosolenia* that contained *Richelia* sp. were *R. cylindrus* (Figure 3d), *R. castracenei*, *R. clevei* and *R. setigera*. In the *R. castacenei* and *R. clevei*, a high numbers (6-16) *Richelia* filament was normally observed in one end of the host cell (Figure 3e-f).

Richelia sp was found in the two species of *Hemiaulus* that is *H. membranaceus* (Figure 3g-h) and *H. sinensis* (Figure 3i). Two filaments of *Richelia sp.* with four vegetative cells and one heterocyst pointing towards the same direction was usually found in each *Hemiaulus* spp. host. All cells of *Hemialus* spp. at all time were found to contain *Richelia* except in some samples collected from site Z in months of March to May 1993. These samples contained *Hemiaulus* spp. cells without *Richelia* sp. or cells with heterocysts only (that is without vegetative cell).

Distribution and abundance

The mean monthly abundance of *R. intracellularis* in *Rhizosolenia* spp. and *Hemiaulus* spp. are shown in Figure 4. The numbers varied from time to time ranging from zero as recorded at site D in January, May and June 2009 and at site Z in June 1993, to mean maximum of 428 ± 105 filaments l^{-1} at site Z in August 1993 (Figure 4). The number of *Richelia* filaments were significantly higher in 1993/4 at site Z as compared to in 2008/9 at site D ($p = 0.044$; $t = 2.1$). Although there were some inter-year variations in the abundance of *Richelia* sp., higher numbers were recorded during the SEM (June to October) while a fewer filaments were detected during the NEM (December to April). Indeed, statistical analysis shows that significant higher number occurred during SEM in 1993/4 at site Z ($p = 0.014$; $t = 3.112$) whereas at site D the differences were not significant ($p = 0.326$; $t = 1.039$). Pooling together all data of 1993/4 and those of 2008/9, the numbers of *Richelia* sp. were on average higher during SEM (131.8 ± 149.5 filaments l^{-1}) than the NEM (73.08 ± 60.95 filaments l^{-1}) but the differences were not significant ($p = 0.242$; $t = 1.206$).

Distinct high numbers (blooms) of *Richelia* sp. were observed during the SEM period in July to October 1993 at site Z. For example, in July 1993, the number of filaments of the *Richelia* in *Rhizosolenia* spp. increased rapidly from none on 5th July 1993 to 262 filaments per liter on 12th July 1993. During this period, about 75% of the encountered *R. styliformis* contained the symbiont *Richelia*. The high concentration lasted for about three weeks with a maximum of 1554 filaments l^{-1} at a depth of 10 m recorded on 2nd August 1993. Thus, the samples collected after one week on 10th August 1993 had no *Richelia* sp. in *Rhizosolenia* spp. at all. However, in addition to the high number of *Richelia* sp. in *Rhizosolenia* spp., there was also higher number of *Hemiaulus* spp. and all contained endosymbiont *Richelia* sp. during this period. Sampling was repeated on the following year (1994) on the same period (July/August) but the numbers of *Richelia* were low as compared to the same period of previous year that is 1993.

The depth distribution pattern down to 20 m of *Richelia* species was assessed during 1993/4 and the results are as presented in Figure 5. There were no systematic pattern with depth and it was observed that the cells were distributed throughout the water column down to 20 m depth. As depicted from the graphs, the higher numbers were recorded during SEM. Indeed, statistical comparison using Friedman test (non-parametric randomized block ANOVA) showed insignificant differences with depth ($p = 0.67$; $MCV = 21.99$).

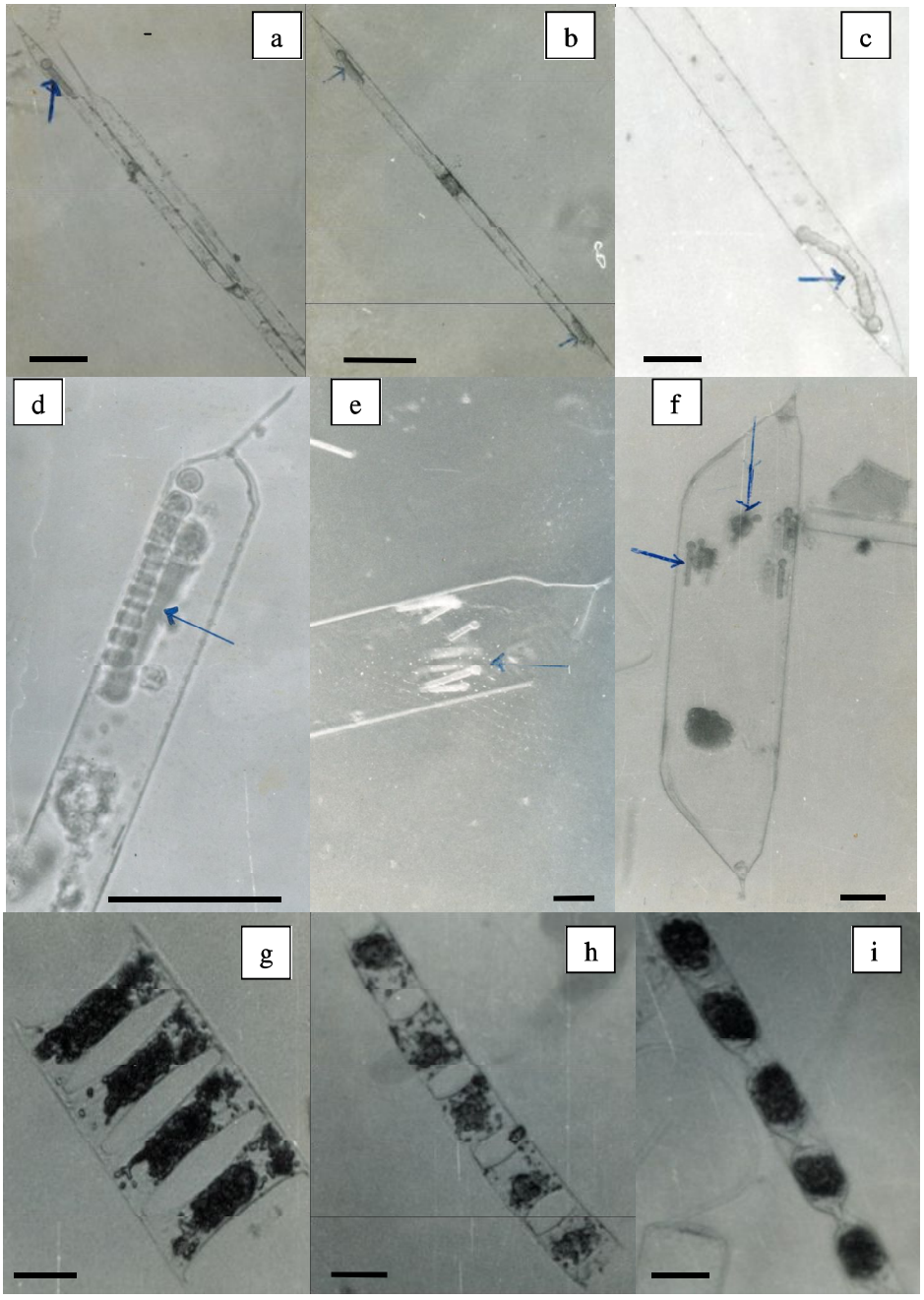


Figure 3. Light micrographs of *Rhizosolenia* spp. and *Hemiaulus* spp. with *Richelia* sp. (arrows) encountered during this study. a-c, *Richelia* sp. in *Rhizosolenia styliformis*; d, in *R. cylindrus*; e, in *R. clevei*; f, in *R. castracenei*; g-h, in *Hemiaulus membranaceus*; i, in *H. sinensis* (note: in *Hemiaulus* spp. trichomes of *Richelia* sp. can not be seen without flourescent light). (Bar = 50 µm).

Nitrogen fixation

The rates of nitrogen fixation by cyanobacteria species in surface water was estimated in 1993/4 samplings at site Z only. The estimated values using a ratio of C_2H_4 produced to N_2 fixed of 4:1 (Capone, 1993), and the type and number of cyanobacteria in assay bottles are shown in Table 1. High rates of nitrogen fixation occurred during NEM (January to May 1994) with maximum value (2.753 ± 0.033 nmol N $h^{-1}l^{-1}$) in February 1994 corresponding to high numbers of *Trichodemium* spp. Lower values were obtained during SEM (June to August) with the lowest value (0.030 ± 0.005 nmol N $h^{-1}l^{-1}$) recorded in August1994 when the number and type of cyanobacteria

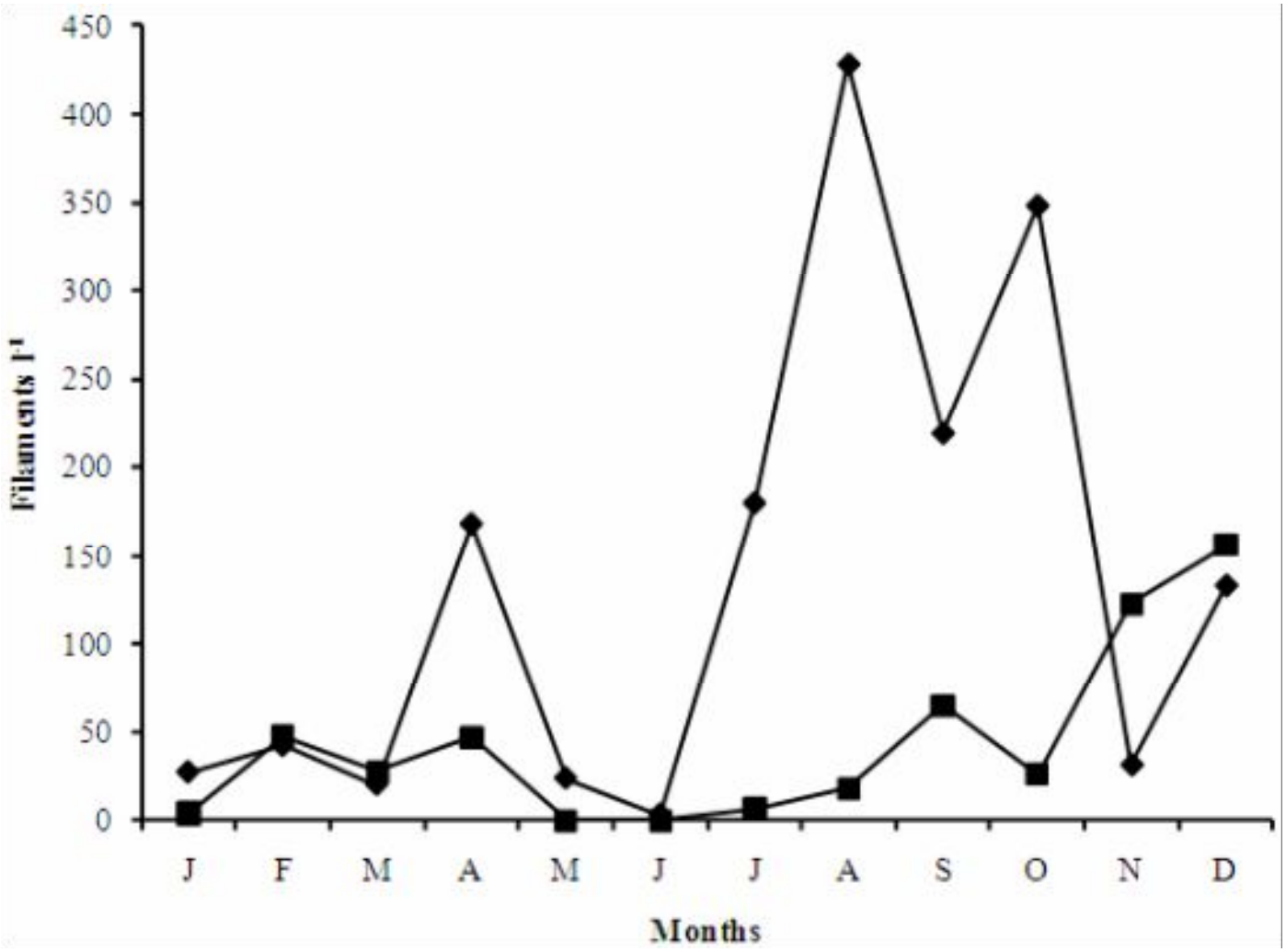


Figure 4. Seasonal variation of *Richelia* sp. mean abundance in Tanzanian coastal waters collected from site Z (Diamond), and from site D (squares).

including *Richelia* sp. was very low. Seawater filtrate (500 ml) that was concentrate by membrane filter did not show detectable rates of acetylene reduction which may indicate that the contribution by bacteria to N_2-fixation in the pelagic water of WIO region is very low as compared to that of cyanobacteria.

DISCUSSION

The various physical and chemical factors measured during this study were within the range of other studies in the tropics and in the Western Indian Ocean Region in particular (Francis et al., 2001; Lugomela et al., 2002; Hamisi et al., 2004). The monthly variations were minimum, a typical characteristic of tropical waters. The result also shows that the surface oceanic water was oligotrophic (nutrient-poor), which is common phenomenon in the tropical and subtropical waters. Thus, in such nutrient-poor environments, symbiotic relationships may be expected to enhance survival via mutual benefits for both partners (Venrick, 1974; Gomez et al., 2005; Zeev et al., 2008).

This is the first study to report total abundance of *Richelia* (that is including in *Hemiaulus*, epiphytic and free living) in the coastal waters of Tanzania, following the abundance estimated by Lugomela (2002) in *Rhizosolenia* spp. only in Zanzibar coastal waters. In addition, the morphological variations of and number in various hosts was studied using epifluorescent light microscopy. The presence of *Richelia* sp. as endosymbiont to some *Rhizosolenia* spp., *Hemiaulus* spp., epiphyte to *Chaetocerous* spp. as well as freely floating correspond with previous findings in the coastal waters of Tanzania (Bryceson, 1977; Lugomela, 2002). It is also possible that the freely observed *Richelia* may

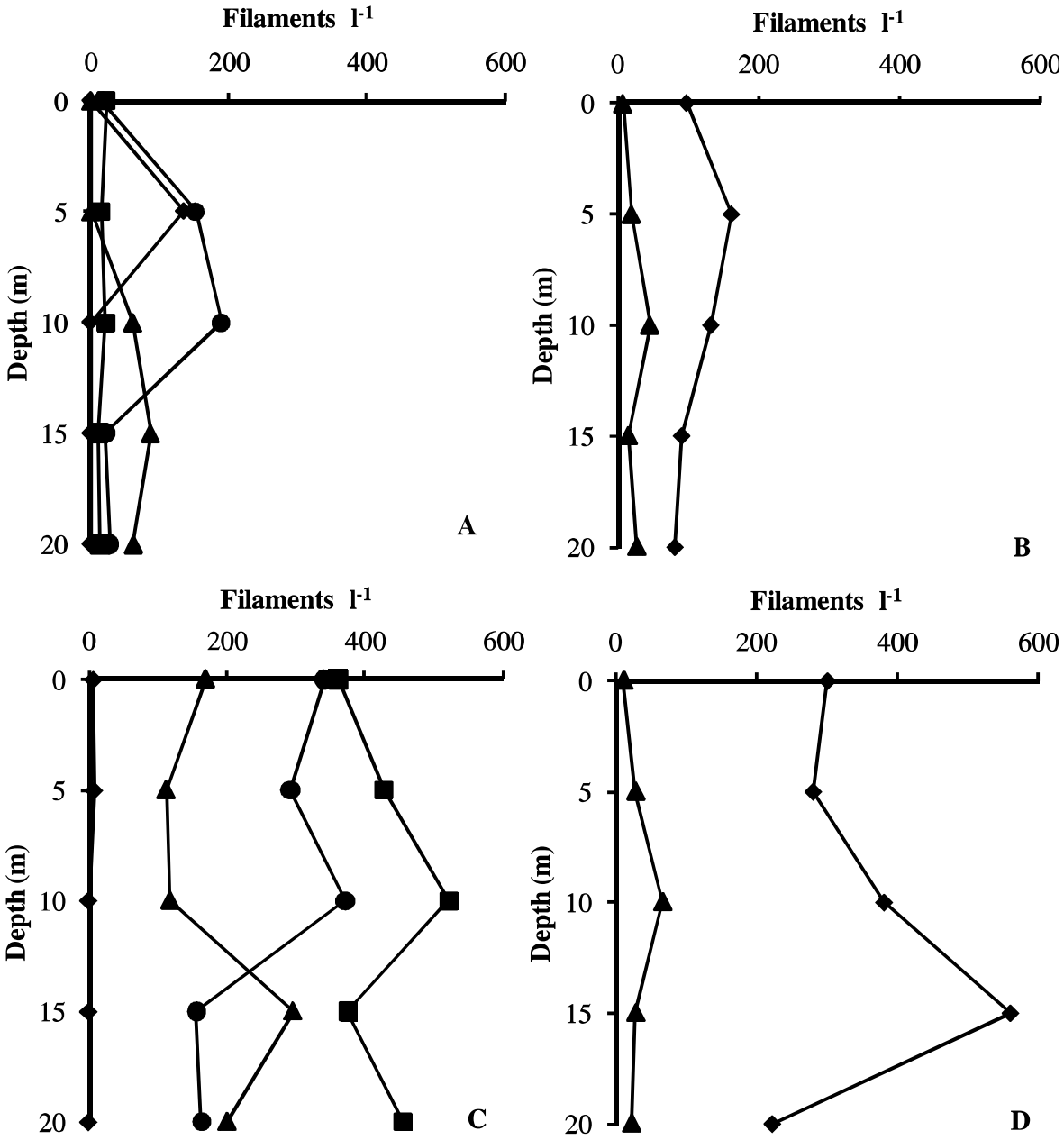


Figure 5. Vertical distribution of *Richelia* sp. at site Z, (A) During the NE monsoon period in December (circles), January (diamond), February (triangles), and March (squares), (B) During the inter-monsoon period in April (diamond) and May (triangle), (C) During the SE monsoon period in June (diamond), July (triangle), August (squares) and September (circle) and (D) During inter monsoon period in October (diamond) and November (triangles). The values represent the average of 3 to 4 replicate samples collected at 1 to 2 week intervals each month.

have been originated from broken diatom or were detached from *Chaetocerous* species. In this study it was observed that the *Richelia* sp. occurring in various hosts diatom species vary in number per host and orientation of the heterocysts (Figure 3). The morphology and sizes of *Richelia* sp. did not show big variations but the number of vegetative cells per filament ranged from 4 to 14 cells, corresponding to other reports (Lugomela et al., 2001). In some samples, only heterocyst of *Richelia* sp. was seen in *Hemiaulus* spp. It was assumed that there is only one species of *Richelia* and as hypothesized by Gomez et al. (2005), the unattached filaments of *Richelia* are able to colonize new hosts. This has also been demonstrated by Janson et al. (1999) who reported that hetR gene

Table 1. The mean rate of acetylene reduction per volume of seawater collected from Zanzibar (site Z) in 1994.

Date	Fixation rate (nmol N $h^{-1}l^{-1}$)	Cyanobacteria species present			
		Trichodesmium	*Richelia* in *Rhizosolenia*	*Richelia* in *Hemiaulus*	Others cyanobacteria
11 Jan 1994	0.813±0.023	692±122	1.52±1.07	12.1±15.6	5.69±0.93
07 Feb 1994	2.753±0.033	5333±265.2	22.5±22.5	200±40.0	0
29 Mar 1994	0.213±0.118	104±16.0	8.00±7.00	0	0
26 Apr 1994	0.273±0.115	717±95.6	0	14.7±25.4	0
04 May 1994	0.258±0.200	258±227	3.50±4.95	0	0
27 Jun 1994	0.043±0.018	5.77±6.87	0	233±121	0
29 Jul 1994	0.053±0.013	5.25±9.09	6.13±5.47	227±102	0
01 Aug 1994	0.030±0.005	7.60±7.23	2.80±4.85	24.0±41.6	0

sequence of *Richelia* growing endosymbiotically in *R. clevei* are closely related to those growing epiphytically on *Chaetoceros* species.

The higher numbers of *Richelia* sp during SEM than NEM especially in 1993/4 at Zanzibar site indicate that the *Richelia* bloom occurred during that time, however further analysis is needed for continued monitoring as the result was contrary to repeated sampling on the same period in the following year 1994 as well as to previous report in 1998 (Lugomela, 2002). Pooling together results from 1993/4 and 2008/9, it was shows that the abundance was higher (though not significant) during SEM and still in contrary to the observation by Lugomela (2002), again suggesting for periodic monitoring and consider the changes in physical chemical parameters particularly nitrogen availability. In addition, the observed variation in number and morphology of *Richelia* sp. in *Hemiaulus* pp. during March to May 1993, suggested that the diatom (hosts) may have being stimulated and sustained by the nitrogenous nutrient provided by *Richelia* sp. and thus they were employed during low concentrations of the nitrogeneous nutrient only while during high concentration (NEM) *Richelia* filaments were probably reduced by host cells, simply because *Richelia* sp. is able to fix molecular nitrogen (Mague et al., 1974). Elsewhere in tropical Atlantic Ocean for example, large blooms of the diatom-cyanobacteria association have been described where heterocyst counts inside the bloom area ranged from hundreds to more than 1000 filaments l^{-1} (Villareal, 1994; Capenter et al., 1999; Jahnke et al., 1999).

The reason for the evenly distribution of cyanobacterium *R. intracellularis* over depth down to 20 m is due to the facts that phytoplankton distribution with depth is species specific. This depends on how certain species are adopted to various environment and biological and physical conditions of that depth. In most cases higher numbers are at the surface because of higher light intensities for photosynthesis and thus light controlled. Since the studied waters were oligotrophic, light is expected to penetrate well beyond 20 m deep and will not be limiting (Lugomela et al., 2002). In addition, the currents and waves in these sites are high enough to thoroughly mix the surface water to the bottom (Francis et al., 2001).

The symbiotic association between *Richelia* sp. and diatoms seems to be mutualistic in nature. Observation on *Richelia* sp. growing as an endosymbiont in *R. clevei* showed that gas-vesicles were absent and therefore the cyanobacterium cell might be unable to regulate their position in the water column (Janson et al., 1995). Consequently unattached filaments should not be viable and a host would be required. The replication would therefore be after contact with host diatom and that is why more frequently the cyanobacterium is found living symbiotically. It is also hypothesized that fixation of molecular nitrogen by the cyanobacterium may stimulate the development of host diatom in oligotrophic waters where nitrogen is often a limiting factor (Venrick, 1974). Indeed, the high numbers of *Rhizosolenia spp.* and *Hemiaulus* spp. with *Richelia* were observed in the coastal waters of Zanzibar during the months of July to October when the nitrate concentration were beyond detection limits. Although nitrogen fixation rates estimation during this study were done while the total number of *Richelia* sp. was low, the results indicate active nitrogen fixation. The amount of nitrogen fixed when the number of *Trichodesmium* was very low, may be associated by the presence of *Richelia* species as has been hypothesized in other studies (e.g. Zeev et al., 2008). Furthermore it is possible that the presence of the autotrophic endosymbiont enhances photosynthesis in highly vacuolised large diatoms such as *Rhizosolenia* spp. with poorly developed chloroplasts and limited cytoplasm. In addition, the fragile filaments of *Richelia* secure protection offered by the rigid diatom frustules (Kimor et al., 1992).

In conclusion, this study showed that, the endosymbiotic *R. intracellularis* present–abundantly in coastal waters of Western Indian Ocean, might be important source of nitrogen to their host and in turn productivity of marine ecosystem. Their abundance

varied between months and with season which might be controlled by various environmental factors particularly nitrogen source.

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