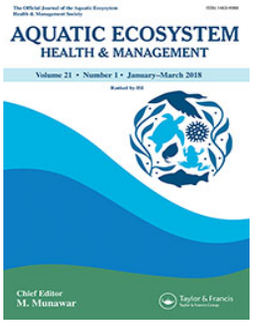

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Community structure of coralline algae and its relationship with environment in Sanya reefs, China

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Coralline algae are an important functional group in coral reef ecosystems. Despite the importance of coralline algae, little is known about their abundance and community structure, especially within Sanya reefs. It was fundamental to study coralline algae species abundance and distribution, and evaluate the effects of environmental factors on the species composition and abundance in Sanya reefs. A total of 24 species in 10 genera were identified based on 11 sampling stations, with the family Corallinaceae being dominant within the study area. The 7 dominant species, which constituted 62.4% of the overall collection, were Amphiroa ephedraea *(16.8%),* Mesophyllum simulans *(11.1%),* Porolithon onkodes *(9.8%),* Neogoniolithon fosliei *(7.5%),* Mesophyllum mesomorphum *(6.6%),* Pneophyllum conicum *(6.6%) and* Hydrolithon boergesenii *(4.0%). There was significant spatial variability in the species composition and abundance of coralline algae (ANOSIM: $R = 0.356$,* P *$= 0.013$). The correlation analysis between biotic and abiotic variables indicated that the turbidity had a negative effect and salinity had a positive correlation on the pattern of coralline algae assemblages (global $\rho = 0.486$, BIOENV analysis). The living cover of coralline algae was greater in deep water than in shallow water at the same sites. This suggests that physical disturbance, either natural or anthropogenic, is more important in regulating the coralline algae community structure in Sanya reefs.*

Keywords: environmental factors, species abundance, spatial variability, effects, correlation

Introduction

Coralline algae are slow growing multicellular plants (Corallinales, Rhodophyta), heavily calcified by calcite crystals embedded in the cell walls (Johansen, 1981; Adey et al., 1982). They distribute globally and are abundant in many types of marine environments, from tropical to polar, and in different light intensity ranging from those of shallow tropical intertidal reef flats to nearly darkness (Adey et al., 1982). Usually, they serve two key roles in coral reef environments. The first is the well-known role of their significant contribution to limestone formation and cementation of the

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reef (Barnes and Chalker, 1990). The second is as settlement substratum for many types of reef benthos. They induce the larval settlement and metamorphosis of many invertebrates, including some hard corals (Heyward and Negri, 1999), gorgonian corals (Lasker and Kim, 1996) and the abalone (Williams et al., 2008). As mentioned above, coralline algae are usually considered as a fundamental group in coral reef ecosystem.

In coral reefs, the clarity of sea water, temperature, and light are major factors influencing the growth rate of coralline algae (Littler and Littler, 1984; Iryu and Matsuda, 1988; Klumpp and McKinnon, 1992). Due to their greatly varied morphologies and diagnostic in different environment (Woelkerling, 1988; Harvey and Woelkerling, 2007), individual species and genera were very difficult to be distinguished in rapid ecological field assessment. Species identification requires optical and scanning electron microscopy techniques (Woelkerling, 1988; Braga et al., 1993).

Coral reefs are currently under serious risks from human and natural disturbances. Surveys on coral reefs suggested that changes in coralline algae abundance in reefs could indicate changes in the structure and function of coral reef ecosystems (Steneck and Testa, 1997). However, the abundance and community structure of coralline algae are still relatively unknown in the reef of South China Sea, except for 8 early known coralline algae collections from Sanya reefs which were only made by Zhang and Zhou (1987). Consequently, there were few comprehensive lists of the species currently available. This investigation was carried out in order to determine the species composition and community structure of the coralline algae of the Sanya reefs. The relationship between the coralline algae spatial characteristic and the environmental factors, including turbidity, salinity, temperature, total suspended material and light attenuation coefficient were investigated as well. In addition, the environmental variables which could best explain the community structure patterns were also studied.

Materials and methods

Site description

Sanya is located at the south of Hainan Island (109°20′∼109°30′E, 18°11′∼18°18′N), southern China. It is one of the typical tropical bays in China exposing to the northern South China Sea. There are many rocky and sandy shores and mudflats and many kinds of tropical habitats such as coral reefs and mangroves. There are abundant reef-associate organisms, especially the hard corals, most of which distributed in Xidao (XD), Dongdao (DD), Xiaozhoudao (XZD), Luhuitou (LHT), Xiaodonghai (XDH), Dadonghai (DDH), Yezhudao (YZD), Dongpai (DP), Xipai (XP), Taiyangwan (TYW) and Shendao (SD) (Figure 1). This reef area was designated as the first national marine protection area for coral reef in China in 1990 (Lian et al., 2010). The sites of XZD, LHT, XDH, TYW and SD on the fringing reef along the shore were defined as inshore sites (IS), while

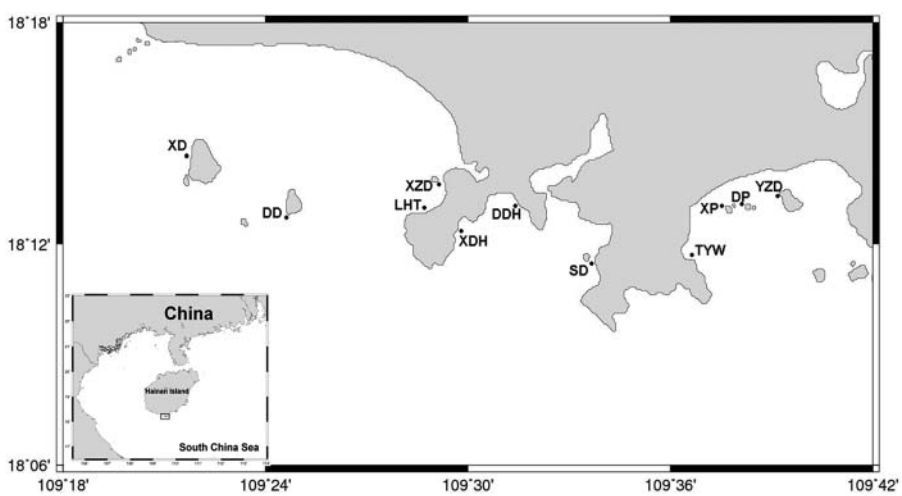

Figure 1. Study sites in Sanya reefs (XD: Xidao; DD: Dongdao; XZD: Xiaozhoudao; LHT: Luhuitou; XDH: Xiaodonghai; DDH: Dadonghai; YZD: Yezhudao; DP: Dongpai; XP: Xipai; TYW: Taiyangwan; SD: Shendao).

the other sites on the islets not far from the shore were treated as offshore sites (OS).

Ecological assessments

The fringing reef in Sanya was mainly distributed above 10 m (Lian et al., 2010), so the quantitative ecological assessment data of the benthic communities were collected along three different transect lines, namely, 3 m, 6 m, 9 m at each of the same sites. Methods of benthic community survey were modified after Vroom et al. (2010) and Preskitt et al. (2004). Specifically, SCUBA divers were summoned to take about 100 photoquadrats (35 cm × 25 cm) along the 60 m transects with a SEA and SEA DX-2G digital still camera (Sea and Sea SUNPAK Co., Ltd., Tokyo, Japan). Each photoquadrat was taken randomly within 2 m on both sides of each transect. All the photoquadrats were analyzed using Coral Point Count with Microsoft Excel Extensions (CPCe) (Kohler and Gill, 2006). Percentage cover of the benthic organisms or substrate type was estimated by identifying items under each point. Data from every quadrat of each transect were treated as individual replicate within a research site.

Sample collection and identification

Total of 226 samples of coralline algae were collected using a hammer and chisel from 11 study sites of Sanya reefs. Each sample was then preserved in 95% alcohol. Specimens were first examined with a Leica dissecting microscope to record the anatomical characteristics of the hypoothallus, perithallus and trichocytes (size, shape and arrangement). Species with similar features were divided into form-functional groups following the methods of Steneck and Paine (1986). The samples were described according to the vegetative and reproductive features (Woelkerling, 1988), and their growth forms were classified as encrusting and branched, following the classification of Woelkerling et al. (1993). Molecular tools were also being used to identify some species that morphology variable greatly, according to the methods of Vidal et al. (2003). Specimens for SEM examining, portions were rinsed in distilled water, air-dried, fractured and mounted on aluminum stubs with conductive cement, and then the samples were gold-coated. They were examined with a Hitachi S-3400N scanning electron microscope (SEM), at an accelerating voltage of 20 kV, following the methods of Garbary and Johansen (1982). Each coralline sample was assigned a species name according to the descriptions and keys from a number of sources (Zhou and Zhang, 1987; Woelkerling, 1988; Tseng, 1983; Adey et al., 1982; Woelkerling and Harvey, 1993; Littler and Littler, 2003; Farr et al., 2009). All the specimens were stored in the Marine Biodiversity Collection of South China Sea, Chinese Academy of Sciences.

The turbidity of water from multi depth were taken by turbidity profiler (AQUAlogger, UK), and the salinity and temperature were measured by an Orion 013010MD conductivity probe (Thermo [Eutech], US). Total suspended material (TSS, mg l^{-1}) at different depth were obtained by filtering a certain volume of water (1∼2 L) through the 0.5 μm cellulose filters (GF/F) and then measuring the weight of the filtrates. The light attenuation coefficient (LAC, K) was analyzed according to Yentsch et al. (2002). Both the biological and environmental surveys were carried out simultaneously in November 2014.

Data analysis

Multivariate analysis was used to detect any spatial differences in the species composition and abundance of the coralline algae assemblages, and to assess which taxon mainly contributed to the spatial variability (Software PRIMER v6). The formulations of diversity index employed in this study were shown as follows:

Shannon-Weaver's index:

$$H' = -\sum_{i=1}^{s} P_i \log_2 P_i,\ P_i = \frac{n_i}{N} \tag{1}$$

Species evenness:

$$J = \frac{H'}{\log_2 S} \tag{2}$$

Margalef species richness:

$$M = \frac{n-1}{\ln N} \tag{3}$$

Species dominance:

$$Y = \frac{n_i}{N} f_i,\ f_i = \frac{n_i}{11} \tag{4}$$

where, n_i was the number of individuals of the ith species in a sample of N; S was the total number of the species.

Therefore, the species abundances were treated as replicates for each station and the statistical analysis was performed between sites at the same level. Similarity matrices were constructed using Bray-Curtis similarity (Clarke and Warwick, 2001). Non-metric multidimensional scaling (nMDS) was also applied to determine the similarity of sites with respect to coralline algae composition. Following the cluster analysis, the species having the greatest contribution to the division of samples into cluster were determined using the similarity percentage program (SIMPER) (Clarke and Gorley, 2006).

ANOSIM was run to detect the effect of sampling sites (IS and OS) on the composition and distribution of the coralline algae. Where required, a square root transformation function was applied to normalize the data and make the variance constant.

The BIOENV procedure was used to determine which set of environmental variables (similarity calculated with the Euclidean distance coefficient) best explains the biological matrices (Clarke and Gorley, 2006). The environmental variables included turbidity, salinity, temperature, TSS and LAC. Prior to analysis, environmental data was averaged and a draftsman plot (scatter plots between pairs of environmental variables) was used to assess the linearity of the data and the inter-correlation between variables. Variables with high degree of correlation (Spearman's correlation coefficient) were omitted from the BIOENV analysis. All parameters were transformed with the log (x+1) and multivariate analysis were carried out by PRIMER software (Clarke and Gorley, 2006).

Results

The species composition and diversity index

The simplest measure of species diversity was a count of the number of species found in the samples. In this work, 24 species belonging to 3 families and 10 genera were identified on the 226 samples, including Corallinaceae (*Amphiroa, Lithophyllum, Neogoniolithon, Lithoporella, Porolithon, Hydrolithon, Pneophyllum*), Hapalidiaceae (*Lithothamnion, Mesophyllum*) and Sporolithaceae (*Sporolithon*) (Table 1). Among the species identified, 16 coralline algae species were newly recorded in this study area, while the other 8 had been recorded before (Zhou and Zhang, 1987). In terms of species number, species in the family Corallinaceae were the most dominant taxa (17 species), followed by family Hapalidiaceae with 6 species and family Sporolithaceae with 1 species. Except for the site LHT, species in the family Corallinaceae were also the most dominant taxa in other sites (Figure 2).

The species dominance (Y), relative species density (P_i) and frequency (f_i) varied within the following ranges, i.e. 0.09~0.73, 0.004~0.168, and 0.09~0.45, respectively, at the sample sites. According to the criteria of dominant species (Xu and Chen, 1989), there are 8 dominant species in the 26 identified ones ($Y > 0.02$). The 7 species which made up 62.4% of the overall collection were *Amphiroa ephedraea* (16.8%), *Mesophyllum simulans* (11.1%), *Hydrolithon onkodes* (9.8%), *Neogoniolithon fosliei* (7.5%), *Mesophyllum mesomorphum* (6.6%), *Pneophyllum conicum* (6.6%), and *Hydrolithon boergesenii* (4.0%). And 2 of them are the most dominant species, one is the *A. ephedraea* ($Y = 0.076$) (articulate), another is *M. simulans* ($Y = 0.07$) (non-articulate), and the highest specie density in the study area is *A. ephedraea* ($P_i = 0.168$). Even so, the maximum specie frequency is *N. fosliei* ($f_i = 0.727$) and *M. mesomorphum* ($f_i = 0.727$), not the *A. ephedraea* ($f_i = 0.455$) (Figure 3).

The species richness (S) and Shannon-Wiener diversity (H' ($\log_2$)) were not high in the studied area, which ranged from the highest 12 species at site XP to the lowest 2 at site DP, and diversity (H') varied between 0.47 at site DP and 3.32 at site XP (Figure 4). According to the sites OS and IS, species richness and diversity index were much higher in the OS sites (average $S = 8.80 \pm 3.96$; $H' = 2.52 \pm 1.18$) than those of IS (average $S = 8.17 \pm 1.83$; $H' = 2.50 \pm 0.29$), while the evenness (J) was the inverse (average $J_{OS} = 0.81 \pm 0.20$, $J_{IS} = 0.83 \pm 0.03$). The low evenness in the OS sites probably indicated that the coralline algae community was disturbed due to the frequent human activities. Moreover, the Margalef species richness index (M) was also ranged from the lowest 0.43 at site DP to the highest 3.56 at site XP. Additionally, in order to check the effect of species richness on the diversity index, the relationship between M and H' was counted.

Table 1. Species list in the study area.

Order	Family	Genus	Species
Corallinales	Corallinaceae	*Amphiroa*	*Amphiroa ephedraea*
			Amphiroa anceps
			Amphiroa beauvoisii
		Lithophyllum	*Lithophyllum pygmaeum*
			**Lithophyllum kotschyanum*
		Neogoniolithon	*Neogoniolithon trichotomum*
			Neogoniolithon megalocystum
			Neogoniolithon fosliei
			Neogoniolithon variabile
			Neogoniolithon megalocystum
			Neogoniolithon setchellii
		Lithoporella	**Lithoporella melobesioides*
		Porolithon	**Porolithon onkodes*
		Mastophora	**Mastophora pacifica*
		Hydrolithon	**Hydrolithon reinboldii*
			Hydrolithon boergesenii
		Pneophyllum	*Pneophyllum conicum*
	Hapalidiaceae	*Lithothamnion*	*Lithothamnion pacificum*
			Lithothamnion intermedium
			Lithothamnion sp.
		Mesophyllum	**Mesophyllum mesomorphum*
			**Mesophyllum simulans*
			Mesophyllum crassiusculum
	Sporolithaceae	*Sporolithon*	**Sporolithon erythraeum*

"*" existed record species in the study area.

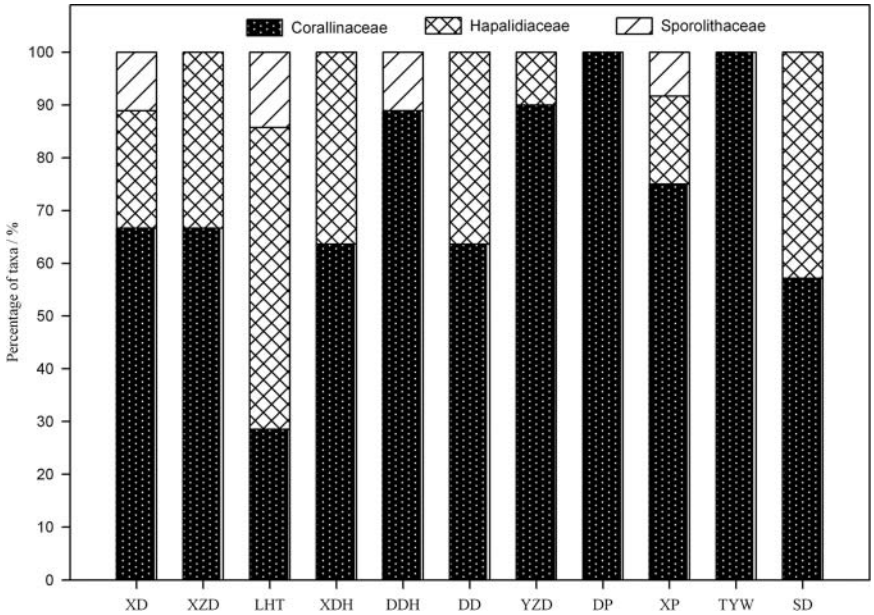

Figure 2. Contribution of each taxa group (percentage of total) collected from each of the study site.

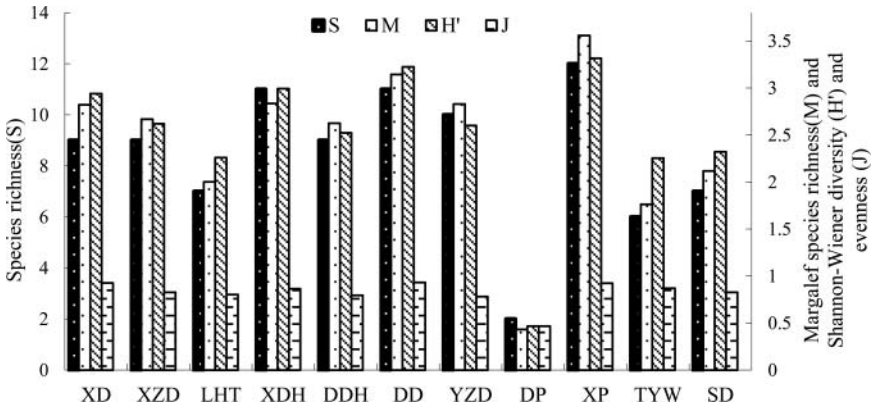

Figure 3. Species richness (*S*), Margalef species richness (*M*) and Shannon-Wiener diversity (H') and evenness (*J*) of coralline algae community of the study sites.

Spatial characteristics of the population structure

Classification and ordination analysis separated sampling stations, at 23.3% of similarity, into two main groups, which reflected high heterogeneity between the IS and OS research sites (Figures 5 and 6). The ANOSIM analysis based on the abundance of coralline algae confirmed the significant difference in sites (ANOSIM: $R = 0.356$, $P < 0.05$). Due to the homogeneity of different sampling sites and dominance of coralline algae, the IS group comprised higher similarities (33.13% average similarity) than the OS group (26.62%

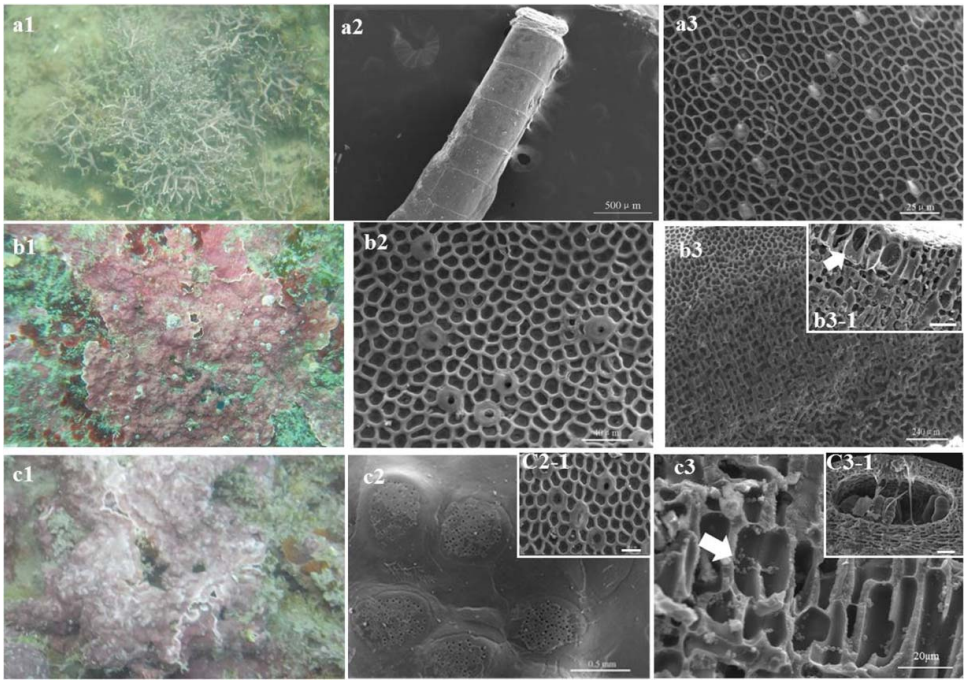

Figure 4. Three species (*Amphiroa ephedraea*: (a1) image of thallus, (a2) the SEM image of intergenicula; (a3) thallus surface pattern of Corallina-type; *Neogoniolithon fosliei*: (b1) image of thallus, (b2) thallus surface pattern of Corallina-type (SEM), (b3) cell fusion in longitudinal section (SEM), (b3-1) arrow showing trichocytes arranged parallel in epithallus [Bar = 20 μm]; *Mesophyllum simulans*: (c1) image of thallus, (c2) multiporate conceptacle with flat roof (SEM), (c2-1) arrow showing scattered trichocytes in surface view [Bar = 20 μm], (c3) arrow showing cell fusion in perithallus (SEM), (c3-1) longitudinal section of tetrasporangial conceptacle with zonate tetrasporangia [Bar = 20 μm]).

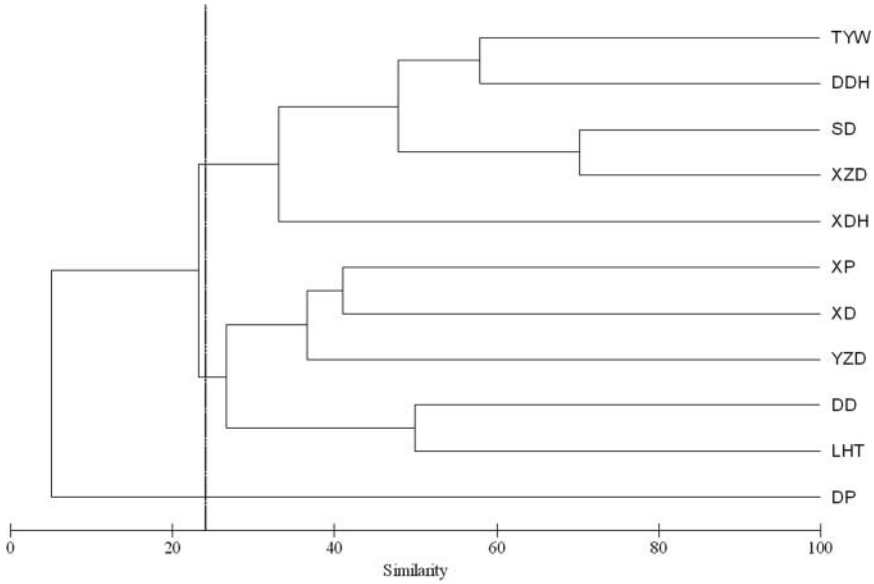

Figure 5. Cluster analysis using the hierarchical agglomerative method employing group average linking of Bray-Curtis similarities (similarity = 24%).

average similarity) (Figure 5). According to the SIMPER results, the species contributing the greatest to the division of samples into different groups were *Amphiroa ephedraea, Mesophyllum simulans, Mastophora pacifica, Lithophyllum pygmaeum* and *Hydrolithon onkodes* in sites (SIMPER, 50% cutoff).

The living cover of coralline algae

The percentage living cover of coralline algae varied greatly both horizontally and vertically, from the highest 38.45% at 3 m of the site of XP to the lowest 0.22% at 6 m of the site of SD (Figure 7). Except for the sties DD and DP, the living cover was greater in deep water than in shallow water at the same sites. The lower living cover in much deeper water sites either might be due to the substrate of sand and/or mud (e.g. XZD, DDH, SD, YZD) or near the waterway (e.g. SD). As for the sites of DD and DP, the coralline algae community in shallow water was disturbed by the intensive tourism and other human activities. The living cover of hard coral in this research area also varied greatly, from the highest 40.0% at 3 m of the site of XZD to the lowest 3.0% at 6 m of the site of SD (Figure 7).

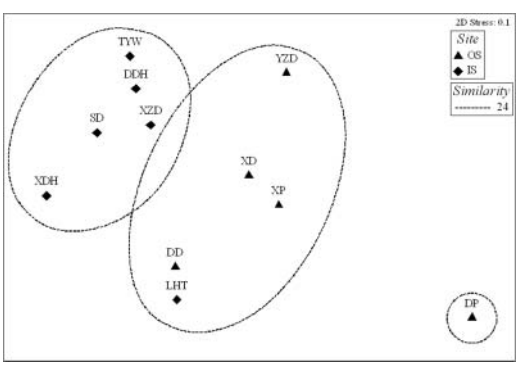

Figure 6. Non-Multidimensional scaling ordination (nMDS) plots showing similarities between samples with no transformation based on coralline algae abundance data. Symbols indicate different sampling sites, IS: inshore; OS: offshore.

Environmental factor gradients

There were significant differences in environmental factors between the research sites IS and OS (ANOSIM: $R = 0.593$, $P = 0.01$). The more anthropogenic activities there were, the higher the turbidity. The average turbidity, salinity, temperature and TSS of the IS sites were 3.98FTU, 32.68, 25.30°C and 3.55 mg l^{-1}, while in the OS sites were 2.79FTU, 32.75, 25.13°C and 2.42 mg l^{-1} respectively (Table 2). And the higher the turbidity there was, the higher the light attenuation coefficient. However, regardless of whether the sites

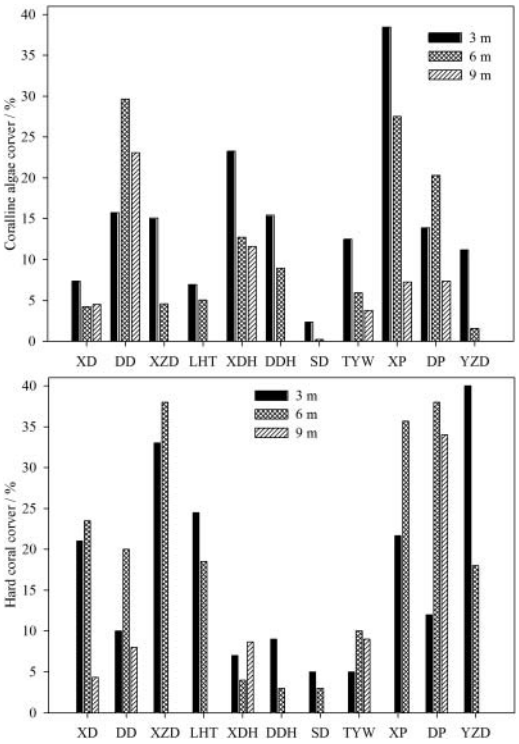

Figure 7. Living cover of coralline algae and hard coral of each study site in three different depths.

were near or far from (e.g. XZD) the mouth of Sanya River (e.g. XP), there was no significant difference between these parameters (ANOSIM: $R = -0.008$, $P = 0.1$), which indicated the high efficient water exchange in the reefs of Sanya.

BIOENV analysis results

The subset of environmental variables showed different correlations with coralline algae patterns. The turbidity displayed a negative correlation with coralline algae patterns (BIOENV: global $\rho = 0.486$, $P < 0.05$), while salinity displayed a positive correlation with that. This analysis revealed that turbidity and salinity were responsible for nearly 50% of variation in coralline algae assemblage structure between the IS and OS sampling sites. The relation between the resemblance matrix of species abundance of Bray-Curtis similarity and resemblance matrix of environmental factors of Euclidean distance was also significant (RELATE: $\rho = 0.355$, $P < 0.05$) (Figure 8). In terms of single environmental factor, the salinity showed the highest positive correlation ($\rho = 0.43$), followed by TSS ($\rho = 0.046$), and the turbidity indicated reverse correlation ($\rho = -0.049$), followed by temperature ($\rho = -0.047$) (Table 3). Therefore, the best 2-variable combination (turbidity and salinity) showed a significant effect on the pattern of coralline algae. The other environmental factor gradients, however, were not important for the current study.

Discussion

Species composition

Compared with the previous 8 records (Zhou and Zhang, 1987), the 16 new records enriched the coralline algae species greatly in Sanya reefs. The 10 genera represent all the three families of Corallinales, i.e. Corallinaceae, Hapaliiaceae and Sporolithaceae and the 7 dominant species which made up 62.4% of the overall specimen, all of them contributing greatly in dividing the samples into two different groups. The most dominant species, *Amphiroa ephedraea*, found in the IS sampling sites, and the second dominant species, *Mesophyllum simulans*, were mainly found in OS sites. This indicated that the niche selection varied widely in different species.

The distribution of coralline algae species varies in depth of each study site due to the differences of the environmental condition in the

Table 2. Averaged environmental factors in each study site.

	XD	DD	XZD	LHT	XDH	DDH	SD	TYW	XP	DP	YZD
Turbidity (FTU)	4.21	3.66	4.28	3.23	2.69	11.984	4.90	2.355	1.95	2.66	1.50
Salinity	32.7	32.8	32.8	32.8	32.6	32.8	32.7	32.8	32.8	32.2	32.9
Temperature (°C)	26.08	25.26	25.82	26.14	25.67	24.73	24.80	24.64	24.67	24.649	24.99
TSS (mg l^{-1})	2.6	2.85	3.3	4	3.65	5.8	3.7	0.87	1.3	2.15	3.2
LAC	0.33	0.31	0.44	0.33	0.25	0.42	0.38	0.28	0.30	0.24	0.25

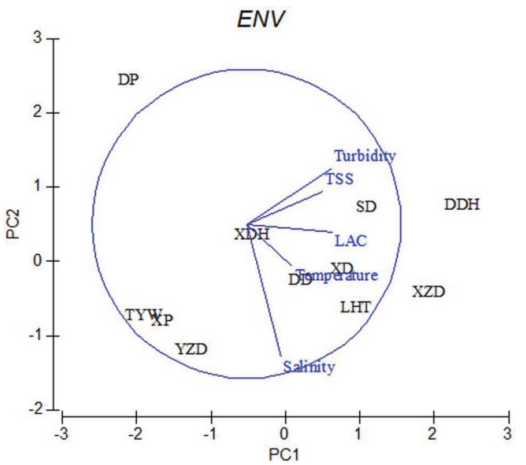

Figure 8. PCA analyse between environmental factors and research sites.

research stations. In the IS sites, both the articulate and non-articulate coralline species distributed in the sites above 6 m depth, while in the sites of 9 m depth, only the non-articulate coralline species distributed there. However, in the IS sites of SD, there were only non-articulate coralline species distributed. In the OS sites, there were also only non-articulate coralline species distributed.

Relationship between population structure and environmental factors

Currently, coral reefs are under intensive disturbance of human activities such as tourism and marine environmental changes. The abundance of both coral (Anthony et al., 2008; Meij et al., 2010; Lian et al., 2010) and coralline algae (Anthony et al., 2008; Kuffner et al., 2008) were gradually decreasing. Among the numerous physical gradients, water motion and sedimentation were thought to be the major factors responsible for diversity patterns of coralline algae (Steller and Foster, 1995; Fabricius and De'ath, 2001). However, they appear to be less sensitive than Corals to moderate organic pollution and nutrient increase (Walker and Ormond, 1982; Szmant, 2001), and the good water condition in the studied area (Huang et al., 2003) provided suitable surrounding to their growth. So in this study, we did not consider the water chemistry as the main environmental factors.

The spatial variability of coralline algae community were observed in the Sanya reefs, with two major groups (representing the IS and OS sites) clearly defined at a statistical significant level (cluster and nMDS ordination). Particularly, BIO-ENV analysis identified the best combination of physical properties (turbidity and salinity), which were most responsible for the observed structure of the coralline algae community in Sanya reefs. It was characterized by a continuum of coralline algae assemblages along these major environmental gradients, as the turbidity and salinity distinguished the samples between IS and OS sites (Figures 3 and 4). With the addition of other factor, such as the temperature, the position of the samples would be displaced, reflecting the interactions among the environmental elements. Multivariate statistical analysis thus provided a new point of view in studying the coralline algae community structure in relation to ambient factors.

In this study area, the sea water condition was similar among the research station, while the IS sites undergone more intensive human impact than the OS sites. This resulted in the higher turbidity in IS sites than in OS sites. The turbidity caused by suspended particles and colloidal matter in the water would reduce the light penetration into the water column (Oliver et al., 2010), thus could inference the physiology of coralline algae. Our results showed that coralline algae community

Table 3. Correlation triangular matrix (Rank correlation method: Spearman, D1 Euclidean distance, *Global Test* $\rho = 0.486$, $P < 0.05$).

	Salinity	TSS	LAC	Temperature	Turbidity
Salinity	0.430				
TSS	0.447	0.046			
LAC	0.416	−0.011	−0.023		
Temperature	0.462	−0.033	−0.037	−0.047	
Turbidity	0.475	0.003	−0.026	−0.004	−0.049

was negatively correlated with water turbidity. A previous survey had reported the similar result (Minton et al., 2011). As for the average species richness (S) and diversity index (H'), the OS sites are higher than that of the IS sites. Different degree of anthropogenic disturbance and turbidity may be attributed as the causes. Coralline algae can survive the salinity varied from 3 to 40 (Wilson et al., 2004), but there was no information available about the relationship between salinity and coralline algae assemblage or their distribution. In the present study, coralline algae assemblages had a positive correlation with salinity. This indicated once more that the sea salinity was important to their growth and distribution.

Therefore, physical process either natural or anthropogenic within the hydrological distinct zone was an important factor to control the coralline algae population structure and distribution in the Sanya coral reefs. Also, this result may be applied to other reef areas.

Conclusions

Coralline species were distinguished at 3 m, 6 m and 9 m water depth, and two distinct groups related to the different sampling sites based on the species composition and abundance were defined. The OS sites which were of lower turbidity were mainly dominated by an assemblage of Corallinaceae. The IS sites with a higher turbidity had a more diverse representation of dominant taxa which included both Corallinaceae and Hapalidiaceae. The species *Amphiroa ephedraea* and *Mesophyllum simulans* were dominant present throughout the reefs. Under the cumulative effects of environmental factors, the multivariate analysis BIOENV procedure identified the best combination of variables (water turbidity and salinity), which were most responsible for the variability in the structure of coralline algae community in Sanya reefs. As the Sanya reefs are located in the tropical marine area and are among the coral reef systems in the world, the results in this study, on a worldwide basis, may provide a similar experience to tropical reef ecology research.

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