17-year change in species composition of mixed seagrass beds around Santiago Island, Bolinao, the northwestern Philippines

Yoshiyuki Tanaka^{a,*}, Gay Amabelle Go^b, Atsushi Watanabe^c, Toshihiro Miyajima^d, Masahiro Nakaoka^e, Wilfredo H. Uy^f, Kazuo Nadaoka^c, Shuichi Watanabe^a, Miguel D. Fortes^b

^a Mutsu Institute for Oceanography, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 690 Kitasekine, Mutsu, Aomori 035-0022, Japan

^b Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, the Philippines

^c Graduate School of Information Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguroku, Tokyo 152-8552, Japan

^d Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwanoha 5-1-5, Kashiwa 277-8564, Japan

^e Akkeshi Marine Station, Field Science Center for Northern Biosphere, Hokkaido University, Aikappu, Akkeshi, Hokkaido 088-1113, Japan

^f Institute of Fisheries R&D, Mindanao State University, 9023 Naawan, Misamis Oriental, the Philippines

*Corresponding author: Mutsu Institute for Oceanography, JAMSTEC, 690 Kitasekine, Mutsu, Aomori 035-0022 Japan. Tel: +81-175-45-1041 (direct); fax: +81-175-45-1079. E-mail address: y-tanaka@jamstec.go.jp

Abstract

Effects of fish culture can alter the adjacent ecosystems. This study compared seagrass species compositions in 2012 with those in 1995, when fish culture was less intensive compared to 2012 in the region. Observations were conducted at the same four sites around Santiago Island, Bolinao: (1) Silaqui Island, (2) Binaballian Loob, (3) Pislantan, and (4) Santa Barbara and by using the same methods as those of Bach et al. (1998). These sites were originally selected along a siltation gradient, ranging from Site 1, the most pristine, to Site 4, a heavily silted site. By 2012, fish culture had expanded around Sites 2, 3, and 4, where chlorophyll a (Chl a) was greater in 2012 than in 1995 by one order of magnitude. *Enhalus acoroides* and *Cymodocea serrulata*, which were recorded in 1995, were no longer present at Site 4, where both siltation and nutrient load are heavy.

Keywords: seagrass, tropical, long-term change, species composition, fish culture, Bolinao

Introduction

Coastal ecosystems in Southeast Asia have been deteriorating rapidly due to various types of human-induced stresses (Fortes 1988). Among them, excess nutrient and organic matter derived from fish culture has particularly affected adjacent ecosystems through the alteration of water quality and sediment conditions (Holmer and Kristensen 1992; Holmer et al. 2002, 2003). Ruiz et al. (2001) reported that the distribution and growth of temperate monospecific seagrass beds deteriorated in response to fish farm nutrient loading. In the case of tropical multispecific seagrass beds, however, quantitative observation of each species is needed to detect temporal variations. Because the responses differ among species, depending on their environmental

requirements, such as nutrient (Agawin et al. 1996; Udy and Dennison 1997), light (Agawin et al. 2001; Pollard and Greenway 1993; Tanaka and Nakaoka 2007), and sediment conditions (Bach et al. 1998; Tanaka and Nakaoka 2006; Terrados et al. 1998, 1999; Uy et al. 2001). Moreover, when multiple environmental factors are simultaneously affected *in situ*, it is difficult to evaluate the effects of each factor without long-term observation of species-specific changes.

In 1995, the culture of milkfish was introduced in the northwestern part of the Philippines, especially around Santiago Island, Bolinao (Holmer et al. 2002; Verceles et al. 2000). This practice has negatively affected both the planktonic community (Reichardt et al. 2006; San Diego-McGlone et al. 2008; Yap et al. 2004) and the benthic community (Nacorda et al. 2012; Reichardt et al. 2006). In the rainy season, during June and August of 1995, just after the fish culture introduction, when the impact of the nutrient load was small, Bach et al. (1998) recorded the variation of tropical seagrass species composition along the siltation gradient around Santiago Island. Both species richness and the lower depth limit of all seagrass species declined along the siltation gradient (Bach et al. 1998). Siltation strongly affects the light availability for seagrasses. Light availability is considered to control the maximum depth penetration of seagrass species (Duarte 1991).

This study was conducted in the rainy season, in September of 2012, after the proliferation of fish culture in the region, at the same sites and by using the same methods as those of Bach et al. (1998). The objective of this study was to detect the temporal variation of tropical seagrass species composition under different environmental conditions. In 1995, the main factor was siltation, whereas in 2012 the environmental factors were mainly siltation and nutrient loading from fish culture.

Materials and Methods

Study site

This study was conducted at four sites around Santiago Island, Bolinao, Pangasinan, northwestern Philippines (Fig. 1): Site 1, Silaqui Island (16°26'28.11"N, 119°55'35.37"E); Site 2, Binaballian Loob (16°23'04.30"N, 119°54'43.91"E); Site 3, Pislantan (16°22'04.19"N, 119°57'44.53"E); and Site 4, Santa Barbara (16°20'43.16"N, 119°55'39.43"E). The four sites were selected based on the geographic information shown on the map of Bach et al. (1998). Around the sites shown, if several candidates existed at 0, 0.5, 1, 2, and 3 m depth from mean low tide (MLT), then we selected the points with the highest species richness. During this study, there was no mariculture facility around Site 1 (Table 1). The distance between the closest floating fish cage or fish net-pen and Sites 2, 3 and 4 was about 300 m, 400 m and 30 m, respectively.

Seagrass density and species composition

The census was conducted from 11 to 14 September 2012, and we strictly followed the method of Bach et al. (1998) to measure the shoot density and species composition. Shoot density of *Enhalus acoroides* was counted at 20 locations at each depth using a 0.5 × 0.5 m frame, because this species has large shoots. For other species, a 0.5 × 0.5 m frame that was divided into 25 quadrats of 0.01 m² was used, and shoot densities in 10 haphazardly selected quadrats in each of five frames were counted at each depth. Originally, Bach et al. (1998) did not observe *Halodule pinifolia*. In this study, *H. pinifolia* was not distinguished from *Halodule uninervis*, because these two species may hybridize (Ito and Tanaka 2011; Waycott et al. 2006).

Environmental conditions

At each site, down-welling irradiance was measured under stable light conditions (clear or overcast) on 12 September 2012 using a cosine-corrected underwater quantum sensor equipped on the water quality profiler AAQ 177 (JFE Advantec Co., Ltd, Kobe, Japan). At least five up-and-down repetitions were conducted to measure light intensity, and then light attenuation coefficients, K_d (m^{-1}), were calculated for each repetition from the standard light attenuation equation of Lambert-Beer (Kirk 1994). In case the site was too shallow for light attenuation measurements, the data measured at adjacent deeper points (at least 2 m depth) were employed. Especially, Site 3 was shallow, and we moved about 700 m distance for the original point.

Water samples were collected on 12, 20, and 21 September 2012 at each site using dark plastic containers. Following Bach et al. (1998), suspended organic and inorganic solids were measured by filtering 1 to 4.5 L of seawater, depending on the concentration of the particles, onto pre-ignited and preweighed 47-mm Whatman GF/C filters. Filters and captured solids were weighed in wet condition for later adjustment of salt content. The filters were dried at 80°C for 24 h and reweighed for dry weight. Particulate organic matter (POM) was determined as weight loss upon ignition at 550°C for 8 h, and particulate inorganic matter (PIM) as the ash content remaining. The contribution of salt to PIM was estimated from the salinity of the water contained in wet filters. Salinity was measured with a refractometer (S/Mill-E, ATAGO Co., Ltd, Tokyo, Japan).

Concentrations of water-column chlorophyll a (Chl a) were determined by filtering 50 to 100 ml of seawater through 25-mm Whatman GF/C filters, as used by Bach et al. (1998). However, rather than using the 90% acetone employed by Bach et al. (1998) as the solvent, we instead used N,N-dimethylformamide, which is not volatile at room temperature.

Statistical analyses

The 2012 values of PIM, POM, Chl a, and K_d were compared among the four sites using one-way ANOVA. When significant variation was detected by ANOVA, post-hoc comparisons were carried out with Scheffé's F method. In addition, the PIM, POM, Chl a, and K_d values at each site were compared with those measured in 1995 using a Welch's t -test. Powers for the t -test were calculated using free software G*POWER. Statistical analyses were conducted at a significance level of 0.05. Before these analyses, the normality was tested by Kolmogorov-Smirnov test and homoscedasticity was tested using Cochran's C -test

Results

Seagrass density and species composition

At Site 1, all seven species reported in 1995 were still observed in 2012, although *C. serrulata* and *S. isoetifolium* decreased and observed only one depth level within site 1 (Table 2). Species richness decreased from six to four at Site 2 and from five to three at Site 3. At Site 4, two species, *E. acoroides* and *Cymodocea serrulata*, were recorded in 1995, but they were not found in 2012. *C. rotundata* showed the highest shoot density within each site in 2012, Site 1, depth 0 m : 2396 shoot m^{-2} , Site 2, depth 0 m : 812 shoot m^{-2} and Site 3, depth 0.5 m : 1360 shoot m^{-2} . On the other hand, other species were the highest in 1995, Site 1, depth 0 m : *T. hemprichii* 464 shoot m^{-2} , Site 2, depth 0 m : *H. uninervis*, 544 shoot m^{-2} , Site 3, depth 0 m : *H. uninervis*, 1064 shoot

m⁻² and Site 4, depth 0 m : *C. serrulata* 214 shoot m⁻². The decline of the lower depth limit of species along the siltation gradient observed in 1995 was not obvious in 2012.

Environmental conditions

The average values of all the measured parameters tended to be highest at Site 4 and lowest at Site 1 (Table 3), as also reported by Bach et al. (1998). PIM was low in this study, and no significant difference was detected among sites. POM and Chl a were significantly lower at Site 1 as compared to values at the other sites. K_d did not show a significant difference between Sites 1 and 2.

In our comparisons of environmental conditions between 1995 and 2012, we found significant differences in all cases except Chl a at Site 1 and K_d at Sites 2 and 4 (Table 3). PIM was smaller in 2012 at all sites, whereas POM was larger in 2012 at Sites 2, 3, and 4. Chl a values were larger than those in 1995 by one order of magnitude at Sites 2, 3, and 4. K_d values were larger in 2012 at Sites 1 and 3. When significant differences were detected by Welch's *t*-test, powers were more than 0.9 for Chl a and K_d . For PIM and POM, those were relatively low, but still more than 0.75 except for site 1 and 2 of POM.

Discussion

This study compared seagrass species compositions before and after the proliferation of fish culture around Santiago Island, the Philippines. We detected major deterioration of species richness at the three sites adjacent to the fish net-pens and/or floating fish cages (Table 2), where both siltation and nutrient load affected the environment simultaneously. In 1995, *E. acoroides* and *C. serrulata* were observed at Site 4 (Table 2). These two seagrasses have been categorized as the species most tolerant against siltation and burial (Bach et al. 1998; Duarte et al. 1997; Terrados et al. 1998). Under the siltation effect alone, morphological characteristics such as the long leaves of *E. acoroides* and the long vertical rhizomes of *C. serrulata* appear to enhance their survival (Bach et al. 1998; Duarte et al. 1997; Tanaka and Nakaoka 2006; Terrados et al. 1998; Vermaat et al. 1997). In 2012, however, after 17 years of fish culture and the resulting increase in nutrient and organic matter loads, no seagrass was observed at Site 4.

Just before the introduction of fish culture, seagrass around Santiago Island was in a nutrient-limited condition. An *in situ* fertilization experiment conducted from September 1994 to January 1995 revealed that *E. acoroides* was experiencing substantial nitrogen deficiency, and *Thalassia hemprichii* had moderate phosphate deficiency (Agawin et al. 1996). Adequate concentrations of nutrients can enhance the photosynthetic activity of seagrasses, although the degree of deficiency and required nutrients vary, depending on the seagrass species.

Excess nutrient input deteriorates light penetration by increasing the population of phytoplankton. In this study, Chl a showed a massive increase as compared to concentrations 17 years ago at Sites 2, 3, and 4 (Table 3). Suzuki and Ishimaru (1990) compared the Chl a contents of marine diatoms, a raphidophycean, and a green alga extracted by both N,N-dimethylformamide and 90% acetone, and the former showed higher extraction efficiencies in all 14 kinds of samples (mean 117%, minimum 102%, and maximum 151% as compared with extraction with 90% acetone). Because the difference of extraction efficiency between N,N-dimethylformamide and 90% acetone is less than a factor of 1.5, the difference in Chl a concentrations between 1995 and 2012 is considered to indicate an actual increase of phytoplankton. Compared to the massive increase in Chl a, the increase of POM and deterioration

of K_d seem relatively moderate. In our study area, severe red tides have been observed and sometimes cause fish kills (Reichardt et al. 2006; San Diego-McGlone et al. 2008; Yap et al. 2004). An outbreak of red tide occurred in November 2012, just after this study was conducted, and in March 2013 (pers. comm. with staff of the Bolinao Marine Laboratory, University of the Philippines). Under those conditions, the light environment should be worse than that measured in this research and may have affected seagrasses..

Excess nutrient input also altered sediment conditions and benthic communities in a milkfish mariculture zone around Santiago Island. In 2008, sediment surfaces adjacent to mariculture facilities were laminated by a sulfur-oxidizing bacterium of the genus *Beggiatoa*, and no macro-infaunal organisms were observed. The relative redox potentials around fish net-pens and floating cages always showed negative values, except those at the sediment surface, and they were apparently lower than those of control sites (Nacorda et al. 2012). Uy et al. (2001) reported that the respiratory demand of belowground parts of two seagrass species increased under a reduced sediment condition. The belowground respiratory demand of *T. hemprichii* and *H. uninervis* were 1.2–1.5 and 1.7–2.3 times, respectively, that of oxidized sediment conditions. High respiratory demand of belowground parts deteriorates the carbon balance of the seagrass shoots (Tanaka and Nakaoka 2007; Uy et al. 2001), making it difficult for them to survive.

Although neither *E. acoroides* nor *C. serrulata* survived at Site 4, these two species showed quite different distribution patterns at the other sites. In 2012, *E. acoroides* was still dominant at Sites 1, 2, and 3, whereas *C. serrulata*, the most widely distributed species in 1995, was observed at only one depth level at Site 1 (Table 2). Different nutrient demands may help to explain the different distribution patterns. The nutrient demand of *E. acoroides* is high (Agawin et al. 1996), whereas that of *C. serrulata* is relatively low (Udy and Dennison 1997). Thus, the extra nutrient load caused by fish culture may not benefit *C. serrulata* enough to overcome the detrimental effects of the reduced sediment condition.

However, it should be noted that the shoot density of *C. serrulata* decreased not only at the sites adjacent to the mariculture facilities, but also at Site 1, which was the least affected by siltation and nutrient load among the four sites (Table 3). Based on our results, Site 1 near Silaqui Island still seems pristine compared to other sites around Santiago Island. However, Senal et al. (2011) detected high nutrient concentrations at a well on Silaqui Island (range of $\text{NO}_3 + \text{NO}_2$, 28.0 – 1644.1 μM) and in seepage water around the island (average NH_3 , 60.4 μM), and they noted the possible effect of submarine groundwater discharge on the coastal ecosystems. This high concentration of nutrients might affect the seagrass distribution and species composition. Future research is needed to track this situation around Silaqui Island, as well as to elucidate how the nutrient load and other factors affect the temporal change in seagrass species composition that was detected in this study.

Seagrass are supposed to show temporal variability of distribution, even under the natural environmental fluctuation. Nakaoka et al. (2007) recorded the total seagrass coverage almost once in a year from 2001 to 2006, at 6 sites in Andaman sea coast of Thailand. At the 5 sites without serious Tsunami impact, the coefficient of variation (CV) of total seagrass coverage was between 0.02 and 0.29. The Global seagrass monitoring network, SeagrassNet conducted the monitoring at Sabang and Paniquian, Mindoro Is. in the Philippines, 22 times from 2001 to 2009 (per comm. with M. Fortes). CV of total seagrass coverage for both sites was 0.26, which is similar level with Nakaoka et al. (2007) Compared to these results, disappearing of seagrasses at Site 4 is considered to be the greater event beyond the natural temporal fluctuation of seagrass distribution.

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Captions

Fig. 1. Study sites (Star marks) around Santiago Island, the Philippines. Site 1: Silaqui Island; Site 2: Binaballian Loob; Site 3: Pislatan; and Site 4: Santa Barbara. The aquaculture facilities (cages and pens) around study sites were traced from a satellite image (Google Earth, checked in May 2014). Fish pens are gray squares, while fish cages are white circles.

Site	Distance from site (m)							
	500	1000	1500	2000	2500	3000	3500	4000
Fish Pen								
Site 1	0	0	0	0	0	0	0	0
Site 2	0	0	0	11	20	24	39	66
Site 3	1	17	40	63	100	121	153	222
Site 4	8	31	96	167	240	326	361	398
Fish Cage								
Site 1	0	0	0	0	0	0	0	0
Site 2	14	74	116	172	183	183	183	183
Site 3	0	8	81	100	101	104	142	183
Site 4	0	0	20	41	53	65	68	111
Total (Pen + Cage)								
Site 1	0	0	0	0	0	0	0	0
Site 2	14	74	116	183	203	207	222	249
Site 3	1	25	121	163	201	225	295	405
Site 4	8	31	116	208	293	391	429	509

Table 1. The number and distance of mariculture facilities (Pens and Cages) from the four study sites
Based on the positions shown on the Fig. 1, the number was counted using free software Q-GIS.

Table(s)

Site	Depth (m)	<i>Enhalus acoroides</i>		<i>Cymodocea serrulata</i>		<i>Halodule uninervis</i>		<i>Halophila ovalis</i>		<i>Thalassia hemprichii</i>		<i>Cymodocea rotundata</i>		<i>Syringodium isoetifolium</i>	
<i>Species composition in 2012</i>															
Site 1	0	0.6 ±	0.3	○		30.0 ±	17.9	○		○		2396.0 ±	141.7	○	
	0.5	45.4 ±	6.4	88.0 ±	18.4	74.0 ±	17.8	24.0 ±	20.3	686.0 ±	41.2	42.0 ±	11.5	188.0 ±	38.9
	1	21.2 ±	3.6	○		666.0 ±	109.3	248.0 ±	66.2	260.0 ±	24.9	○		○	
	2	14.4 ±	2.1	○		162.0 ±	30.9	14.0 ±	9.9	428.0 ±	46.3	○		○	
	3	2.8 ±	1.8	○		144.0 ±	41.9	94.0 ±	24.0	14.0 ±	7.6	○		○	
Site 2	0	22.9 ±	7.6	○		★		○		162.0 ±	52.0	812.0 ±	85.1	○	
	0.5	3.0 ±	2.4	○		○		○		70.0 ±	31.2	○		○	
	1	2.8 ±	1.6	○		○		○		○		○		○	
	2	1.6 ±	1.1	○		○		○		○		○		○	
Site 3	0	○		○		○		○		○		○		○	
	0.5	4.8 ±	3.3	○		○		○		○		1360.0 ±	47.8	○	
	1	58.0 ±	8.9	○		○		○		176.7 ±	25.5	○		○	
Site 4	No seagrass														

<i>Species composition in 1995 (cited from Bach et al. 1998)</i>															
Site 1	0	16.0 ±	1.7	2.0 ±	14.0	42.0 ±	38.0	388.0 ±	79.0	464.0 ±	59.0	78.0 ±	36.0	4.0 ±	24.0
	0.5	12.6 ±	1.3	80.0 ±	24.0	150.0 ±	28.0	332.0 ±	52.0	394.0 ±	41.0	108.0 ±	25.0	396.0 ±	59.0
	1	8.8 ±	1.4	138.0 ±	13.0	80.0 ±	27.0	50.0 ±	36.0	316.0 ±	43.0	106.0 ±	30.0	108.0 ±	25.0
	2	5.8 ±	1.2	62.0 ±	19.0	★		186.0 ±	53.0	190.0 ±	23.0	○		32.0 ±	18.0
	3	○		54.0 ±	32.0	16.0 ±	29.0	★		90.0 ±	28.0	○		○	
Site 2	0	★		14.0 ±	28.0	544.0 ±	99.0	52.0 ±	51.0	176.0 ±	54.0	474.0 ±	70.0	○	
	0.5	5.4 ±	1.3	64.0 ±	49.0	502.0 ±	80.0	38.0 ±	45.0	398.0 ±	50.0	16.0 ±	31.0	○	
	1	3.0 ±	1.6	12.0 ±	26.0	194.0 ±	74.0	184.0 ±	68.0	354.0 ±	58.0	○		○	
	2	○ ±		★		340.0 ±	83.0	188.0 ±	78.0	220.0 ±	56.0	○		○	
Site 3	0	8.0 ±	1.8	○		1064.0 ±	103.0	12.0 ±	26.0	160.0 ±	53.0	○		○	
	0.5	5.2 ±	1.3	160.0 ±	59.0	8.0 ±	34.0	○		○		○		○	
	1	○		58.0 ±	52.0	○ ±		○		○		○		○	

Table 2. Mean and standard error of seagrass shoot density per square meter at various water depths at four sites around Santiago Island, Bolinao.
(★) Species present at the site but at densities below those that could be analyzed quantitatively
(○) Species absent

Site	PIM (mg L ⁻¹)			POM (mg L ⁻¹)			Chl a (μg L ⁻¹)			K _d (m ⁻¹)		
<i>Water quality in 2012</i>												
Site 1	0.3 ±	0.1 (3)	* 0.84	1.8 ±	0.1 (3)	a *	0.7 ± 0.2	(3)	a	0.54 ±	0.01 (5)	a *
						0.29			0.59			0.99
Site 2	1.0 ±	1.0 (3)	* 0.87	5.1 ±	0.4 (3)	b *	13.8 ± 2.4	(3)	b *	0.55 ±	0.01 (5)	a
						0.51			0.99			0.26
Site 3	1.1 ±	0.3 (3)	* 0.99	6.4 ±	0.4 (3)	b *	15.9 ± 1.1	(3)	b *	0.83 ±	0.02 (5)	b *
						0.76			1.00			0.93
Site 4	2.3 ±	0.8 (3)	* 0.77	6.8 ±	0.5 (3)	b *	16.3 ± 0.9	(3)	b *	1.23 ±	0.01 (5)	c
						0.94			1.00			0.74
<i>Water quality in 1995 (cited from Bach et al. 1998)</i>												
Site 1	3.5 ±	0.57 (16)	a	2.6 ±	0.3 (16)	a	0.34 ± 0.04	(16)	a	0.31 ±	0.03 (16)	a
Site 2	4.8 ±	0.5 (16)	b	3.5 ±	0.4 (16)	ab	0.8 ± 0.1	(16)	b	0.43 ±	0.06 (16)	b
Site 3	4.6 ±	0.3 (16)	b	3.7 ±	0.5 (16)	b	1.14 ± 0.16	(16)	b	0.61 ±	0.04 (16)	c
Site 4	8.2 ±	1.1 (16)	c	4.3 ±	0.3 (16)	b	2.04 ± 0.28	(16)	c	0.91 ±	0.08 (16)	d

Table 3. Mean concentration and standard error of suspended particulate inorganic matter (PIM), particulate organic matter (POM), phytoplankton biomass (Chl a), and mean light attenuation coefficient (*K_d*) in the water colum at four sites around Santiago Island, Bolinao. Sample numbers are shown in parentheses. Different letters within a column indicate that values differed significantly (*P* < 0.05) by multiple comparisons of means.

Figure(s)