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Research Paper

# Potential Suitabilty of Coenocytic Green Algae as an Indicator of the Coastal Environment in the Kuroshio Region.

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## Abstract

Potential suitability of coenocytic green algae (CGAs) such as *Valonia* spp. and *Caulerpa* spp as indicator species of the coastal environment in the Kuroshio Current region was examined by means of biological and socio/economical approaches. The investigations included the following: 1) field surveys in the five study areas in the Philippines, Taiwan and Japan; 2) fixed point observation in northern Luzon; 3) laboratory experiments using culture strains of collected specimens; 4) a case study in Green Island, Taiwan. Nine CGA species were recognized in the study areas and the results of the above investigations indicated that CGAs were potentially suitable species as indicators of coastal environments in the Kuroshio region.

Key words: *Caulerpa*, Claveria, Lagonoy Gulf, Ludao (Green Island), *Valonia*

## Introduction

The ecosystem in coastal waters depends on production by primary producers such as seaweed/sea grass beds and coral reefs and its maintenance is very important for sustainable use of fishery resources along the coast (Campos and Aliño 2008, Mok 2008). Recently, large-scale degradation of the coastal ecosystem has been reported in various regions along the Kuroshio Current, which is now one of the most serious problems in local communities (e.g., Morooka *et al*. 2012). However, the cause of such degradation has not been understood and, therefore, effective methods for recovering the degraded coastal condition remain to be taken in many cases. Accurate estimation of environmental degradation using reliable environmental indicators is an indispensable step for the solution of this problem. Since coastal ecosystem degradation is occurring beyond national boundaries in the Kuroshio Current region, the coastal environment indicators should be used commonly among the countries in the region.

A number of marine animals living in coastal waters have served as environmental indicators so far (Niemi and McDonald 2004) and moreover, recent studies also revealed that the vegetation of seaweed is also influenced by changes in the coastal environment such as temperature and nutrition. For example, barren ground in the ocean, called as "isoyake", has been one of the most severe problems in the coastal ecosystem around Japan and it is widely believed that disappearance of seaweeds in the isoyake area was caused by changes in environmental conditions such as temperature and nutrition (Fujita 2010). Recently, it was reported that the species composition of large brown seaweeds, i.e., kelps and *Sargassum* spp. along the coast of Tosa Bay, Japan has changed according to the increase in surface seawater temperature (Haraguchi *et al*. 2009, Tanaka *et al*. 2012).

Species of one seaweed group, the coenocytic green

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algae (CGAs), have been used frequently for many physiological experiments using laboratory culture strains because of the characteristic structure of their giant cells (reviewed by Mine *et al*. 2008). Moreover, some CGA species are reported to be potential environmental indicators (Barile 2004, Titlyanov *et al*. 2011). There are several conditions for "good indicator species", e.g. being easy to collect in the field, having easily recognizable morphologies, and being easily classified and treated in the laboratory. We started a research project in 2011 to evaluate the suitability of CGAs as an environmental indicator in the Kuroshio region by examining algae in the above conditions. The project comprises the following four parts of both biological and socio/economic studies:

1) Selection of study areas in the Philippines, Taiwan and Japan, where seaweed vegetation including CGAs was rich and local communities are established.
2) Field survey: CGA specimens are collected in the study areas for species identification, culture strain production, molecular-biogeographical analyses, and physical and biological conditions around the CGA populations are examined.
3) Laboratory culture experiments where the effects of light, temperature and nutrition conditions on the growth and reproduction are investigated.
4) Socio/economic studies on problems in coastal degradation and the political situation connected with its protection, in particular the local residents' interests on the CGAs and their relationships with local societies.
5) Comparative studies of CGA abundance and environmental conditions among different study areas.

Here we report an outcome of the project concerning the CGAs belonging to the genus *Valonia* C. Agardh and genus *Caulerpa* J.V.Lamouroux

## Materials and methods

### Field survey trips

Following several preliminary survey trips in the coastal regions along the Kuroshio Current in the Philippines (southern, central and northern Luzon, and Batanes Islands), Taiwan (Kending, Ludao (Green Island), Keelung) and Japan (Ohtsuki and Tosa Bay) carried out in 2009 to 2011, the following five study areas were selected: 1) Lagonoy Gulf (southern Luzon), 2) Claveria (northern Luzon), 3) Green Island, 4) Keelung and 5) Ohtsuki town (Kashiwa-jima Island and Nishidomari) on the basis of criteria that seaweed vegetation including CGAs are rich and a community of local people has been established in the neighborhood.

In Lagonoy Gulf, surveys were made on San Miguel Island (N 13º 23' 21.620", E 123º 48' 32.605"), Batan Island (N 13º 16' 55.275", E 123º 58' 26.173"), San Lorenzo (N 13º 20' 14.481", E 123º 44' 26.340"), and Cawayan (N 13º 17' 52.180", E 123º 47' 52.130"). The collection records of a practical course held in Bicol University, Tabaco Campus were provided by Mr. Alex Camaya and also incorporated in the present study (Table 1). Surveys in Claveria were conducted in Taggat Lagoon (N 18º 36' 43.876", E 121º 02' 48.897") where a few large rocks are present between the lagoon and open sea (Fig. 1A), and seaweeds were growing inside the rocks.

Green Island has population of about 4,000 and area about 15 km$^2$ and is surrounded by rocky shore composed of limestone. The seashore around the villages Nanliao (N 22º 39' 52.563", E 121º 28' 18.649"), Zhongliao (N 22º 40' 30.795", E 121º 28' 13.184") and Chaikou (N 22º 40' 27.433", E 121º 28' 43.449") was investigated in the present study because of the presence of a relatively large local community. Keelung is a large harbor city near the Taiwanese capital Taipei. Terrestrial areas are fully developed and most of the seashore has been reclaimed. Natural rocky shores in Heping Island (N 25º 09' 47.473", E 121º 45' 45712") and Chaojing (N 25º 08' 28.337", E 121º 48' 08.542") were surveyed in the present study.

The seashore along Ohtsuki town has not reclaimed except for the areas for ports. Because of the establish-

**Table 1. *Valonia* spp. and *Caulerpa* spp. found in the study areas during the present study.**

| Study areas[1)] | (I) | (II) | (III) | (IV) | (V) |
|---|---|---|---|---|---|
| Genus *Valonia* | | | | | |
| *V. aegagropila* | + | + | + | + | |
| *V. utricularis* | +[2)] | + | + | + | + |
| Genus *Caulerpa* | | | | | |
| *C. brachypus* | | | | | + |
| *C. peltata* | + | + | + | + | |
| *C. macrophysa* | +[2)] | + | + | + | |
| *C. webbiana* f. *tomentella* | + | + | | | |
| *C. serrulata* f. *lata* | | + | | | |
| *C. sertularioides* f. *longipes* | + | + | | | |
| *C. cupressoides* var. *lycopodium* | | + | | | |

1) Study areas are (I) Lagonoy Gulf, (II) Claveria, (III) Ludao (Green Island), (IV) Keelung and (V) Ohtsuki.
2) Identified by photographs taken in the practical courses held in Bicol University, Tabaco Campus provided by Mr. Alex Camaya.

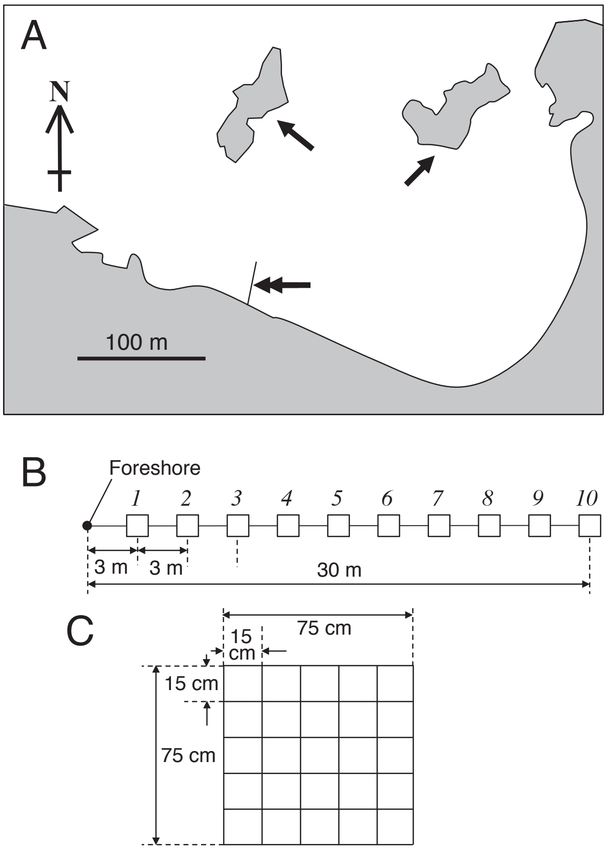


**Fig. 1.** Field survey in Taggat Lagoon, Claveria. A. Map of Taggat Lagoon. Arrows indicate two large rocks located along the edge of lagoon. A double arrow indicates the 30-meter line laid for line census observations. B. A diagram of arrangement of quadrats used in line census observations. C. A diagram of the quadrat used in line census observations showing the arrangement of 25 segments in it.

ment of a local community and of rich seaweed vegetation, we surveyed Kashiwa-jima Island (N 32º 46' 02.805", E 132º 37' 38.035") and Nishi-domari (N 32º 46' 45.314", E 132º 43' 57.435").

Trips for field surveys were conducted in the following dates and study areas: 13-20, August, 2011 (Lagonoy Gulf, Claveria); 18-19, March, 2012 (Keelung); 18-23, May (Green Island, Keelung); 19-20, June (Ohtsuki); 3-4, December, 2012 (Lagonoy Gulf); 15-19, March, 2013 (Claveria, Green Island), 8-10, June (Green Island) and 21-23, November, 2013 (Green Island) .

**Seaweed vegetation characterization**

The seaweed populations growing in the intertidal and upper subtidal zones along the study areas were approached via snorkeling. The status of CGAs and other seaweeds present in the neighborhood were observed and photographed by a digital camera (TG-810, Olympus, Tokyo). Some of CGAs were collected, cleaned with a paint brush and fragments of a few tens of millimeter were excised, transferred to a plastic bottle containing 50 mL of filtered seawater added with a small amount of the enrichment for the culture medium Provasoli's Enriched Seawater (PES) (Provasoli 1966) and carried back to the laboratory. Other collected materials were processed for herbarium specimens, formalin-fixed specimens for species identification. In addition, some of the collected plants were thoroughly dried by gently mixing with an excess amount of silica gel (Wakogel C-100, Wako Pure

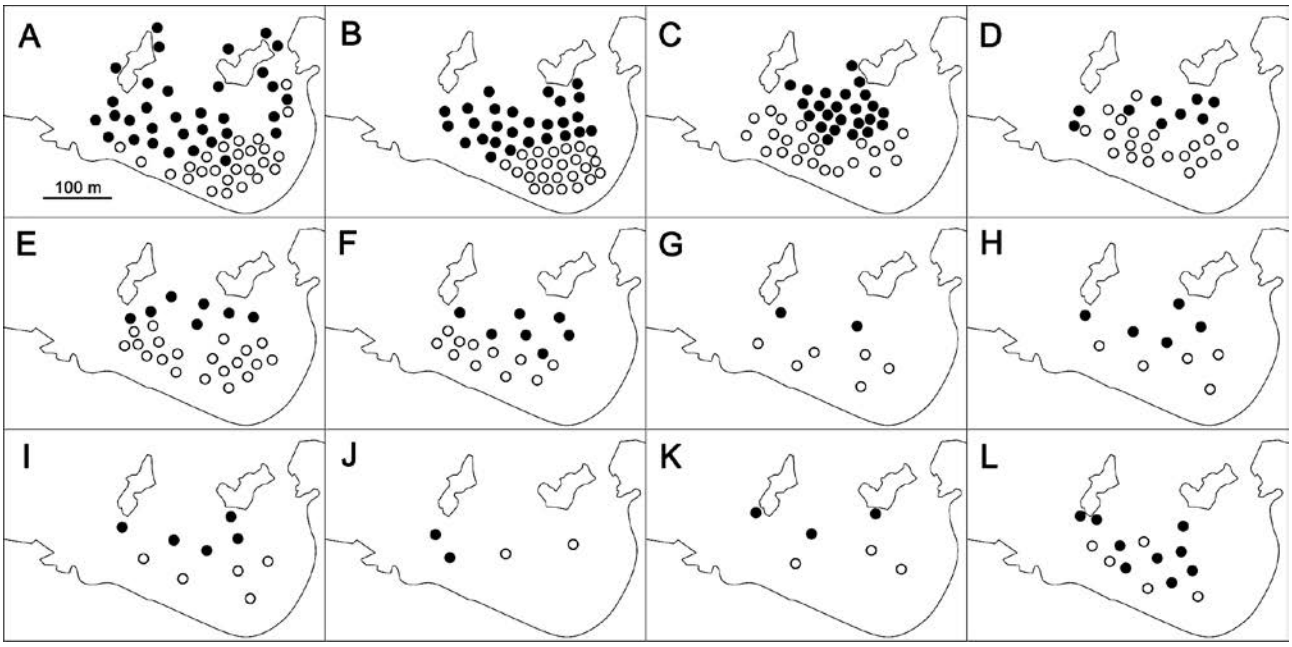


**Fig. 2.** Results of the monthly whole area observations in Taggat Lagoon. Black and white circles represent the points where individuals of *Valonia aegagropila* and *Caulerpa sertularioides* f. *longipes* were present. Fig. 2A-L shows the results of observation conducted in January to December, 2013, respectively.

Chemicals, Osaka) for molecular analysis.

Furthermore, vegetation of the CGAs and seaweeds growing in the vicinity of CGAs were surveyed by whole area and line census observations, in each fixed point set in Taggat Lagoon, Claveria (Fig. 1A). In order to record the changes in horizontal distribution of CGAs according to the seasons, the lagoon was surveyed monthly and places where *Valonia* spp. and/or *Caulerpa* spp. was found were recorded on a map as shown in Fig. 2. The dates of surveys were 18 January, 18 February, 18 March, 18 April, 16 May, 18 June, 19, July, 24 August, 18 September, 18 October, 18 November and 18 December, 2013. For line census observations, a 30-m line was drawn from the foreshore of the western part of the lagoon; ten quadrats, each of which was 75 cm × 75 cm in size, were placed along the line at intervals of 3 meters (Fig. 1A, B). Each quadrat was divided into 25 segments, 15 cm × 15 cm in size (Fig. 1C). The quadrats were observed monthly and the number of segments where each macroscopic seaweed species was found was recorded. The dates of surveys were 30 May, 30 June, 28 July, 24 August, 17 September, 7 October, 23 November and 10 December, 2013, and 31 January, 7 February, 15 March and 19 April, 2014.

For analyses of water quality, seawater was collected from the CGA habitats in a polypropylene airtight container (capacity 250 mL) and kept in a refrigerator until it was sent to Stone & Resource Industry R/D Center, Hualien, Taiwan. Substances analyzed were chemical oxygen demand (COD), inorganic phosphate and ammonium ion.

The climate classification of the study areas was determined by C/P and $[I/H]_{RCP}$ indices (Tomizuka *et al*. 2012) based on the previous reports of seaweed flora around the study areas (Huang 2000, Kitayama and Lin 2012, Lin 2012, Silva *et al*. 1987). Scientific names of seaweed taxa and authorities follow AlgaeBase (Guiry and Guiry 2015).

**Laboratory culture experiments**

The living materials of *Valonia* spp. were cleaned by sterilized paintbrush until no other organisms were detectable under the dissecting microscope. Cleaned portions were then excised by sterilized scissors, transferred to fresh PES medium and incubated under illumination provided by cool fluorescent lamps at 16 $\mu$mol s$^{-1}$ m$^{-2}$ in a 12 h:12 h light : dark photoperiod and the temperature was 20± 2°C. *Valonia* spp. produces new cells by lenticular cell formation (Okuda *et al*. 1997, Mine *et al*. 2008). Cells for experiments were isolated by excising newly formed lenticular cells with a fragment of the cell wall of the parent cell using a razor blade.

Each isolated cell was transferred to a well of a tissue culture plate (35-3043, 12 well, flat bottom with low evaporation lid, Becton Dickinson Labware, Franklin Lakes) containing 3 mL of the test medium, in which the volume of enrichment of PES medium added to 1 L of autoclaved seawater was changed by 0.75 7.5 or 75 mL. Three plates containing isolated cells were stacked and placed directly under the illumination provided by four white light emitting diodes (OSW4XME1C1S-100, OptoSupply Ltd., Hong Kong) from above. Two layers of black cheese cloths was placed under the top and middle plates to reduce the intensity of incident light in the middle and bottom plates, stepwise resulting in photon flux densities in the wells of the top, middle and bottom plates, which were 400, 80 and 20 $\mu$mol s$^{-1}$ m$^{-2}$ (Fig. 3A). The light : dark cycle of illumination was 12h: 12h.

The culture plates with illumination were incubated at 15, 20 or 25 ºC for three weeks and the bottom view of the plates was captured using an image scanner (GT-X700, Seiko Epson Corp., Suwa) at 1200 dpi. The

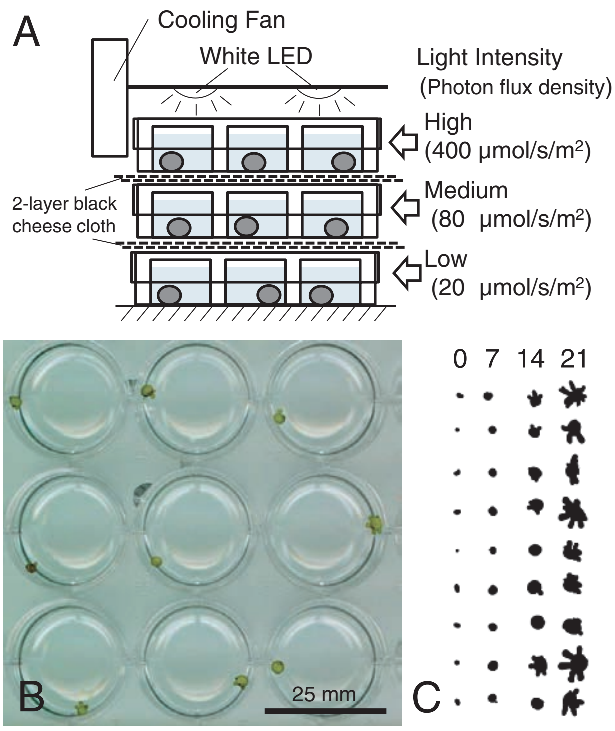


**Fig. 3.** Laboratory culture experiments. A. A diagram of stack of three tissue culture plates containing test medium and isolated cells of *Valonia utricularis*. under illumination provided by white light-emitting diodes. Note the intensity of incident light in the stacked plates was reduced stepwise by the 2-layer black cheese cloths. B. Scanned image of bottom view of tissue culture plate incubated under a light intensity of 80 $\mu$mol s$^{-1}$ m$^{-2}$ at 20ºC for 14 days in different nutrient conditions. C. Outlines of nine cells shown in Fig. 3B. Numbers represent days after the beginning of incubation.

scanned image was opened by image analysis software ImageJ (U. S. National Institutes of Health, Bethesda, http://rsb.info.nih.gov/ij/index.html), the outline of each cell was traced by a "wand" tool and the area of the cell was measured and considered as the size of the cell. Finally the cell growth during the incubation period was calculated by the following equation:

$$\text{Growth (\%)} = \frac{(A_t - A_0)}{A_0} \times 100,$$

where $A_0$ is the area of the cell at the beginning of incubation and $A_t$ is the area of the cell at the end of incubation.

**Molecular analyses**

Due to the intraspecific variation of erect shoot morphology (e.g., Ohba *et al.* 1992), the species identification is often difficult in *Caulerpa* spp. and, therefore, molecular analyses using plastid *tuf*A gene were carried out. The *tuf*A is a preferred marker for species identification and phylogeny of green algal taxa including *Caulerpa* spp. (e.g. Saunders and Kucera 2010, Belton *et al.* 2014). For DNA extraction, amplification and sequence reaction of the *tuf*A region, most of the procedures followed Sauvage *et al.* (2013). Decided DNA sequences were compared with published sequences of the same and related species using a BLAST (Basic Local Alignment Search Tool) search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to understand the species identity of the *Caulerpa* spp. collected from Taggat lagoon, Claveria, in the present study.

**Surveys of socio/economic status in the study areas**

To understand current problems in the degradation of coastal environment and the political situation in regard to its protection, interviews with local stakeholders, e.g., coast guards, leaders of local communities, divisions of local government in charge of environment, sanitation, fisheries, etc., were taken through an interpreter.

## Results

**Field survey of CGA vegetation**

Nine taxa belonging to the genus *Valonia* and *Caulerpa* have been collected and identified so far during the field surveys, i.e., *Valonia aegagropila* C. Agardh, *V. utricularis* (Roth) C. Agardh, *Caulerpa brachypus* Harvey, *C. peltata* J.V.Lamouroux, *C. macrophysa* (Sonder ex Kützing) G.Murray, *C. webbiana* f. *tomentella* (Harvey ex J. Agardh) Weber-van Bosse, *C. serrulata* f. *lata* (Weber-van Bosse) C.K. Tseng, *C. sertularioides* f. *longipes* (J. Agardh) Collins and *C. cupressoides* var. *lycopodium* Weber-van Bosse (Table 1). Morphological variability in *C. peltata* and *C. macrophysa* is high and specimens with peltate ramuli are regarded as *C. peltata,* and those with spherical ramuli are classified as *C. macrophysa* in the present study. The identification of taxonomic entity *C. chemnitzia,* firstly identified as *C. peltata* and *C. macrophysa*, was also supported by molecular data (see below).

Species of *Valonia* and *Caulerpa* were readily found along the coasts in the areas. The two species of *Valonia* were found commonly in most study areas. Although *C. peltata* was commonly found, the species composition of *Caulerpa* was different among the areas. These algae were found along the seashore in various physical environments including those facing the open sea under a strong wave influence and those sheltered from waves in locations such as lagoons. In all cases, they were growing in the subtidal zone always covered by seawater even in low tide and were surrounded by a rich vegetation of other seaweed species.

The fixed-point observations in Taggat Lagoon, Claveria, further support the above observations. Whole area observations on CGA vegetation showed that vegetation of both *Valonia aegagropila* and *Caulerpa sertularioides* f. *longipes* was rich from winter to spring and they especially spread over the lagoon from January to March (Fig. 2). In addition, *C. sertularioides* f. *longipes* was found in the areas near the foreshore of the lagoon whereas *V. aegagropila* was mainly growing in the central areas of the lagoon more than 20 meters apart from the foreshore (Fig. 2).

Nineteen taxa of seaweeds, including two CGA species, have been found in the line census observations: red algae, *Galaxaura* sp., *Halymenia* sp., *Amphiroa foliacea* J.V.Lamouroux, *Gracilaria* sp. and *Hypnea valentiae* (Turner) Montagne; Brown algae, *Dictyota dichotoma* (Hudson) J.V.Lamouroux, *Padina australis* Hauck and *Sargassum aquifolium* (Turner) J.Agardh, and Green algae, *Ulva lactuca* Linnaeus, *Anadyomene plicata* C. Agardh, *Chaetomorpha crassa* (C. Agardh) Kützing, *Dictyosphaeria cavernosa* (Forsskål) Børgesen, *Valonia aegagropila*, *Caulerpa sertularioides* f. *longipes*, *Codium geppiorum* O.C.Schmidt, *Halimeda opuntia* (Linnaeus) Lamouroux, *Chlorodesmis fastigiata* (C. Agardh) Ducker, *Neomeris vanbosseae* M.Howe and *Acetabularia dentata* Solms-Laubach. Table 2 shows the monthly occurrence of these seaweeds in the quadrats placed along the 30-m line drawn in the lagoon. The observed areas were rich in seaweed vegetation from

**Table 2. The number of segments where the seaweed species were found in each quadrat (25 segments in total) during line census observations in Taggat Lagoo.**

| Month | Quadrat number | *Galaxaura* sp. | *Halymenia* sp. | *Amphiroa foliacea* | *Gracilaria* sp. | *Hypnea valentiae* | *Dictyota dichotoma* | *Padina australis* | *Sargassum aquifolium* | *Ulva lactuca* | *Anadyomene plicata* | *Chaetomorpha crassa* | *Dictyosphaeria cavernosa* | *Valonia aegagropila* | *Caulerpa sertularioides* f. *longipes* | *Codium geppiorum* | *Halimeda opuntia* | *Chlorodesmis fastigiata* | *Neomeris vanbosseae* | *Acetabularia dentata* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2013 | 1 | | | | | 2 | | 16 | | 7 | | | | | | | | | | |
| | 2 | | | 3 | | | | 9 | 3 | 5 | | 1 | | | | | | | | |
| | 3 | | | | | | | 2 | | 25 | | | | | | | | | | |
| | 4 | | | 1 | 1 | | | 2 | | 16 | | 1 | | | | | | | 1 | |
| | 5 | | | 1 | | | | 1 | 22 | 1 | | | | | | | | | | |
| | 6 | 2 | | 3 | | | | 7 | 16 | | | | | | | | | | | |
| | 7 | | | | | | | | 25 | | | | | | | | | | | |
| | 8 | | | | | | | | 25 | | | | | | | | | | | |
| | 9 | | | | | | | | 25 | | | | | | | | | | | |
| | 10 | | | | | | | | 25 | | | | | | | | | | | |
| Jun 2013 | 1 | | | | | | | | 6 | | | | | | | | | | | |
| | 2 | | | | | | | | 1 | 1 | | | | | | | | | | |
| | 3 | | | | | | | | 25 | | | | | | | | | | | |
| | 4 | | | | | | | | 25 | | | | | | | | | | | |
| | 5 | | | | | | | | 25 | | | | | | | | | | | |
| | 6 | | | | | | | | 25 | | | | | | | | | | | |
| | 7 | | | | | | | | 25 | | | | | | | | | | | |
| | 8 | | | | | | | | 25 | | | | | | | | | | | |
| | 9 | | | | | | | | 25 | | | | | | | | | | | |
| | 10 | | | | | | | | 25 | | | | | | | | | | | |
| Jul 2013 | 1 | | | | | | 4 | 3 | 15 | | | 2 | | | | | | | | 1 |
| | 2 | | | | | | 3 | 5 | 13 | | | | | | | | | | | |
| | 3 | | | | | | | | 25 | | | | | | | | | | | |
| | 4 | | | | | | | | 25 | | | | | | | | | | | |
| | 5 | | | | | | | | 25 | | | | | | | | | | | |
| | 6 | | | | | | | | 25 | | | | | | | | | | | |
| | 7 | | | | | | | | 25 | | | | | | | | | | | |
| | 8 | | | | | | | | 25 | | | | | | | | | | | |
| | 9 | | | | | | | | 25 | | | | | | | | | | | |
| | 10 | | | | | | | | 25 | | | | | | | | | | | |
| Aug 2013 | 1 | | | | | | | 4 | 14 | | | | | | 6 | | | | | 1 |
| | 2 | | | | | | | 4 | 14 | | | 2 | | | 2 | | | | | |
| | 3 | | | | | | | 1 | 25 | | | | | | | | | | | |
| | 4 | | | | | | | | 25 | | | | | | | | | | | |
| | 5 | | | | | | | | 25 | | | | | | | | | | | |
| | 6 | | | | | | | | 25 | | | | | | | | | | | |
| | 7 | | | | | | | | 25 | | | | | | | | 1 | | | |
| | 8 | | | | | | | | 25 | | | | | | | | | | | |
| | 9 | | | | | | | | 25 | | | | | | | | | | | |
| | 10 | | | | | | | | 25 | | | | | | | | | | | |
| Sep 2013 | 1 | | | | | | 1 | | | | | | | | 2 | | | 3 | | 1 |
| | 2 | | | | | | | 3 | 14 | | 1 | 1 | | | 5 | | | | | |
| | 3 | | | | | | | 1 | 25 | | | | | | | | | | | |
| | 4 | | | | | | | | 25 | | | | | | | | 1 | | | |
| | 5 | | | | | | | | 25 | | | | | | | | | | | |
| | 6 | | | | | | | | 25 | | | | | | | | | | | |
| | 7 | | | | | | | | 25 | | | | | | | 1 | | | | |
| | 8 | | | | | | | | 25 | | | | | | | | | | | |
| | 9 | | | | | | | | 25 | | | | | 1 | | | | | | |
| | 10 | | | | | | | | 25 | | | | | | | | | | | |
| Oct 2013 | 1 | | | | | | 2 | 3 | | | 1 | | | | | | | 1 | | |
| | 2 | | | | | | | 3 | 8 | | | | | 1 | 2 | | | 4 | | |
| | 3 | | | | | | | 4 | 6 | | | 4 | | | | | 1 | | | |
| | 4 | | | | | 3 | | | 5 | | 1 | 2 | | | | | 2 | | | |
| | 5 | | | | | | | | 25 | | | | | | | | | | | |
| | 6 | | | | | | | | 25 | | | | | | | | | | | |
| | 7 | | | | | | | | 25 | | | | | | | | 1 | | | |
| | 8 | | | | | | | | 25 | | | | | | | | | | 1 | |
| | 9 | | | | | | | | 25 | | | | | | | | | | | |
| | 10 | | | | | | | | 25 | | | | | | | | | | | |

**Table 2. (continued)**

| | Quadrat number | *Galaxaura* sp. | *Halymenia* sp. | *Amphiroa foliacea* | *Gracilaria* sp. | *Hypnea valentiae* | *Dictyota dichotoma* | *Padina australis* | *Sargassum aquifolium* | *Ulva lactuca* | *Anadyomene plicata* | *Chaetomorpha crassa* | *Dictyosphaeria cavernosa* | *Valonia aegagropila* | *Caulerpa sertularioides* f. *longipes* | *Codium geppiorum* | *Halimeda opuntia* | *Chlorodesmis fastigiata* | *Neomeris vanbosseae* | *Acetabularia dentata* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nov 2013 | 1 | | | | | | | 2 | | | | | | | | | | 1 | | |
| | 2 | | | | | | | | | | | 1 | | | | | | | | |
| | 3 | | | | | | | | 1 | | | | | | | | | | | |
| | 4 | | | | | | 1 | | | | | | | | | | | | | |
| | 5 | | | | | | | | | | | | | | | | 1 | | 1 | |
| | 6 | | | | | | | | 1 | | | | | | | | | | | |
| | 7 | | | | | | | | | | | | | | 3 | | | | | |
| | 8 | | | | | | | | 1 | | | | | | | | | | | |
| | 9 | | | | | | | | | | | | | 1 | | | | | | |
| | 10 | | | | | | | | 2 | | | | | | | | | | | |
| Dec 2013 | 1 | | | | | | 1 | | | 3 | | | | | | | | 1 | | 1 |
| | 2 | | | | | | | 4 | | 2 | | 2 | | | | | | 4 | | |
| | 3 | | | | | | | | 3 | | | 4 | | 1 | 3 | | | | | 1 |
| | 4 | | | | | | 1 | | | | | | 2 | | | | 3 | | 2 | |
| | 5 | | | | | | | | 2 | | | | | 1 | 4 | | 1 | | | |
| | 6 | | | | | | | | | | | 2 | | | | | | 3 | | |
| | 7 | | | | | | | | 2 | | | | | | | | 4 | | | 1 |
| | 8 | | | | | | | | | | 5 | 1 | | 1 | | | 4 | | | |
| | 9 | | | | | | | | 3 | | | | 1 | | | | 1 | | | |
| | 10 | | | | | | | | 2 | | 1 | 3 | | | | | | 4 | | |
| Jan 2014 | 1 | | | | | | | 4 | | 20 | | | | 2 | | | | | | |
| | 2 | | | | | | 1 | | | 15 | | 2 | | | | | | | | 1 |
| | 3 | | | | | | | | 1 | 17 | | 3 | | | | | | | 1 | 2 |
| | 4 | | | | | | | | | 4 | | 1 | | | 3 | | | | | |
| | 5 | | 1 | | | | | | | 10 | | | | 1 | 2 | | | | | |
| | 6 | | | | | | | | | 15 | | 1 | | | | | 1 | | | |
| | 7 | | | | | | | | | | | | | | | | | | | |
| | 8 | | | | | | | | | 18 | | | | 1 | | | 1 | | | |
| | 9 | | | | | | 1 | | 2 | 5 | | 1 | | | | | | | | |
| | 10 | | 2 | | | | | | | 8 | | | | 1 | 3 | | | | | |
| Feb 2014 | 1 | | | | | | | 6 | | 25 | | | | | | | | | | |
| | 2 | | | | | | | 7 | 1 | 25 | | 1 | | | | | | | | |
| | 3 | | | | | | | 4 | | 14 | | 1 | | | | 1 | | | | 1 |
| | 4 | | | | | | | 2 | | 18 | | | | | 3 | | | | | |
| | 5 | | | | | | | 1 | | 25 | | | | | | | | | | |
| | 6 | | 2 | | | | | | | 23 | | 2 | | 1 | 4 | | | | 1 | |
| | 7 | | | | | | | 3 | | 10 | | | | 1 | | 1 | | | | 1 |
| | 8 | | | | | | | | | 14 | | 2 | | | | | | | | |
| | 9 | | | | | | | | 1 | 7 | | 5 | | | 3 | | | | | |
| | 10 | | | | | | | 1 | | 18 | | | | 1 | | | | | | |
| Mar 2014 | 1 | | | | | | | 6 | | 25 | | | | | | | | | | 1 |
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| | 3 | | | | | | | | | 25 | | | | | 4 | | | | | 2 |
| | 4 | | | | | | | | 4 | 25 | | 1 | | 2 | | | | | | |
| | 5 | | | | | | | 5 | 4 | 23 | | | | | 3 | | | | | |
| | 6 | | | | | | | | 3 | 25 | | 1 | | | | | | | | 1 |
| | 7 | 3 | | | | | | 1 | 4 | 13 | | | | 1 | 3 | | | | | |
| | 8 | | | | | | | 3 | 5 | 24 | | | | | | | | | | |
| | 9 | | | 1 | | | | | 4 | 18 | | | | | 4 | 3 | | | | |
| | 10 | | | | | | | 3 | 8 | 13 | | | | 2 | | | | | | 1 |
| Apr 2014 | 1 | | | | | | 4 | | | | | | | | | | | 1 | | 3 |
| | 2 | | | | | | | 3 | 14 | | | | | | | | | 5 | | |
| | 3 | | | | | | | 3 | 17 | | | | | | | | | 1 | | |
| | 4 | | | | | | | | 25 | | | | | | | | | | | |
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January to October and mainly occupied by the brown alga *Sargassum aquifolium* from April to October and the green alga *Ulva lactuca* from January to March. Thus CGAs in Taggat Lagoon were growing concomitantly with other leaf-like green algae when the macroscopic brown algae had disappeared.

The C/P and $[I/H]_{RCP}$ indices of the seaweed vegetation in northeastern Taiwan, southern Taiwan and the Pacific coast of Luzon were 1.27/6.28, 2.56/10.6 and 3.10/13.0, respectively, showing that northeastern Taiwan is classified as a subtropical region whereas southern Taiwan and the Pacific coast of Luzon are tropical.

**Laboratory culture experiments**

The relative growth of the culture strain of *V. utricularis* under various environmental conditions is shown in Fig. 4. They exhibited the fastest growth at 25ºC whereas they did not show significant growth at 15ºC and even died under strong illumination (400 $\mu$mol s$^{-1}$ m$^{-2}$). Changes in the nutrients of the medium by addition of PES enrichment did not affect the growth significantly under the culture conditions used in the present study. The cell divisions, the lenticular cell formation, were commonly observed in the well-growing cells (Fig. 3C). The growth was not significantly different between those cells cultured under medium (80 $\mu$mol s$^{-1}$ m$^{-2}$) and strong illumination (Fig. 4) indicating that growth of this alga does not require incident light stronger than medium illumination.

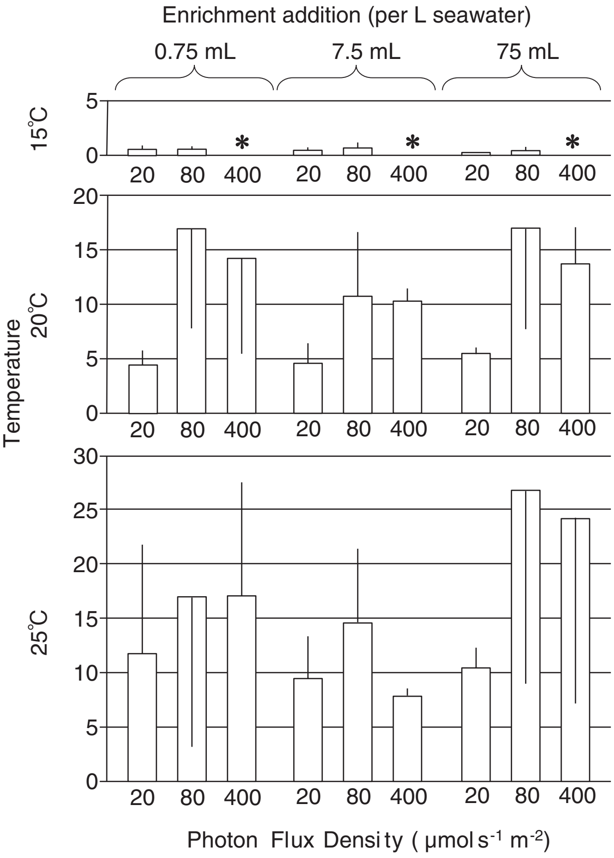


**Fig. 4.** Growth of *Valonia utricularis* strain established from living materials collected in Green Island during laboratory culture experiment under different temperature, light intensity and nutrient concentration. White bars and black lines represent average of percent growth observed after 21-day incubation calculated from triplicate experiments according to the equation in the text and its standard deviation, respectively. Asterisks indicate the culture conditions under which cells did not survive.

**Molecular analyses**

Among DNA sequences of plastid *tuf*A genes obtained from four specimens of Taggat Lagoon, sequences of *Caulerpa sertularioides* f. *longipes* and *C. cupressoides* var. *lycopodium* were consistent with those published of respective species so far. On the other hand, obtained sequences from *C. peltata* and *C. macrophysa* were identical, and even had 99% similarity with published ones that are assigned to *C. chemnitzia* as Lineage 6 in Belton et al (2014).

**Case study in Green Island, Taiwan**

During the field survey in the present study, we found an example of neighboring shores with a different abundance of CGAs on the northwest coast of Green Island (Figs. 5 and 6). Zhongliao Village and Chaikou Village are neighboring villages, only 800 m apart, both of which are facing to the north and surrounded by a 150 m-wide, complicated rocky shore composed of limestone (Fig. 5). Species of CGAs such as *Valonia aegagropila*, *V. utricularis*, *Caulerpa macrophysa*, and *C. peltata* were considerably abundant in the upper subtidal zone of the seashore around Zhongliao Village (Fig. 6A). In contrast, although the vegetation of other seaweed species was also rich, no CGAs were found in the seashore around Chaikou Village during three field surveys conducted in the present study (Fig. 6B).

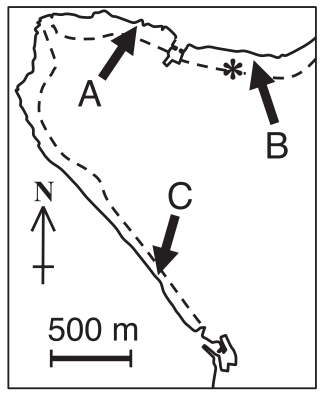


**Fig. 5.** Map of northeastern area of Ludao (Green Island). A. Zhongliao Village, B. Chaikou Village, C. Nanliao Village. An asterisk indicates the location of sewage outlet. Solid lines indicate outline of rocky shore near the low tide line and broken lines indicate boarder between rocky shore and land near the high tide line.

Interviews with leaders of the two villages

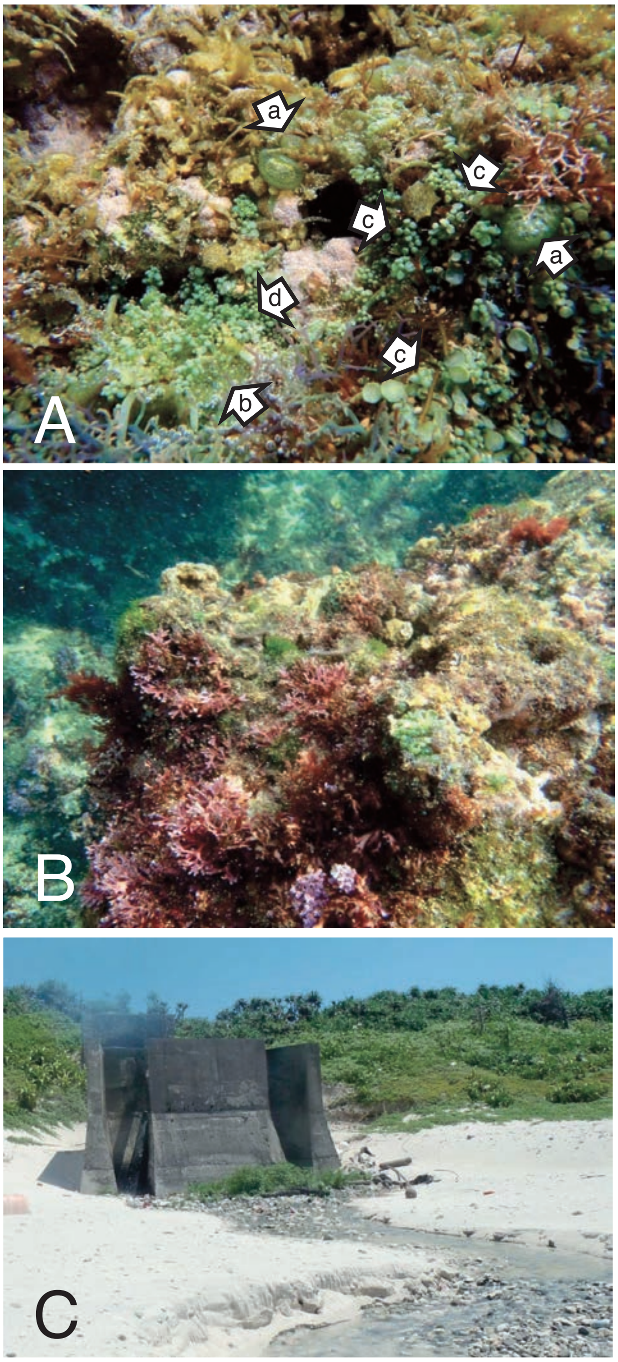


**Fig. 6.** Photographs of the seashore along the northeastern area of Green Island. A and B. Seaweeds growing in the rocky shore in upper subtidal zone. A. Seashore in Zhangliao. Arrows with symbol indicate species of coenocytic green algae (CGAs). a) *Valonia aegagropila*, b) *V. utricularis*, c) *Caulerpa peltata*, and d) *C. macrophysa*. B. Seashore in Chaikou. No CGAs are detectable. C. Outlet of sewage found in the middle of seashore in Chaikou.

were made and they pointed out that there were intermittent discharges of sewage derived from local communities from the drain near the Chaikou into the seawater (Figs. 5 and 6C). Seawater samples from the seashore around two villages and that around Nanliao (Fig. 5), the largest village in Green Island, where CGAs were readily found, were subjected to water analyses for COD, inorganic phosphate and ammonium ions, and the results are shown in Table 3. The concentration of the three substances was considerably higher in the sewage than in the seawater samples. Moreover, although COD and ammonium ion concentration in seawater were similar among three villages, inorganic phosphate was higher in Chaikou than in the other two villages.

**Table 3. Results of water analyses of seawater from seashore and sewage of three villages in Green Island[1)].**

| Locality | COD | Ammonium ion | Inorganic phosphate |
|---|---|---|---|
| Zhongliao | 2.0 | N.D.[2)] | 0.08 |
| Chaikou (seashore) | 2.2 | N.D.[2)] | 0.17 |
| Chaikou (sewage) | 24.0 | 3.96 | 23.8 |
| Nanliao | 2.2 | N.D.[2)] | 0.12 |

[1)] Results were obtained from water samples (250 mL each) collected from each locality on 9, June 2013.
[2)] less than the lower limit of measurement (0.01 mg $mL^{-1}$).

## Discussion

The field surveys along the five study areas carried out in the present study showed that CGAs belonging to the genera *Valonia* and *Caulerpa* were readily detectable in the upper subtidal zone in many types of seashore where the seaweed vegetation is rich. The results of the fixed-point observations in the Taggat Lagoon in the present study are consistent with the above observations. Interviews with local people revealed that CGA species were recognized by the people because of their conspicuous morphology and this was also supported by the fact that the CGAs had specific names in the native languages in the region; *Valonia* and *Caulerpa* are called “ Bola-bola” and “Lukay-lukay “ in the Philippines (Tagalog), and “法囊藻 (Fanangzao)” and “蕨藻 (Juezao)” in Taiwan (Taiwanese), respectively.

There were also species commonly found in most study areas, such as *Valonia* spp., *Caulerpa peltata* and *C. macrophysa*. The two species of *Caulerpa*, *C. peltata* and *C. macrophysa,* were often growing near to each other as in Fig. 6A in most of study areas in the present study. An early laboratory culture experiment revealed the plasticity of branches of *C. peltata* according to culture conditions such as temperature and light cycle (Ohba and Enomoto 1987, Ohba *et al.* 1992). The fact that *tuf*A sequences of *C. peltata* and *C. macrophysa* from Taggat Lagoon are identical in the present study indicated that the phenotypic differences of the two species were indistinguishable using the marker for species identification. *Caulerpa* species with variable shapes of ramuli including *C. peltata* and *C. macrophysa*, the so-called *C. racemosa-peltata* complex, can be divided into 11 species in the Indo-Pacific Ocean (Belton *et al.* 2014), which also implies the possibility that several different species can be found among *C. peltata* and *C. macrophysa* observed in the present study.

It has been shown that the effect of environmental conditions on the growth and reproduction of *Valonia* cells can be evaluated conveniently using the method of the laboratory culture experiments adopted in the present study. The result obtained from the experiments that the growth was not promoted by light stronger than medium light (80 µmol $s^{-1}$ $m^{-2}$; approximately similar to the cloudy weather in brightness) might be due to the fact that the photosynthesis saturated at this illumination and is possibly related to the habitats of the species limited to the subtidal zone.

The above results all confirm the potential merits of CGAs as indicators of the coastal environment in the Kuroshio Current region because of the following factors: 1) the existence of common species throughout the region; 2) their distribution in shallow, intertidal to upper subtidal zone accessible by ordinary people; 3) the availability of culture strains for laboratory experiments on growth and reproduction. Moreover, instances showing the significant relationships between CGA vegetation and conservation of the coastal environment were found in Taiwan and the Philippines. In the case study on Green Island conducted in the present study, we found two neighboring villages with distinct CGA vegetation. Seaweed vegetation would possibly be affected by a number of physical and chemical factors in its environment including salinity (e.g. Karsten 2007). However, the environmental conditions in nature seem to be similar in the two villages on Green Island because there are no considerable differences in the geographical features and fresh water supplies between them. Therefore, the CGA vegetation in Green Island could possibly be a typical example artificially affected by the water quality and further analyses to explore the substances inhibiting the algal growth and reproduction by laboratory culture experiments will be necessary. In addition, the authors have reported their findings regarding the effects on water quality to the village officials. The village officials paid much attention to these results because due to the rapid increase in the numbers of visitors (350,000 - 400,000 persons per year) to this area for recreational purposes, there is the growing concern that water contamination within the island will influence the ecosystem surrounding the entire area. In order to cope with this situation, the village agency has taken several actions, and plans for installing sewage purification systems are being gradually carried out.

In Lagonoy Gulf, due to the recent increase in the population, six marine protected areas (MPAs) have been established to date and many local people are involved in the activities for conservation of the coastal environment. The term MPA indicates a sanctuary where fishing activities are strictly prohibited according to specific ordinances, and the surrounding sea area is managed by a vigilant group often consisting of local residents. In Bicol region, MPAs are being eagerly established, and some of them have been in existence for nearly 20 years. Mendoza and Soliman (2013) recently published a survey of the seaweed growing in the 12 different localities in the gulf indicating that all the localities generally showed similar C/P and $[I/H]_{RCP}$ indices but CGA vegetation was considerably different among the localities and CGAs are remarkably abundant in Catanduanes Island where the pressure on the environment due to an increasing population has been significantly weaker than in other localities. For further investigations on the usefulness of CGAs as an environmental indicator in the Kuroshio Current region, it would be important to find more examples where the abundance of CGA vegetation is related to the coastal environment, including social conditions in the local community.

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