

# Pigment Composition in Different-colored Scleractinian Corals before and during the Bleaching Process

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Lee-Shing Fang, Chih-Wei Liao and Ming-Chin Liu (1995) Pigment composition in different-colored scleractinian corals before and during the bleaching process. *Zoological Studies* **34**(1): 10-17. Scleractinian corals have many different color types in water. All the colors become bleached when the coral is under stress. The pigment changes behind these phenomena were investigated in the study. Analysis of pigment composition in four color types of seven stony coral species showed chl. a, chl. c<sub>2</sub>, peridinin, diadinoxanthin and dinoxanthin comprised more than 95% of the total amount of pigments. There was little compositional difference between these pigments in corals of different color. During bleaching caused by salinity change, both the number of zooxanthella in each polyp and the amount of total pigment in each zooxanthellae decreased with time. The rate of decline of porphyrins coincided with the level of stress that was created by lowering the salinity of the incubation sea water. The rate of decline of carotenoids was less sensitive. This suggests that the rate of change of porphyrins could reflect the bleaching status of stony coral.

Key words: Scleractinian coral, Pigments, Bleaching, Salinity.

Corals are very colorful organism. Many colors can be seen on coral in situ including brown, green, yellow, red, orange, blue, purple, and black. The coloration of Gorgonacea, Coenothecalia, and Stolonifera is particularly rich and it has been suggested that the coloration results from carotenoid or chromoproteins in their skeletons (Goodwin 1968, Kennedy 1979). However, the skeletons of most corals are white and their colors are due to the presence of photopigments of zooxanthellae in the polyps (Jaap 1979, Sumich 1980). Zooxanthellae in scleractinian corals were generally regarded as the species Symbiodinium microadriaticum (Freudenthal) (Taylor 1969, Falkowski and Dubinsky 1981, Blank and Trench 1985), although, there was evidence of different physiological, biochemical, and genetic variations among varieties of this symbiotic alga (Chang and Trench 1982, Chang et al. 1983, Blank and Trench 1985). Could the photopigments in this single species of zooxanthellae have such drastic variations which would account for the numerous color appearances of

scleractinian coral? If so, what is the pigment corresponding to each particular coloration? Or is it just the variation of the ratio between different pigments in the zooxanthellae that result in color changes? Furthermore, since bleaching of coral has become more common recently due to various environmental impacts. Accordingly, measuring the amount of photosynthetic pigment per unit of zooxanthellae in bleaching corals could offer new insights into these events in nature (Hoegh-Guldberg and Smith 1989). It is thus of interest to investigating the process of pigment change during bleaching. In this study, pigment composition in zooxanthellae of stony corals with different colorations was analyzed first. The relative amounts of each pigment were determined in order to look for the possible mechanism of their color differences. Bleaching experiments were performed and the daily pigment changes in the samples were monitored with respect to the variation in the number of zooxanthellae and the amount of photopigment in each unit of zooxanthellae. The findings of this

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study could provide better understanding of coral coloration as well as the mechanism of color change under environmental stress.

#### MATERIALS AND METHODS

#### Sample preparation

Samples of Acropora selago, A. nasuta, A. secale, Montipora undata, M. foliosa, M. aequituberculata, Symphyllia recta, colored brown, green, red, or orange, were collected from the Hong-chai area ( $21^{\circ}56'$  N,  $120^{\circ}44'$  E) at the southern tip of Taiwan. They were taken from water at the depth of 10 to 15 meter, stored at 0°C in an ice box and shipped back to the laboratory for pigment analysis as quickly as was possible. Those samples which could not be processed immediately were sealed in plastic bags filled with nitrogen gas and stored at  $-20^{\circ}$ C until analysis.

#### **Pigment extraction**

Coral polyps were washed off by dental care water pike (Johannes and Wiebe 1970). The wash water was collected and centrifuged at 4°C, 3×10<sup>4</sup> g for 15 min after which the supernatant was discarded. Cold acetone (90%) was added to the tube with a few drops of MgCO3. The tube was ultrasonicated (Heat-systems-ultrasonics W375, 50% duty cycle) for 30 min, then shaken vigorously to extract pigment. The procedure was repeated several times until the residue became colorless. An equal volume of diethyl ether was added to the acetone solution, shaken well, then washed with 15 volumes of 10% NaCl solution. The diethyl ether layer was collected by use of a separatory funnel and blown to dryness with nitrogen gas. The pigment residue was now ready for chromatographic analysis.

#### Pigment separation, identification, and analysis

The pigment residue was re-dissolved in a few drops of ether and applied to a thin layer chromatography (TLC) plate (Merck Kiesel gel COF 254), developed with a solution of petroleum ether:ethyl acetate:diethyl amine (60:30:10, v/v), vacuum-dried in darkness after running, and then redeveloped with 80% n-propyl alcohol for a second running in the same direction. The R<sub>f</sub> of each developed pigment was recorded, then the pigment was eluted with 90% acetone (chlorophyll a), methanol (chlorophyll  $c_2$  and peridinin) or alcohol (diadinoxanthin and dinoxanthin). The absorption spectrum of each pigment was taken from 400 to 700 nm to identify other characteristics of the pigment in addition to the R<sub>f</sub> identification (Parsons and Strickland 1963). After identification, the concentration of each pigment was calculated by its absorption at 440 nm using Bear's law as described in Mantoura and Llewellyn (1983).

## High performance liquid chromatography (HPLC)

Twenty  $\mu$ I of pigment sample in acetone was injected into a dual pump HPLC system. Separation was performed on a steel column (250 mm × 4 mm) packed with C-18 reverse phase matrix using methanol-water as the mobile phase. The running program was set at 0-50 min with a gradient change from 80% to 100% methanol at a flow rate of 1 ml/min. The eluate was monitored and detected at a wavelength of 440 nm. Identification and concentration calculation of each pigment were achieved by comparison with standards obtained from TLC analysis as specified above.

#### Pigment change during bleaching

Freshly collected A. secale colonies were kept in two  $30 \times 35 \times 60$  cm<sup>3</sup> aguariums with constant aeration and circulating filtration at room temperature. Tanks were placed by the window to expose them to natural daylight cycles. The salinities of the sea water in the two tanks were 30‰ and 25% respectively. Small coral samples from the colony were collected daily. Polyp numbers washed off from these samples were first counted, then zooxanthellae were released from the polyps by osmo-shock followed by vigorous shaking. The number of zooxanthellae was estimated using a red blood cell counting vessel. Pigments were then extracted from these zooxanthellae for HPLC analysis. The experiment continued until the time when the coral colony became white to the naked eve.

#### RESULTS

#### Coral species and their coloration

The in situ observation of initial coloration for different coral species are presented in Table 1. Different species had similar or variable coloration. 
 Table 1. The color of different coral observed in situ

Coral species		*		
	BROWN	GREEN	RED	ORANGE
Montipora foliosa	+	+		+
M. incognita	+	+		+
M. undata	+	+		+
Symphyllia agaricia	+		+	*
S. nobilis	+		+	
Acropora nasuta	+	+		
A. delicatula	+	+		

The same species also showed coloration variations in similar habitats. No obvious relationship between species and initial the coloration it presented was found.

#### Pigment isolation and identification

Pigments, which were isolated by TLC and further characterized by absorption spectrum, were determined to be chlorophyll a, chlorophyll c<sub>2</sub>, peridinin,  $\beta$ -carotene, diadinoxanthin mixed with dinoxanthin and an unknown pigment of light pink color. The HPLC elution pattern of these pigments showed four clear peaks (Fig. 1) which were chlorophyll c<sub>2</sub>, peridinin, diadinoxanthin plus dinoxanthin (diadino. + dino.) and chlorophyll a.



Fig. 1. The elution pattern of photopigments extracted from stony coral with HPLC at the detection wave length of 440 nm.

These five species of chromogen accounted for more than 95% of the total amount of pigments extracted from the samples. Therefore, they were chosen as the parameters for further experimental investigation.

#### Pigment composition of stony coral with different color

Figure 2 shows that there was little difference in the composition of photopigments between the variable colored coral species. Chi-square test for independence of pigment ratios between color samples further demonstrated that there was no significant difference between them (Table 2). It is quite clear that there was no higher content of chl. a or chl.  $c_2$  in green colored coral than in other colored coral, nor was there a higher content of peridinin in orange colored coral, as one would expect.

#### Pigment changes during bleaching

Both the number of zooxanthellae in each polyp and the total amount of photopigments per polyp decreased during the bleaching period (Fig. 3). The rate of decrease was faster in 25‰ salinity. Total pigment change per unit of zooxanthellae was much more drastic (Fig. 4). In the 25‰ salinity these values diminished rapidly, but in the 30‰ salinity they declined gradually.



Fig. 2. Pigment composition in different coral species with different coloration.

Specise		A. selago	A. nasuta		M. undata		M. foliosa		sa	M. aequituberculata		S. recta	
col	color	G	В	G	В	0	В	G	0	В	G	В	R
A. selago E	В	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	G		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
A. nasuta	В			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G	G				n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
M. undata	В					n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	0						n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
M. foliosa	В							n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
G	G								n.s.	n.s.	n.s.	n.s.	n.s.
	0									n.s.	n.s.	n.s.	n.s.
<i>M. aequituberculata</i> B G	В										n.s.	n.s.	n.s.
	G											n.s.	n.s.
S. recta	В												n.s.

Table 2. Chi-square test of independence for pigment ratio between color samples of different corals

A.: Acropora; M.: Montipora; S.: Symphilia; B = brown; G = green; O = orange; R = red; \*: significant; n.s.: not significant.



**Fig. 3.** The change of the number of zooxanthellae and the total amount of pigment in each polyp of *Acropora secale* under different environmental stress. — Zooxs./polyp in 25‰ salinity, — Zooxs./polyp in 30‰ salinity, --o--- Pigment/ polyp in 25‰ salinity, --o--- Pigment/polyp in 30‰ salinity.

Detailed analyses of the changes of the five major pigments in these zooxanthellae are shown in Figs. 5A and B. Besides diadino. + dino., the other three pigments exhibited a significant linear decreasing relationship with time in 25‰ salinity. However, concentrations of pigments in zooxanthellae were statistically constant in the 30‰ experimental set except those of chl.  $c_2$ . It was noticed that porphyrins (chl. a and  $c_2$ ) and carotenoids (peridinin, diadino. + dino.) had different



Fig. 4. The change of total pigment per zooxanthellae of *Acropora secale* with time in salinities of 25‰ and 30‰. \* : significant; n.s.: not significant.

response during the bleaching experiments. The porphyrins had decayed to nearly zero by the sixth day but the carotenoids had not (Fig. 5A). Linear regression of chl.  $c_2$  concentrations showed significant decrease in both 25% and 30% experimental sets while the content of diadino. + dino. remained stable (Figs. 5A, B).

Microscopic examination of zooxanthellae showed that diameter of the algal cells were 10

to 12 mm on the first day. After three to four days, some of the diameters were as small as 3 to 5 mm and a few cells were already colorless in the 25‰ salinity set.

#### DISCUSSION

#### Coloration of scleractinian coral

It is generally recognized that the polyps of scleractinian coral are colorless or semi-transparent. The rich coloration they exhibit underwater mainly comes from symbiotic zooxanthellae (Ciereszdo and Karus 1973, Jaaps 1979, Sumich 1980, Chalker and Dunlap 1981). On the other hand, color types in scleractinian coral have been used as markers of genetically different clones within a species (Chornesky 1991) or as behavioral types within a population (Rinhevich and Loya 1983, Hidaka and Yamazato 1984, Hidaka 1985). As many as thirteen distinct color types were recognized in the population of Agaricia tenuifolia at Carrie Bow Cay, Belize (Rutzler and Macintyre 1982). If this much variation come from the single species Symbiodinium microadriaticum which is generally regarded as the major symbiotic algal in stony corals (Taylor 1969, Falkowski and Dubinsky 1981), it would be interesting to know the nature of the variation of the pigments behind these colorful morphs. Unexpectedly, data from this experiment show that there is very little difference in the basic

composition of the chromogens in corals of various colors (Figs. 2, 3). In other words, different colors presented by these stony corals do not originate from the composition of pigments, but from some other reasons.

Photopigments in algae associate with protein to become photosynthetically functional. For example, peridinin-chlorophyll a-proteins (PCPs) are major light-harvesting molecules in marine dinoflagellates (Prizéllin and Haxo 1976, Prizéllin and Sweeney 1978). However, the observed color of a bound protein pigment could vary if the binding status of the chromogen and the protein changed. Carotenoid-protein complex changing from green to red in shrimp shells after protein denaturing is a well known comparable phenomenon. Another example is the variation of the light absorption spectrum of biliverdin (a chromogen of heme metabolism) in the microenvironment of its protein moiety in fish blood which reveals colors from green, blue to purple (Low and Bada 1974, Fang et al. 1986). Moreover, pigments of anthocyanidins complexing with different metals, or forming intermolecular hydrophobic associations, result in colorful change during various flowering stages (Kondo et al. 1992). Such factors should be further investigated since photopigments analyzed in this study were obtained by a procedure which freed them from most other ligends.

In addition to the chemical aspects, there are also biological factors to consider. Zooxanthellae from coral were found to have different isoelectric



Fig. 5. The change of various photopigments in zooxanthellae of *Acropora secale* with time in different salinities. A: 25‰ salinity; B: 30‰ salinity; \*: significant; n.s.: not significant.

forms of PCPs (Chang and Trench 1982), and even different chromosome numbers (Blank and Trench 1985). Three species of zooxanthellae were suggested by Trench and Blank (1987) which could have different colors. Nevertheless, even if different species of zooxanthellae do account for the color variation of corals investigated in this study, their photopigment composition are very similar.

#### The change of photopigments during the bleaching

Despite the fact that bleaching is a common phenomenon of coral under stress, there are few objective parameters to quantify the degree of bleaching, other than surveying of the bleached area on corals. Fang et al. (1987 1991) used ATP concentration to estimate the degree of stress on living coral which, in a sense, is a measurable biochemical parameter. However, coral can be stressed without exhibiting bleaching (Fang et al. 1991). Therefore, the ATP method can not satisfactorily quantify the degree of coral bleaching. On the other hand, examination of Figs. 5A and B in this study reveals that porphyrin concentration is a potential criterion by which to scale the degree of bleaching. It is more useful for this purpose than carotenoids because porphyrin concentration, especially that of chl. c2, decreased significantly according to the level of stress encountered by the coral. On the other hand, when coral was transferred to deep water where light was low, the concentrations of chl. a and, in particular, chl. c2 per zooxanthellae cell, were significantly enhanced (Kaiser et al. 1993).

Early observations on salinity tolerance of corals found arrange of 27% to 40% in the field, but there was variation among species. Acropora was thought to be sensitive to salinity changes, but Porites can survive concentrations up to 48%0 (Kinsman 1964). In the bleaching of A. secale at low salinity (25%), there was not only a decrease of zooxanthellae per polyp (Fig. 3), but also a decrease in amount of photopigment per zooxanthella (Fig. 4). However, sudden exposure to reduced salinity (30%) did not affect Stylophora pistillata or Seriatopora hystrix (Hoegh-Guldberg 1989). On the other hand, exposure to sea water temperatures greater than 30°C reduced the density of zooxanthellae in these corals but not the concentration of photopigment per zooxanthella (Hoegh-Guldberg 1989).

Carotenoids are photopigments that have two

major functions. First, they are supplemental photosynthetic pigments that absorb light energy in the blue region (Lehninger 1982, Gerberding et al. 1991). Second, they are photoprotective pigments that prevent light damage to chloroplasts (Koyama 1991, Young 1991). Especially in dinoflagellates, diadinoxanthin, and dinoxanthin are major pigments for photo-protection (Demers et al. 1991). In this experiment, the decay rate under salinity stress of porphyrins was faster than that of carotenoids under the experimental conditions. The maintaining of carotenoids could be a compensation by the zooxanthellae for the loss of chl. a and c<sub>2</sub> during bleaching in order to sustain photosynthetic ability. Peridinin, diadinoxanthin, and dinoxanthin, studied in this investigation, can be replenished either by being newly synthesized or converted from other accessory pigments through the xanthophyll cycle, as occurs in other dinoflagellates (Demers et al. 1991).

The colorful appearance of scleractinian coral, and the bleaching process when it is under stress, are interesting ecological phenomena. Study of their physiological-biochemical changes not only leads us to an in-depth understanding of these natural phenomena, but also it provides us information, such as using porphyrins to quantify the status of bleaching, that may eventually become useful in the conservation of coral resources.

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### 不同顏色之造礁珊瑚其色素組成及白化過程中之色素變化

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在水中,石珊瑚呈現出各種不同的色彩,但不同的色彩在珊瑚受到外界壓力時都會白化,本研究乃分析白 化過程時珊瑚中各種色素組成的變化。分析四種色彩,七種不同珊瑚的色素組成,結果發現葉綠素a,葉綠素 c<sub>2</sub>,peridinin,diadinoxanthin及dinoxanthin佔95%以上之總色素含量,而具有不同色彩的珊瑚之間,這些 色素組成差別並不大。在白化過程中,單位水螅體之共生藻數目及總色素量都隨時間增長而下降。其中porphyrin類色素之下降速率與外界壓力大小變化一致。類胡蘿蔔素類之含量在中度外界壓力下並不會減少,但在 較大外界壓力下則緩慢下降。由以上結果可知,porphyrin之變化較可反應出石珊瑚白化的程度。

關鍵詞:造礁珊瑚,色素,白化,鹽度。

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