J Appl Biol Chem (2013) 56(1), 43–48
http://dx.doi.org/10.3839/jabc.2013.008

Online ISSN 2234-7941
Print ISSN 1976-0442

Original Article: Bioactive Materials

Analysis of Carotenoids in 25 Indigenous Korean Coral Extracts

Sang Min Kim · Suk-Woo Kang · Eun A Lee · Eun-Kyoung Seo · Jun-Im Song · Cheol-Ho Pan*

Received: 31 July 2012 / Accepted: 7 December 2012 / Published Online: 31 March 2013

Abstract In this study, methanol extracts from 25 indigenous Korean corals were prepared and their carotenoid constituents were analyzed by high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS). Among them, extracts from nine species showed detectable peaks in the HPLC chromatogram at 450 nm and the ultraviolet/visible spectra exhibiting carotenoid-specific characteristics were chosen. The mass data of carotenoid peaks revealed that only peridinin could be identified based on literature comparison and suggested the potential presence of novel carotenoid structures. This is the first reported investigation of indigenous Korean coral carotenoids and further work is needed to explore the carotenoids and their potential roles in the ecosystem of indigenous Korean corals.

Keywords carotenoid · coral · high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry · peridinin · ultraviolet/visible spectrum

S. M. Kim · S.-W. Kang · C.-H. Pan
Functional Food Center, Korea Institute of Science and Technology, Gangneung 210-340, Republic of Korea

E. A Lee
Institute of Eco-Science, Ewha Womans University, Seoul 120-750, Republic of Korea

E.-K. Seo
College of Pharmacy, Ewha Womans University 120-750, Republic of Korea

J.-I. Song
Department of Life Sciences, Ewha Womans University 120-750, Republic of Korea

*Corresponding author (C.-H. Pan: cheolpan@gmail.com)

Introduction

Corals are marine animals in the class Anthozoa; they typically live in compact colonies of many identical individuals and secrete calcium carbonate to form a hard skeleton, referred to as a coral reef (Osinga et al., 2011). Coral reefs are among the largest and most diverse ecological communities on the planet and have high economic value as a source of food and natural products (Osinga et al., 2011). Most corals obtain the majority of their energy and nutrients from photosynthetic unicellular algae, called zooxanthellae, of the genus *Symbiodinium*, that live within the coral's tissue (Apprill et al., 2007). The algae benefit from a safe place to live and consume the polyp's carbon dioxide and nitrogenous waste to survive. Recently, coral pigments have been the subject of interest in relation to their significance in coral bleaching, a phenomenon defined as the loss of color of corals (Venn et al., 2006). The coral symbiont pigments, including chlorophyll *a*, peridinin, chlorophyll c_2, diadinoxanthin, diatoxanthin, and β-carotene, are known to play a role not only as light harvesting components for photosynthesis but also in protecting components from high irradiance (Hochberg et al., 2006). Apprill et al. (2007) reported that visibly healthy corals exhibit variable pigment concentrations and symbiont phenotypes. Coral bleaching occurs as a result of the elimination of symbiotic algal cells or the degradation of algal pigments. Bleaching is triggered by a range of environmental stressors, including temperature extremes and high irradiance, and causes significant impact on the marine ecosystem. There have been numerous studies about environmental effects on symbiont pigment profiles in corals (Mydlarz et al., 2010). In Korea, several coral species have been reported in faunal studies from the sea area off the eastern and southern coasts of Korea (Song and Lee, 1998) and, in particular, the sea area off Jeju Island, Korea, shows the highest variety of coral species (Kang et al., 2005). However, taxonomical studies have been the major focus of previous research and few studies have been reported on the chemical constituents of Korean indigenous corals (Seo et al., 1996; Bae et al., 2000).

The major role of carotenoids in corals is the protection of the photosynthetic apparatus of the zooxanthellae from irreversible light-induced photoinhibition that may lead to the loss or expulsion of the algal symbiont from corals (Venn et al., 2006). Thus, understanding of carotenoid pigment profiles in coral species are highly related with the symbiont algal species and the growth of corals. However, those studies have not yet been carried out on Korean indigenous corals. The purpose of this investigation was to screen major carotenoids from Korean indigenous corals and obtain schematic understanding of carotenoid profiles. For that, 25 corals were collected from the sea off the coast of Jeju island and their methanolic extracts were prepared. In addition, carotenoids pigment profiles were explored by high-performance liquid chromatography/photodiode array detector/atmospheric pressure chemical ionization-mass spectrometry (HPLC-PAD-APCI-MS) and compared with literature.

Materials and Methods

Collection of samples. Twenty-five coral species were collected from Munseom, Beomseom, and Supseom, Jeju Island, and from Geokumdo between November 2008 and July 2010 by SCUBA diving, and were identified by Prof. Jun-Im Song (Korea Coral Resources Bank; Table 1). Among them, 24 species belonged in a class Anthozoa and a phylum Cnidaria, and one species, *Herdmania momus*, was from subphylum Urochordata of the phylum Chordata. Voucher specimens with barcode numbers have been deposited at the Korea Coral Resources Bank, Ewha Womans University.

Extraction. Each marine organism collected was quickly washed with cold water to remove salt contained in its body. After washing the sample, its whole body was measured (wet weight) and ground. The ground sample was suspended in methanol (MeOH) (three times the volume of the ground sample), sonicated for 2 h at room temperature, and then extracted for 20 h at room temperature, finishing with the third sonication treatment for 1 h. The extracted sample was filtered and evaporated *in vacuo*, to afford a methanol-soluble extract. Each extract was further freeze-dried and weighed (to 10 mg) in an amber wide crimp top vial with a barcode number to produce the standard marine organism extract for aliquotting (Table 1).

Carotenoid analysis. HPLC-APCI-MS analysis was performed with the extract solutions at 10 mg/mL in ethanol using a Varian

Table 1 Indigenous Korean coral extracts

Species name	Barcode No.[1)]	Family name	Wet weight (g)[2)]	Extract weight (g)[3)]	Extraction efficiency (%)[4)]
Scleronephthya gracillimum	EWZS1909	Nephtheidae	282	5.28	1.87
Umbellulifera spiculosa	EWZS2881		1200	31.37	2.61
Dendronephthya gigantea	EWZS1911		713	12.32	1.73
Dendronephthya suensoni	EWZS2337		1000	25.09	2.51
Dendronephthya mollis	KCRB87		780	12.96	1.66
Dendronephthya putteri	EWZS2331		613	9.65	1.57
Dendronephthya spinulosa	KCRB86		1150	21.05	1.83
Dendronephthya castanea	EWZS2313		1500	26.26	1.75
Acalycigorgia grandiflora	KCRB88	Acanthogorgiidae	640	14.22	2.22
Euplexaura crassa	KCRB89		700	10.77	1.54
Bebryce thomsoni	KCRB94		237	4.39	1.85
Calicogorgia granulosa	EWZS2343		1000	20.62	2.06
Astrogorgia sp.	EWZS3003	Plexauridae	1500	27.77	1.85
Villogorgia antillarum	KCRB93		137	1.85	1.35
Plexauroides praelonga	EWZS2325		1000	8.94	0.89
Anthoplexaura dimorpha	EWZS3002		2300	34.95	1.52
Entacmaea quadricolor	EWZS3006	Actiniidae	600	27.52	4.59
Nemanthus nitidus	EWZS3009	Nemanthidae	2200	19.80	0.90
Montipora trabeculata	EWZS3005	Acroporidae	900	10.53	1.17
Psammocora profundacella	EWZS3007	Thamnasteriidae	2100	20.09	0.96
Alveopora japonica	EWZS3008	Poritidae	2700	31.10	1.15
Myriopathes japonica	EWZS3004	Myriopathidae	900	24.27	2.70
Myriopathes ulex	KCRB95		337	9.17	2.72
Palythoa sp.	KCRB91	Sphenopidae	510	15.50	3.04
Herdmania momus	KCRB90	Pleurogona	1200	31.09	2.59

[1)]Barcode number refers to the sample number of voucher specimens deposited at the Korea Coral Resources Bank, Ewha Womans University.
[2)]Wet weight of the harvested coral sample.
[3)]Extract weight after extraction of whole harvested coral sample with methanol and freeze-drying.
[4)](Weight of extract/Wet weight of sample)×100

HPLC-hyphenated MS system (USA) as described in our previous work with slight modifications (Kim et al., 2012). Sample (20 μL) was injected for each experiment and the separation was carried out with an YMC carotenoid column (250 mm×4.6 mm i.d. with a 3 μm particle size; Waters, USA). The mobile phase consisted of methanol/*tert*-butyl-methyl ether (10:90, v/v, Solvent A) and methanol/water (95:5, v/v, Solvent B) with a flow rate of 1 mL/min at 40°C. In the gradient condition, Solvent A was increased to 50% over 20 min after 0% solvent A for an initial 10 min and then increased to 90% over 10 min and kept for 10 min. The chromatograms were assessed at 450 nm for carotenoids. Mass spectral data were acquired from *m/z* 200–1000 with APCI ion source (positive mode) under the following conditions: 12 psi of drying gas at 150°C, 55 psi of nebulizing gas (N_2), 18 psi of vaporizer gas pressure, a corona current of 5 μA, and a housing temperature of 50°C. The analytical data were processed by using Varian MS Workstation software (ver. 6.9; USA). The putative carotenoid peaks showing λ_{max} peaks around 450 nm were selected in the HPLC chromatogram at 445 nm and their UV/visible spectra were acquired at the range of 250–700 nm by photodiode array detector (PDA). MS spectra of the putative carotenoid peaks were obtained and compared with literature data.

Results and Discussion

HPLC/PAD/APCI-MS analysis was applied to obtain carotenoid pigment profiles from coral extracts and identify major carotenoids since this analytical method has been found to be one of the most powerful techniques for the rapid identification of natural products, especially carotenoid compounds without purification step (Maoka et al., 2002). Even though it is difficult to identify the carotenoid only with APCI-MS spectral data, HPLC/ultraviolet (UV)-visible absorption spectrometry by PAD can give the chromophore information of the separated carotenoids which can not be provided from APCI-MS. In the result, the Korean indigenous coral species showed 0.89–4.59% extraction efficiency of wet sample with the methanol as solvent. Among 25 coral extracts, nine extracts, from *Scleronephthya gracillimun*, *Calicogorgia granulosa*, *Astrogorgia sp.*, *Anthoplexaura dimorpha*, *Entacmaea quadricolor*, *Montipora trabeculata*, *Psammocora profundacella*, *Alveopora japonica*, and *Palythoa sp.*, showed distinguishable peaks in the HPLC chromatograms at 450 nm (Fig. 1). There was no detectable peak in the chromatograms from the other coral extracts, indicating that carotenoid levels were very low or zero in those coral extracts. However, we could not reach a conclusion as to whether these species possessed symbiont algae with no carotenoids or did not possess symbiont algae at all. Among 25 coral species in this study, *S. gracillimun*, *Dendronephthya gigantea*, *D. putteri*, and *D. suensoni* are known to be dominant species in the seas off Jeju Island and an artificial propagation study has been attempted with them to preserve coral species (Kim et al., 2004; Choi and Song, 2007). Among these four species, carotenoid peaks were detected only in *S. gracillimun*. However, no investigation about the pigments or symbiont algae has been reported with these Korean indigenous coral species to date.

The potential carotenoids were marked with numbers in each

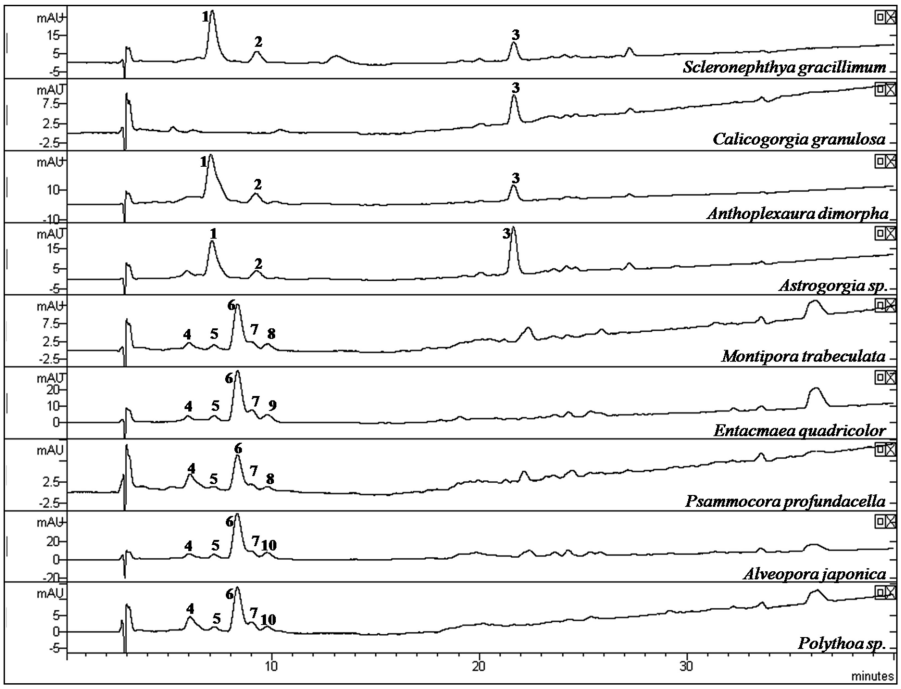

Fig. 1 HPLC chromatograms of carotenoid-containing coral extracts. Among 25 Korean indigenous coral species, nine species showed significant peaks at 450 nm. The presumed carotenoid peaks were determined by UV/visible spectrum at 210–750 nm and numbered.

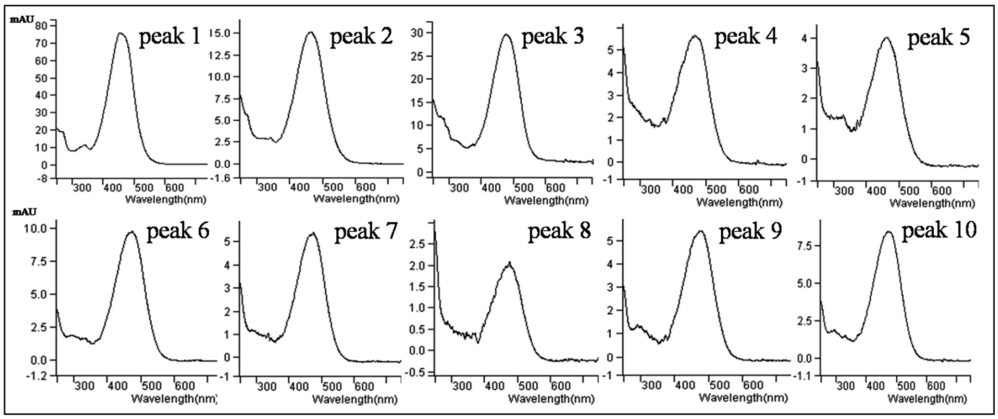

Fig. 2 UV/visible spectra of the presumed carotenoids in indigenous Korean coral extracts. The UV/visible spectra were obtained at 210–700 nm by photodiode array detector from the HPLC. The peak numbers indicate the presumed carotenoids in the HPLC chromatograms in Fig. 1.

Table 2 Mass data of carotenoids from indigenous Korean coral extracts

Peak number[1)]	1	2	3	4	5	6	7	8	9	10
Mass fragment[2)]	503.5	455.6	597.6	319.4	345.5	553.6	284.4	341.5	443.4	611.7
T. I.[3)]	n.i.[4)]	n.i.	n.i.	n.i.	n.i.	peridinin	n.i.	n.i.	n.i.	n.i.

[1)]Presumed carotenoid peaks in the HPLC chromatograms in Fig. 1.
[2)]Mass data indicate the fragment ion of each carotenoid with 100% abundance from LC/APCI-MS.
[3)]T. I.: tentative identification.
[4)]n.i.: not identified.

chromatogram in Fig. 1 (peaks **1**–**10**). The first clue for the identification of carotenoids was their UV/visible absorption spectra at 210–750 nm. Most carotenoids demonstrate distinguishable UV/visible spectra, with the wavelength of maximum absorption (λ_{max}) around 450 nm (Rodriguez-Amaya and Kimura, 2004). Some common food carotenoids can be identified only by characteristics of UV/visible absorption data, including λ_{max}, and the ratio of absorption peak height (Rodriguez-Amaya and Kimura, 2004). The UV/visible absorption spectra at 210–750 nm of the 10 potential carotenoid peaks in Fig. 1 are shown in Fig. 2. The spectra showed in close agreement with the characteristics of carotenoid-specific spectra. However, there was no spectrum corresponding to common food carotenoids, such as β-carotene, lutein, zeaxanthin, or β-cryptoxanthin, which show two or three λ_{max} peaks around 450 nm (Rodriguez-Amaya and Kimura, 2004). Thus, the carotenoids in Korean indigenous coral extracts did not apparently belong to carotenoids common in food.

To identify the carotenoids, MS data of the carotenoid peaks in the HPLC chromatograms were acquired from *m/z* 200–1000 with a positive APCI ion source. The mass fragments of ions with 100% abundance are illustrated in Table 2. Identifications were made primarily by comparison with literature data on coral carotenoids. Unfortunately, we could identify only one carotenoid, peridinin (peak **6**), which shows mass fragments of *m/z* 631, 613, and 553, corresponding to $[M+H]^+$ (6% abundancy), $[M+HH_2O]^+$ (34% abundancy), and $[M+HH_2OAcOH]^+$ (100% abundancy), respectively (Maoka et al., 2002). The chemical structure of peridinin and its mass data are presented in Fig. 3. Peridinin was discovered in five coral species, *M. trabeculata*, *E. quadricolor*, *P. profundacella*, *A. japonica*, and *Palythoa sp.*, as a major carotenoid (Fig. 1) and its relative content in each extract showed six times difference among these coral species as shown in Fig. 4. Peridinin has already been reported as a major carotenoid of several coral species, along with diadinoxanthin, diatoxanthin, and pyrrohoxanthin (Ambarsari et al., 1997; Maoka et al., 2011). Peridinin contents determined by spectrophotometrical measurement following relative quantification by HPLC area were ranged from 0.24–0.43 mg/100 g sample in three *Acropora* corals (Maoka et al., 2011). In the present study, however, exact quantification analysis based on pure compound could not be carried out due to the absence of pure peridinin. Further study is needed for exact determination of peridinin content. Meanwhile, peridinin is also considered a diagnostic pigment for dinoflagellates and high concentrations of this light-harvesting pigment give zooxanthellae their characteristic golden-brown color (Hochberg et al., 2006). For the other carotenoid peaks (peak **1**–**5** and **7**–**10**), we could not identify any carotenoid from literature data, based on mass fragments. Recently, Maoka (2011) reviewed carotenoids in marine animals and suggested various metabolites derived from major carotenoids including diatoxanthin, alloxanthin and peridinin. However, we could not match mass data in this study with those metabolites and find any known compounds or mass fragments from literature data. These results suggested that the coral extracts in this study may contain unusual carotenoids other than carotenoid peridinin, diadinoxanthin, diatoxanthin, and β-carotene that have been found commonly in corals. In fact, an unusual carotenoid, canthaxanthin, was identified in *Corallium rubrum* as a major carotenoid (Cvejic et al., 2007), and a carotenoid of purple color with unknown

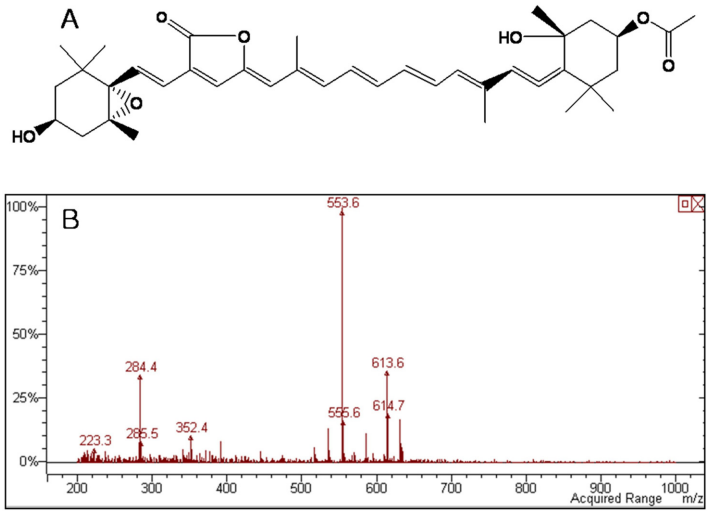

Fig. 3 Chemical structure of peridinin (A) and its mass spectrum (B).

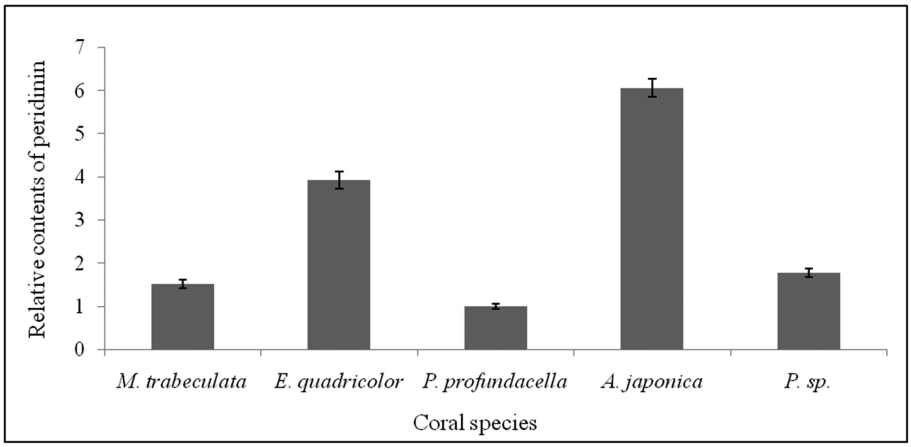

Fig. 4 Relative contents of perinidin in five coral extracts from *M. trabeculata*, *E. quadricolor*, *P. profundacella*, *A. japonica*, and *Palythoa sp*., which contain peridinin as a major corotenoid. The HPLC area of perinidin peak in the chromatogram was obtained at 445 nm and the relative contents of perinidin in each extract were expressed based on the peak area of *P. profundacella.*

chemical structure has been reported in *Gorgonia ventalina* (Leverette et al., 2008). In the investigation of photosynthetic pigments in corals collected from seas off the coast of Japan, 20 species of corals were analyzed by HPLC and 31 pigment types, including various chlorophylls, were identified (Daigo et al., 2008). However, there was no overlap in the coral species with those in this study, indicating that coral communities and their symbiont algae show great diversity, and that the chemical constituents in coral extracts, therefore, may also be diverse, depending on the coral and algal community.

In summary, carotenoids were examined in 25 coral species collected from Jeju Island, Korea. To our knowledge, this is the first report on a carotenoid analysis of indigenous Korean corals. Of the 25 species examined, only nine were shown to possess putative carotenoids and only peridinin was identified by mass spectrometric analyses. Because corals are among the largest and most diverse ecological communities on the planet and have direct and indirect influences on marine organisms, understanding coral biology and their symbiotic relationship with zooxanthellae is important for the preservation of coral ecosystems. To date, research on indigenous Korean corals has been limited to the taxonomy and reproduction of corals and is still in its infancy compared with the world standard. Thus, further research should be focused on aspects of the coral ecosystem, such as the relationship between pigments and bleaching, the major zooxanthellae living in indigenous Korean corals, and photosynthesis effects on coral growth, beginning with the current results.

Acknowledgments This study was supported by a Korea Institute of Science and Technology (KIST) Gangneung Institute intramural research grant (2Z03850) and by a grant from Marine Biotechnology Program Funded by Ministry of Land, Transport and Maritime Affairs of Korean Government.

References

Ambarsari I, Brown BE, Barlow RG, Britton G, and Cummings D (1997) Fluctuations in algal chlorophyll and carotenoid pigments during solar bleaching in the coral *Goniastrea aspera* at Phuket, Thailand. *Mar Ecol Prog Ser* **159**, 303–7.

Apprill AM, Ridigare RR, and Gates RD (2007) Visible healthy corals exhibit variable pigment concentrations and symbiont phenotypes. *Coral Reefs* **26**, 387–97.

Bae BH, Im KS, Choi WC, Hong J, Lee CO, Choi JS et al. (2000) New acetylenic compounds from the stony coral *Montipora sp. J Nat Prod* **63**, 1151–514.

Choi EJ and Song JI (2007) Reproductive Biology of the temperate soft coral *Dendronephthya suensoni* (Alcyonacea: Nephtheidae). *Integra Biosciences* **11**, 215–25.

Cvejic J, Tambutté S, Lotto S, Mikov M, Slacanin I, and Allemand D (2007) Determination of canthaxanthin in the red coral (*Corallium rubrum*) from Marseille by HPLC combined with UV and MS detection. *Mar Biol* **152**, 855–62.

Daigo K, Nakano Y, Casareto BE, Suzuki Y, and Shioi Y (2008) High-performance liquid chromatographic analysis of photosynthetic pigments in corals: an existence of a variety of epizoic, endozoic, and endolithic algae. *International Coral Reef Symposium (ICRS) Proceedings* **11**, 123–7. (http://www.reefbase.org/resource_center/publication/icrs.aspx)

Hochberg EJ, Apprill AM, Atkinson MJ, and Bidigare RR (2006) Bio-optical modeling of photosynthetic pigments in corals. *Coral Reefs* **25**, 99–109.

Kang DH, Song JI, and Choi KS (2005) Image analysis of typhoon impacts on soft coral community at Munseom in Jeju, Korea. *Ocean Polar Res* **27**, 25–34.

Kim SH, Lee J, Kim YT, Kim HC, Yang YS, Suh SJ et al. (2004) Artificial propagation and application on the Korean soft corals. *Underwater Sci Technol* **5**, 17–24.

Kim SM, Jung YJ, Kwon ON, Cha KH, Um BH, Chung D et al. (2012) A potential commercial source of fucoxanthin extracted from the microalga *Phaeodactylum tricornutum*. *Appl Biochem Biotechnol* **166**, 1843–55.

Leverette CL, Warren M, Smith MA, and Smith GW (2008) Determination of carotenoid as the purple pigment in *Gorgonia ventalina* sclerites using Raman microscopy. *Spectrochim A* **69**, 1058–61.

Maoka T (2011) Carotenoids in marine animals. *Mar drugs* **9**, 278–93.

Maoka T, Akimoto N, Tsushima M, Komemushi S, Mezaki T, Iwase F et al. (2011) Carotenoids in marine invertebrates living along the Kuroshio current coast. *Mar Drugs* **9**, 1419–27.

Maoka T, Fujiwara Y, Hashimoto K, and Akimoto N (2002) Rapid identification of carotenoids in a combination of liquid chromatography/UV-visible absorption spectrometry by photodiode-array detector and atmospheric pressure chemical ionization mass spectrometry (LC/PAD/APCI-MS). *J Oleo Sci* **51**, 1–9.

Mydlarz LM, McGinty ES, and Harvell CD (2010) What are the physiological and immunological responses of coral to climate warming and disease? *J Exp Biol* **213**, 934–45.

Osinga R, Schutter M, Griffioen B, Wijffels RH, Verreth JAJ, Shafir S et al. (2011) The biology and economics of coral growth. *Mar Biotechnol* **13**, 658–71.

Rodriguez-Amaya DB, and Kimura M (2004) HarvestPlus Handbook for Carotenoid Analysis, In *HarvestPlus Technical Monograph* (Vol. 2), HarvestPlus, Washington, USA.

Seo Y, Rho JR, Geum N, Yoon JB, and Shin J (1996) Isolation of guaianoid pigments from the gorgonian *Calicogorgia granulose*. *J Nat Prod* **59**, 985–6.

Song JI and Lee IS (1998) Fauna of anthozoans from adjacent waters of Geojedo Island in Korea. *Korean J Syst Zool* **14**, 229–42.

Venn AA, Wilson MA, Trapido-Rosenthal HG, Keely BJ, and Douglas AE (2006) The impact of coral bleaching on the pigment profile of the symbiotic alga, *Symbiodinium*. *Plant Cell Environ* **29**, 2133–42.