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Partial quantification of pigments extracted from the zooxanthellate octocoral *Sinularia flexibilis* at varying irradiances

Mohammad Kazem KHALESI & Packo LAMERS

[1]*Department of Animal Sciences & Fisheries, University of Agricultural Sciences and Natural Resources, P.O. Box 578, Sari, Iran; e-mail: mk_khalesi@yahoo.com*

Abstract: Chlorophyll-*a* (chl-*a*) and carotenoid pigments of the zooxanthellate octocoral *Sinularia flexibilis* were analyzed using high performance liquid chromatography following exposure to three light intensities for over 30 days. From the coral fragments located at different light intensities, a total carotenoid of >41 µg g^{-1} dry weight, including peridinin, xanthophylls (likely diadinoxanthin + diatoxanthin), and chl-*a* as the most abundant pigments, with minor contents of astaxantin and β-carotene were detected. The whole content of chl-*a* weighed 5 µg g^{-1} dry weight in all coral colonies. Chl-*a* and carotenoids contributed 11.2% and 88.2%, respectively, to all pigments detected, and together accounted for 99.4% of the total pigments present. The highest contents of carotenoids and chl-*a* was observed in the coral grafts placed in an irradiance of 100 µmol quanta m^{-2} s^{-1}; they showed lower ratios of total carotenoids: chl-*a* compared to those exposed to 400 µmol quanta m^{-2} s^{-1} after >30 days of incubation. The ratios of peridinin and xanthophylls with respect to chl-*a* from the colonies at 400 µmol quanta m^{-2} s^{-1} were approximately double those observed at irradiances of 100 and 200 µmol quanta m^{-2} s^{-1}. Partial quantification of pigments in this study showed that the carotenoids of *S. flexibilis* showed a decrease at irradiances above 100 µmol quanta m^{-2} s^{-1}, with the exception of an increase in β-carotene at 200 µmol quanta m^{-2} s^{-1}.

Key words: Carotenoids; chlorophyll-*a*; light intensity; *Sinularia flexibilis*

Abbreviations: chl-*a* – chlorophyll-*a*; DW – dry weight; HPLC – high-performance liquid chromatography.

Introduction

The symbiotic soft coral *Sinularia flexibilis* Quoy et Gaimard, 1883, like other symbiotic corals co-exists with photosynthetic, endosymbiotic dinoflagellates of the genus *Symbiodinium* (i.e., zooxanthellae). The zooxanthellae in these corals require to access to ample light in order to provide the coral host with translocated essential nutrients and photosynthetically fixed carbon. Such corals inhabit near surface waters where they are exposed to increased radiation. It has been found that, in order for these photosynthetic corals to protect themselves against deleterious effects of extra irradiance, they have developed some adaptation mechanisms such as pigment production, including carotenoids. Carotenoids are considered to be important photo-protective components of corals in both light harvesting and photoprotection (as natural sunscreens: Bandaranayake 2006). It is well-known that carotenoids potentially originate from endosymbionts and are potent antioxidants that can reduce oxygen radical damage (Frank & Cogdell 1996; Mobley & Gleason 2003).

Zoxanthellae contain pigments, i.e., chl-*a* and *c*, carotene, xanthophylls (diadinoxanthin + diatoxanthin), and a pigment unique to the Dinophyceae: peridinin (Jeffrey & Haxo 1968; Johansen et al. 1974; Liaaen-Jensen 1989). Peridinin has been reported to possess anti-tumor and anti-carcinogenic activities (Suzuki et al. 2003). The majority of xanthophylls are involved in photo-protection the content of which as well as of other pigments is variable, depending on the predominant light conditions (Iglesias-Prieto & Trench 1997). The pigment/chl-*a* ratios also vary between species and groups and are influenced by light conditions (Latasa 1995; Goericke & Montoya 1998; Nicklisch & Woitke 1999). Reported light intensities in coral habitats normally vary from >2000 µmol quanta m^{-2} s^{-1} on the surface with a descending pattern of 60, 20 and 4% attenuation moving downward up to a depth of 150 m (e.g., Lesser & Farrel 2004; Rodolfo-Metalpa et al. 2008).

Carotenoids extracted from symbiotic soft corals (Jeffrey & Haxo 1968; Hallenstvet & Liaaen-Jensen 1979) were found to be identical with those of dinoflagellates and ascribed to zooxanthellae symbionts. Zooxanthellate corals are, therefore, rich in carotenoids (Chalker & Dunlap 1981) and changes in carotenoid concentrations may have important physiological implications for the coral and the coral-algal symbiosis (Klepple et al. 1989). Moreover, the chlorophyll–carotenoid interactions are a subject of biological importance (Gruszecki et al. 2000). It has been found (e.g., Khalesi et al. 2008) that in symbiotic corals as the light intensity increases, the contents of photosynthetic pig-

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ments as well as accessory photosynthetic pigments decrease.

The study of algal pigmentation in some symbiotic corals has already been reported (Kleppel et al. 1989; Brown et al. 2000). However, in addition to lack of data on carotenoids of *S. flexibilis*, changes in intensities of light for their effect on the photoprotective properties of carotenoids in this octocoral have not yet been investigated. Because carotenoid content depends on environmental conditions such as irradiance, in this study changes of chl-*a* and carotenoid contents of *S. flexibilis* at three light intensities were investigated in laboratory conditions.

Material and methods

Corals and experimental conditions

Colonies of the octocoral *Sinularia flexibilis* were obtained from the Burger's Zoo, Arnhem, the Netherlands. These corals all originate from one stock and are, therefore, all genetically clones. These parent colonies were transferred to the laboratory and placed in a main stocking tank (Eco-deco systems, Dymico-Model 1000) containing ~1300 L saltwater, made of Instant Ocean, at 34‰ ± 0.4 salinity, 25.8 ± 0.2 ℃, for rearing the coral stock. The tank was equipped with one VHO Halide, 10,000 HQI lamps (Aqua Medic aqualight 400, Aquaria Veldhuis, Enschede, the Netherlands) adjusted for 12 h light: 12 h dark photoperiod. Cultures of *S. flexibilis* were grown in the laboratory to estimate the influence of light conditions on the pigment content as well as pigment/chl-*a* ratios. To do this, pieces of the coral (nubbins or fragments of 5–7 cm) were cut, fixed on PVC platelets, and attached to the substrate within 1–2 weeks. These nubbins were placed in three experimental tanks ($n = 5$ nubbins per tank) located on the main stocking tank. Each experimental tank exposed to approximate light intensities of 100, 200 and 400 µmol quanta m^{-2} s^{-1} measured as photon flux density (PFD) using an underwater photo sensor (LI-COR, Li250 Light meter). The required light intensity was provided through adjusting the distance of the colonies inside the tanks from the light source. The water in the experimental tanks was circulated through the main stocking tank; therefore, all three tanks had similar environmental conditions except in light intensity. The water flow, which is a vital factor for this coral (Khalesi et al. 2007) was created by air tubes in the experimental tanks. The water velocity was measured by a thermistor to ensure having almost identical water velocities around the microcolonies. The experimental coral colonies were incubated in the tanks for over 30 days, during which their response and development was monitored.

Pigment extraction and quantification

After the incubation period, the coral samples from each tank were separately collected, washed with filtered sea water, and their wet weight was determined by weighing dry-blotted samples. Each group (treatment) was then freeze-dried for dry weight determination. The groups were crushed and extracted separately according to Kinzie (1993) using chilled methanol: tetrahydrafuran (80 : 20 vol : vol) as the solvent at 4 ℃ overnight. This solvent is efficient in extracting both chl-*a* and more polar pigments (such as xanthophylls) and has been recommended for extracting pigments from coral endosymbionts (Chalker & Dunlap 1981). The separate extracts were centrifuged and aliquots of the supernatant from the extracts of each colony (treatment) were qualified using a spectrophotometer (Spectronic 20 Genesys, USA). The solvent from each extracted colony was then dried separately using N gas flow, followed by storing at −20 ℃ for later quantification of pigments. The HPLC procedure was based on Fraser et al. (2000), done at The PRI, Wageningen University, the Netherlands. Pigments were characterized by their retention time and spectral characteristics, compared to those found in the literature (Fraga et al. 1995; Jeffrey & Wright 1997; de Oliveira-Proenca et al. 2001). The pigments concentrations calculated using the equations of Jeffrey & Humphrey (1975), normalized to the dry weights of the coral fragments.

Results and discussion

Photosynthetic pigments

Main photosynthetic pigments found in the colonies of *S. flexibilis* were: the porphyrins chl-*a* with its isomers, and the carotenoids peridinin, xanthopylls (likely diadinaxanthin, diatoxanthin and dinoxanthin), astaxanthin, and β-carotene (Table 1). Total content of the carotenoid pigments was >41 µg g^{-1} DW at all light intensities. Chl-*a* content of all coral colonies totally weighed 5 µg g^{-1} DW. Chl-*a* and carotenoids contributed with 11.2% and 88.2%, respectively, to all pigments detected, and together accounted for >99% of all pigments present. A proportion of >95% was reported for all pigments found in four species of symbiotic hard corals (Fang et al. 1995). Other unidentified minor peaks present in *S. flexibilis* likely consisted of dinoxanthin and isomers of chlorophylls and carotenoids, as by-products of extraction and storage (Jeffery & Wright 1997).

The highest total carotenoids content of 19 µg g^{-1} DW was found in the colonies located at a light intensity of 100 µmol quanta m^{-2} s^{-1}. The coral colonies settled at irradiances of 200 and 400 µmol quanta m^{-2} s^{-1} were almost similar in amounts of peridinin (9 and 10 µg g^{-1} DW) and xanthophylls (1.5 µg g^{-1} DW). Astaxanthin and β-carotene contents were very low (1 µg g^{-1} DW) and seemed unrelated to increased light intensity; they were two times at 100 than at 400 µmol quanta m^{-2} s^{-1} (Table 1). The minor amounts of both astaxanthin and β-carotene are common in brownish cnidarians (Borneman 1997) such as *S. flexibilis.* β-carotene represented 1.1% of all carotenoids; this is similar to 1% detected in eight Alcyonian soft corals (Hallenstvet & Liaaen-Jensen 1979). Astaxanthin accounted for 1.6% of total carotenoids detected in*S. flexibilis*, which is higher than 0.3% of total carotenoids found in free dinoflagellates (Johansen et al. 1974). The high percentage of peridinin (82%) is close to 85.5% reported by Johansen et al. (1974) in free dinoflagellates, and lies in the range of 70–88% in eight soft corals reported by Hallenstvet & Liaaen-Jensen (1979). The carotenoids totally comprised 0.0024% of the freeze-dried coral material, which is comparable to 0.003–0.009% of lyophilized soft corals repre-

Table 1. Contents and ratios of pigments per unit dry weight of *S. flexibilis* colonies at three irradiances.

Irradiance (µmol quanta m^{-2} s^{-1})	Pigments (µg g^{-1} DW)								
	Chl-*a*	Total carot.	Carot./chl-*a*	Chl-*a*/ carot.	Perid.	Xanth.	*β*-carot.	Astax.	WT (g)
100	3	19	6.5	0.15	15	3	0.4	0.3	0.5
200	1.6	11	7	1.14	9	1.5	0.6	0.24	0.65
400	0.8	11.7	15	0.07	10	1.5	0.1	0.14	0.5

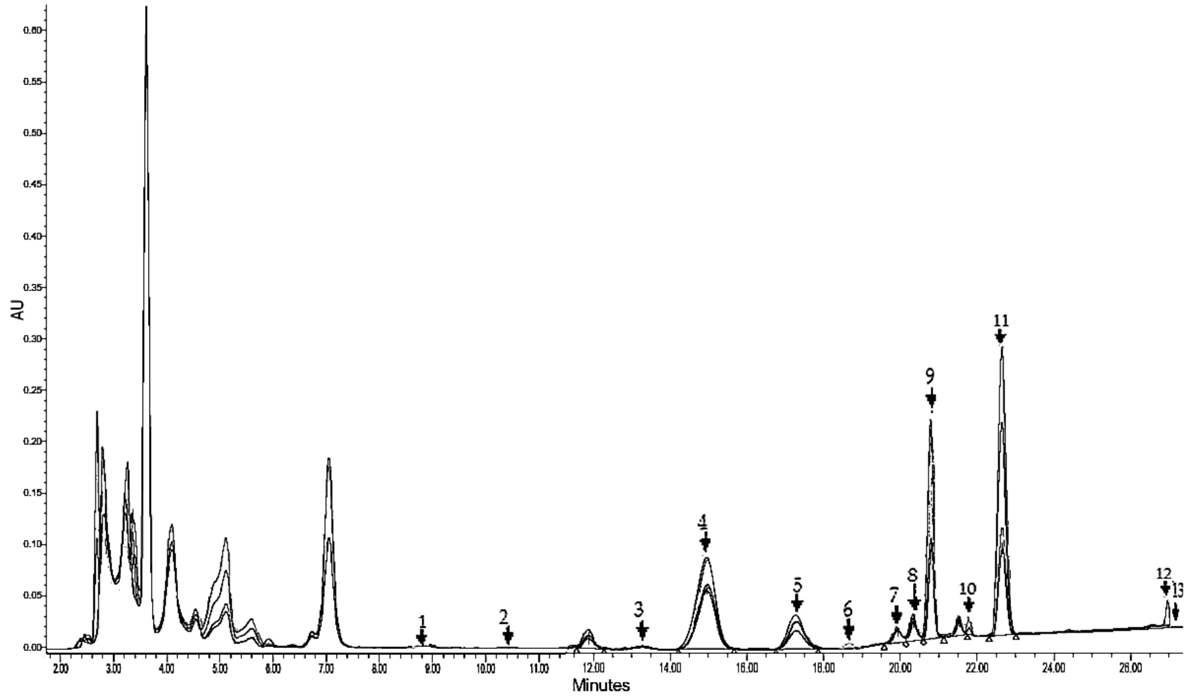

Fig. 1A. *Sinularia flexibilis*. HPLC chromatograms of pigments extracted from the colonies of *S. flexibilis* kept under different regimes of irradiance for >30 days. The multiple lines show partial pigment concentrations at each light treatment (100, 200, and 400 µmol quanta m^{-2} s^{-1}). Pigment identities are: (1) Xanthoplylls; (2) Astaxanthin; (3) Chlorophyll-like compound; (4) Peridinin; (5, 6, 8) possibly carotenoids; (7) Chl-*a*; (9, 10) Chl-*a* isomeres; (11) possibly chlorophyll; (12) Chlorophyll-like compound; (13) *β*-carotene.

sented by Hallenstvet & Liaaen-Jensen (1979). This may suggest species-specificity of carotenoid contents in corals.

Effect of light intensity

Quantitative results from HPLC (Fig. 1) on the amount of both the pigments (peridinin, chl-*a*) and carotenoids [*β*-carotene and certain photoprotecting xanthophylls in smaller quantities (likely diadinoxanthin+diatoxanthin)] showed to be different among the treatments. It appears that increased irradiance caused a descending pattern in the concentration of chl-*a* contents after the >30–d experiment, especially between the colonies at 100 and 400 µmol quanta $m^{-2}s^{-1}$ (Fig. 2). Chl-*a* (g^{-1} DW) was 3.75 times higher in 100 than in 400 µmol quanta m^{-2} s^{-1} and almost two times that of the corals in 200 µmol quanta m^{-2} s^{-1}. The same effect of light intensity on chl-*a* content was also found previously in *S. flexibilis* (Khalesi et al. 2008). This also corroborates the general finding that in zooxanthellate corals, chl-*a* densities tend to diminish in symbiotic dinoflagellates in response to changes in light intensity (Hoegh-Guldberg & Smith 1989; Fitt & Cook 2001). Higher chlorophyll content at lower light levels than at higher intensities (<100 to >200 µmol quanta m^{-2} s^{-1}), by experimentation or in the field, has been reported in various symbiotic cnidarians (e.g., Porter et al. 1984; Muller-Parker 1987; Verde & McCloskey 2002).

The coral samples at 100 and 200 µmol quanta m^{-2} s^{-1} showed almost similar contents of both astaxantin and *β*-carotene; *β*-carotene was slightly higher at 200 µmol quanta m^{-2} s^{-1} (Fig. 2). Lower concentrations of astaxantin and other zooxanthellar pigments, including peridinin, xanthophylls, and chl-*a* was also reported (Kleppel et al. 1989) in the reef coral *Montastrea annularis* Ellis et Solander, 1786 resulting from light-induced bleaching (loss of pigments). The observed decrease in the total carotenoid contents at relatively high irradiance (400 µmol quanta m^{-2} s^{-1}) in this study agrees with De Mora et al. (2000); it is, however, in contrast with general assumption that carotenoids increase at high light intensities (e.g., Demers et al. 1991; Montané et al. 1998). Exposure to artificial light intensi-

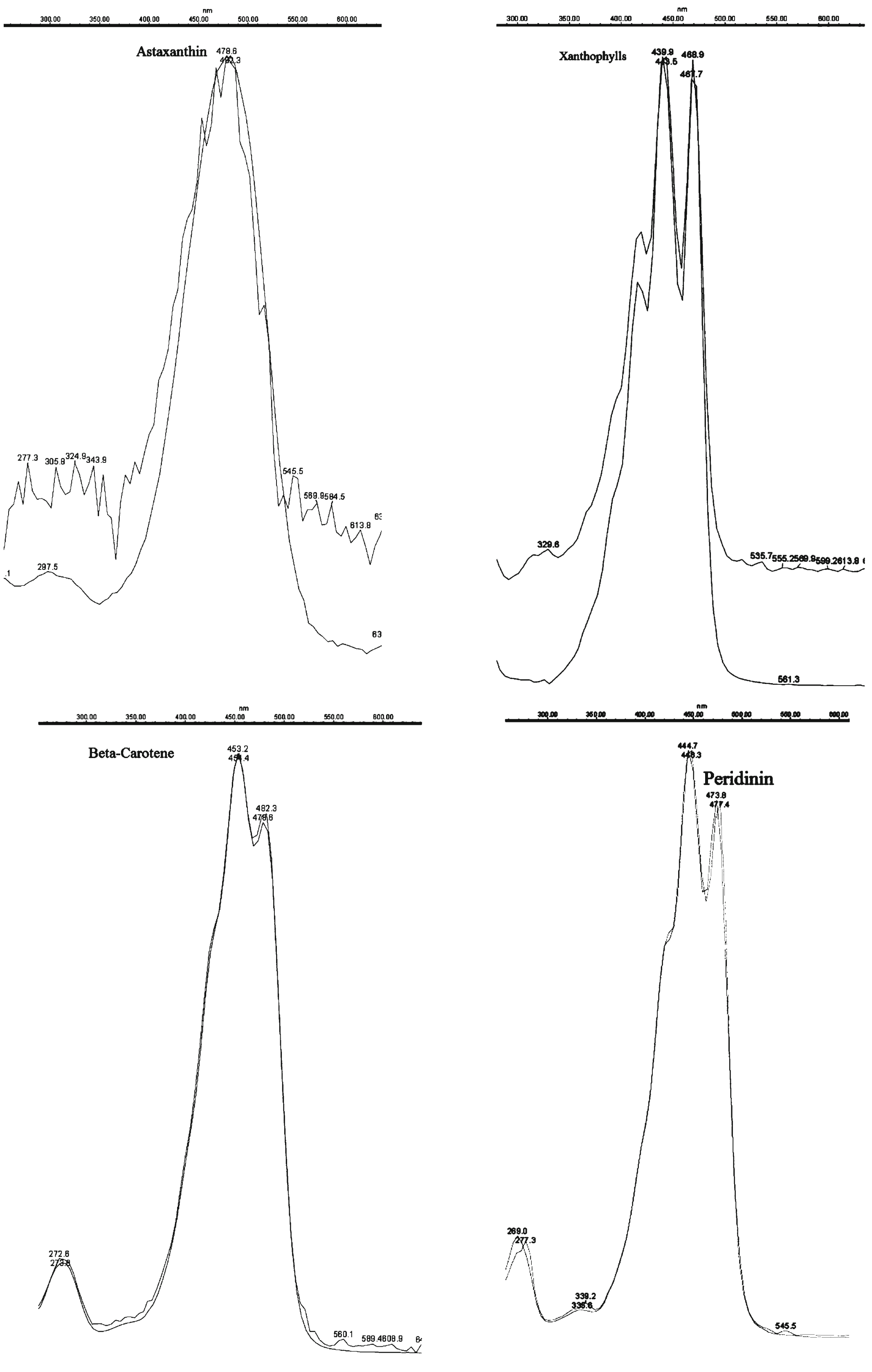

Fig. 1B. *Sinularia flexibilis*. Representative chromatograms illustrating the peaks corresponding to the detected pigments in the coral colonies.

ties (>200 µmol quanta m^{-2} s^{-1}) did not induce any changes in carotenoid levels in zooxanthellae of a symbiotic sea anemone (Verde & McCloskey 2002). Overall, the contents of all detected carotenoids in *S. flexibilis*

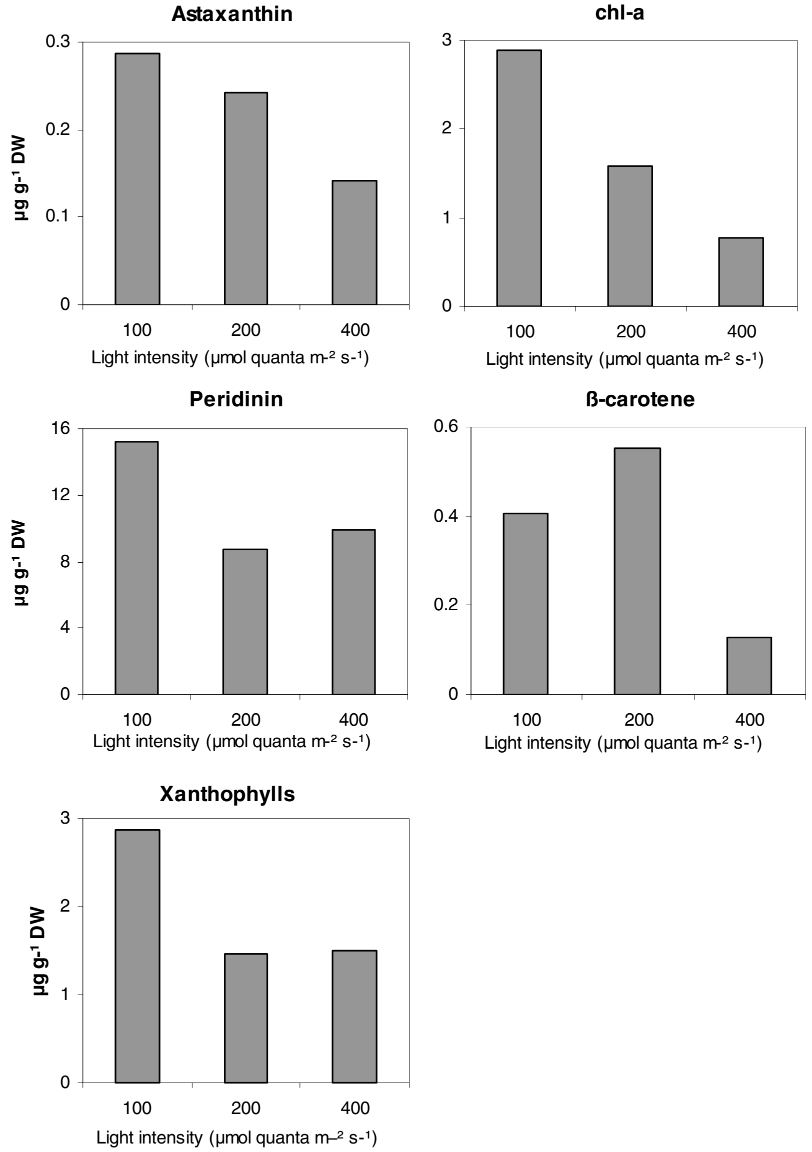

Fig. 2. Contents of detected pigments g^{-1} DW, determined by HPLC, in the colonies of *S. flexibilis* at three light intensities after over 30 days under laboratory conditions.

appeared to be comparatively lower at elevated irradiances (>200 µmol quanta m^{-2} s^{-1}).

Ratios of pigments

The ratio of chl-*a* to peridinin (0.2 : 0.2, w : w) was relatively higher at 100 and 200 µmol quanta m^{-2} s^{-1} compared to a ratio of 0.2 : 0.1 in the corals at 400 µmol quanta m^{-2} s^{-1}; this may be because rates of photosynthesis under lower light conditions tend to be optimized (Myers et al. 1999). The ratios of chl-*a* : total carotenoids also differed between 100 and 400 µmol quanta m^{-2} s^{-1}, but this ratio was almost similar at irradiances of 100 and 200 µmol quanta m^{-2} s^{-1} (Table 1). It, therefore, seems that relatively low and high light intensities (100 and 400 µmol quanta m^{-2} s^{-1}) had an impact on the values of the diagnostic pigment/chl-*a* ratios, with the exception of peridinin and xanthophylls at irradiances of 100 and 200 µmol quanta m^{-2} s^{-1}; in these light intensities, the ratios of peridinin/chl-*a* (5.3 and 5.5) and xanthophylls/chl-*a* (1 and 0.9) were almost similar. A higher ratio of total carotenoids/chl-*a* may be because of faster degradation of chlorophyll that can be taken to suggest photoprotection against raised irradiances (De Mora et al. 2000). This indicates a decrease in chl-*a* in favor of the photoprotective carotenes to respond the increased irradiance stress on the coral.

Conclusion

In conclusion, the results of this study suggest that, for relatively short periods of time, increasing irradiance does not seem to multiply either photosynthetic or photoprotective densities in *Sinularia flexibilis.* Partial quantification of carotenoids of this soft coral showed no increase in the biosynthesis of these pigments by elevated light intensity under experimental conditions after 30 days. The carotenoids showed to decrease at irradiances above 100 µmol quanta $m^{-2}s^{-1}$, except the increase in β-carotene at 200 µmol quanta m^{-2}

s^{-1}. The higher ratio of carotenoids/chl-*a* at relatively high light intensity (400 µmol quanta m^{-2} s^{-1}) indicates that while chl-*a* decreased with increasing irradiance, the proportion of photoprotective carotenoids remained comparatively high to reduce photodamage to the coral. Furthermore, the higher content of peridinin at relatively low light intensity (100 µmol quanta m^{-2} s^{-1}) can be taken as an irradiance in which this species optimizes the production of peridinin as a potential antitumor compound to be utilized for biomedical research. Overall, it appears that *S. flexibilis* prefers rather low light intensities to enhance the biosynthesis of both photosynthetic and accessory pigments. Because very little information is available on the carotenoid composition of soft corals, especially under enclosed situations, this study provides more insights in the carotenoids composition of soft corals. Future studies should concentrate further on this topic.

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