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Study on the year-round fluctuation of total lipid content and lipid classes composition in soft coral *Sinularia flexibilis* from Nha Trang, Khanh Hoa coastal area

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ABSTRACT

Samples of soft coral *Sinularia flexibilis* were collected for one year, from January to December, in the Nha Trang coastal area, Khanh Hoa province, to assess the month-by-month fluctuation of total lipid content and lipid classes composition in this species. Full lipid content reached the highest value from March to May, lower in the period of June-September, and bottomed in October and November. There was a significant decrease in this content between May and June, September and October; the content increased from February to March but was not stable from October to February. The lipid classes composition of *Sinularia flexibilis* includes polar lipids (PL), sterols (ST), triacylglycerol (TAG), monoalkyldiacyl-glycerol (MADAG), wax (W), and free fatty acids (FFA). The concentration of PL in summer (April-August) was lower than that in winter (September-February); this content increased significantly during November-December; meanwhile, it was observed to decrease in April-May, December-January. The content of ST increased from October to March and declined from April to September. The remaining classes, including W, MADAG, and TAG, tend to grow in the summer and decline in the other months. The fluctuation of lipid class composition throughout the year is closely related to zooxanthellae microalgae in corals at different times of the year and is directly influenced by environmental conditions, especially seawater temperature. Besides, this coral's maturation and reproduction effect with the increase or release of reproductive materials was also observed.

Keywords: Lipid, lipid class, total lipid, coral, *Sinularia flexibilis*.

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INTRODUCTION

Sinularia flexibilis is one of the famous soft corals, widely distributed in the different regions around the world, including the coastal areas of Nha Trang, Khanh Hoa, Vietnam. Studies on this coral species' chemical composition and biological activity of this coral species have been conducted since earlier. Various researchers have reported the isolation of compounds with potential antitumor activity [1–4]. Mohammad K. K. has compiled 210 publications from 1975–2007 on secondary metabolites isolated from corals of the genus Sinularia, including 42 publications on *Sinularia flexibilis* species in a published review in 2008 [5]. From the chemical composition of this coral, scientists have isolated cembranoid diterpene, bicembranoid diterpene, steroids, polyhydroxysteroids, and polyamine compounds with wide range of biological activities such as antibacterial, anti-inflammatory, antitumor, cytotoxic, analgesic, neuroprotective, etc. [6–10]. Amongst them, there are many compounds with interesting chemical structures. Based on the obtained results, this coral species is evaluated to have great potential for developing new medicinal products [5].

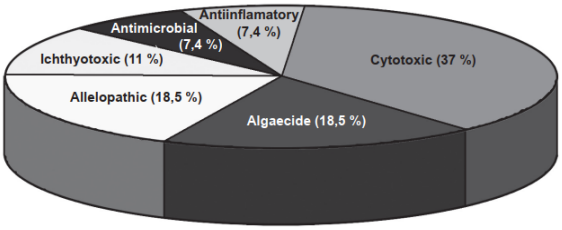

Figure 1. Percentages of biological activities of compounds isolated from *S. flexibilis* [5]

Although many studies have been conducted on the secondary metabolites of the soft coral *Sinularia flexibilis*, in contrast, research on lipids is still limited [11, 12]. Detailed studies on lipid class composition and investigation of its change or stability of this coral species in months year-round have not been performed at all.

MATERIALS AND METHODS

Materials

Samples of soft coral *Sinularia flexibilis* were collected for 12 consecutive months in one year in the coastal of Nha Trang, Khanh Hoa at a depth of 7–8 m. Prof. Hoang Xuan Ben and colleagues identified the sample at the Institute of Oceanography, Nha Trang.

Methods

Total lipid extraction method

From the fresh coral sample obtained, total lipids were extracted according to the method of Folch J. F., using the solvent system $CHCl_3$:MeOH with a ratio of 2:1 by volume [13]. The lipid mass and the dry sample weight obtained calculated the total lipid content.

Method to determine the composition and content of lipid classes

The composition and content of lipid classes were analyzed and determined on pre-coated plates (6 cm × 6 cm, Sorbfil, Krasnodar, Russia); scan images on Epson Perfection 2400 PHOTO machine (Nagano, Japan) with standard resolution and size, combined with image analysis program Sorbfil TLC Videodensitometer, Krasnodar, Russia [14].

RESULTS AND DISCUSSION

Change in total lipid content by months year-round of soft coral *Sinularia flexibilis*

The total lipid content of the studied coral samples was determined based on the total lipid obtained and the dry sample weight. The results are presented in Table 1 and Figure 2.

Table 1. Total lipid content from January to December of the soft coral *S. flexibilis*

Month	Sea temperature (ºC)	Total lipid content (%/dry weight)
1	24.9	21.36 ± 3.44
2	25.4	30.02 ± 3.95
3	25.3	46.90 ±3.50
4	27.4	44.45 ± 3.02
5	28.7	49.57 ± 5.80
6	29.5	31.27 ± 2.24
7	28.4	36.87 ± 3.82
8	28.5	35.88 ± 2.65
9	28.7	32.40 ± 3.59
10	27.7	19.05 ± 3.35
11	26.8	16.60 ± 1.89
12	25.4	27.39 ± 3.88

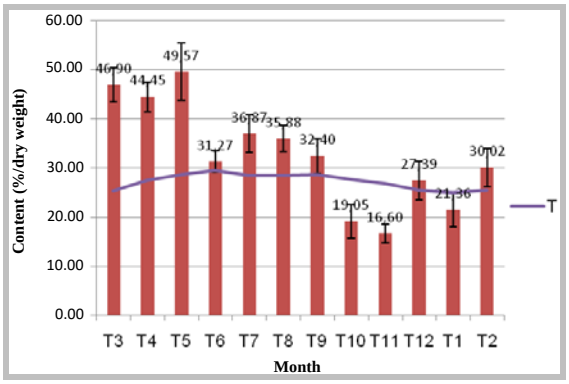

Figure 2. Total lipid content from January to December of soft coral *S. flexibilis*

Figure 2 indicates that the total lipid content of the soft coral *S. flexibilis* is significantly different between the summer and the other months. Full lipid content on dry weight exhibited a high level in March-May (highest maxima 49.57 ± 5.80% on dry weight was recorded in May), lower in June-September and significantly accumulated in October-November (the lowest level in November was 16.6 ± 1.89% on dry weight). Total lipid content showed a significant decrease between May-June and September-October. However, it increased again in February-March. It should be noted that, during the period of October-March, when the lowest sea temperature of the year was recorded, the total lipid content varied in a random manner.

It is known that the annual variation of sea temperature and solar radiation causes coral reef ecosystems to change cyclically. In Nha Trang, sea temperature varied between 28^{o}C and 30^{o}C most of the time in one year (April-September) and dropped to 24–25^{o}C in December-January; meanwhile, the maximum solar irradiance observed in the period from January to April [15]. In addition, storms and monsoons from October to December strongly increase turbulence and turbidity in shallow areas of the coral reefs. Changes in environmental conditions will be the factors that directly affect the fluctuations of total lipid content. In addition, other environmental factors such as reproductive period and growth also need to be considered.

Changes in the lipid class composition by months year-round of soft coral *Sinularia flexibilis*

Obtained results indicated that, similar to other soft coral species reported previously, the structural lipid of *S. flexibilis* contains polar lipid (PL) and sterol (ST) meanwhile storage lipid includes triacylglycerol (TAG), monoalkyldiacylglycerol (MADAG), wax (W) and free fatty acids (FFA) (Table 2). PL, MADAG, W are the main classes in the total lipid of *S. flexibilis*, in which the content of MADAG and W are higher than that of PL. The result is similar to those of other Sinularia species studied previously [16].

The FFA class accounts for an insignificant proportion of total lipids; its content remains lower than 5% of total lipid throughout the year. In addition, there is an unidentified class in minor proportion, which ranged from 3.82% (July) to 9.33% (November) of total lipid. The change in the content of this unidentified class coincided with the variation of structural lipid (PL and ST) through months year-round (Figure 3). The variation in the content of the PL class between months was more evident than that of the ST class. The average level of PL class in summer (April-August) is lower than that in winter (September-February). The level of PL accumulated in July (13.8 ± 1.33% of total lipid); however, it increased significantly from November to December (28.8 ± 1.48% of total lipid). The ST level showed weak maxima in June (5.95 ± 0.07% of total lipid) and reached a peak in December (13.8 ± 0.31% of total lipid). Samples collected in October-March showed a higher level of ST than that collected during April-September although the difference was insignificant.

In contrast to the fluctuation of PL and ST classes, the others, including W, MADAG, and TAG, tend to increase in the summer and decrease in the remaining months (Figure 4). The level of TAG varied from 6.43 ± 0.27% (September) to 14.3 ± 0.67% (February), there was a significant drop from August to September (11.3 ± 0.54% to 6.43 ± 0.27% of total lipid). This class remained at a high level in January-August and declined in September-

December. The MADAG level varied from 18.6 ± 0.64% (December) to 28.9 ± 0.88% (July) of total lipid. The average level of MADAG in October-February is lower than that in March-September. Notably, a significant increase was observed in June-July.

Table 2. Contents of lipid classes (% of total lipids) from January to December of the soft coral *S. flexibilis*

Month	PL	ST	Un-identified	FFA	TAG	MADAG	W	T
1	21.0 ± 1.0	9.45 ± 0.45	9.26 ±1.94	2.56 ± 0.27	11.8 ± 0.41	20.3 ± 1.04	25.7 ± 1.54	25.3
2	19.3 ± 1.16	9.05 ± 0.59	4.31 ± 0.49	2.71 ± 0.47	14.3 ± 0.67	22.7 ± 1.24	27.6 ± 1.61	27.4
3	21.4 ± 1.90	9.21 ± 0.30	5.97 ± 0.43	2.15 ± 0.22	11.2 ± 1.0	25.6 ± 1.31	24.5 ± 1.31	28.7
4	19.0 ± 1.19	8.11 ± 0.50	5.39 ± 0.38	2.45 ± 0.26	13.1 ± 0.49	25.0 ± 1.10	27 ± 3.27	29.5
5	14.3 ± 0.55	6.81 ± 0.30	4.41 ± 0.46	2.91 ± 0.20	12.8 ± 0.41	24.4 ± 0.87	34.4 ± 1.06	28.4
6	15.2 ± 1.06	5.95 ± 0.07	4.82 ± 0.53	3.1 ± 0.32	12.6 ± 0.91	24.5 ± 1.63	33.9 ± 1.0	28.5
7	13.8± 1.33	6.65 ± 0.56	3.82 ± 0.27	2.14 ± 0.19	13.3 ± 0.59	28.9 ± 0.88	31.4 ± 1.76	28.7
8	17.6 ± 0.91	7.08 ± 0.22	5.33± 0.54	2.61 ± 0.11	11.3 ± 0.54	24.4 ± 0.61	31.7 ± 1.22	27.7
9	20.5±1. 26	7.66 ± 0.18	7.87 ± 0.24	2.65 ± 0.20	6.43 ± 0.27	25.8 ± 0.53	29 ± 1.34	26.8
10	20.0 ± 1.49	8.87 ± 0.75	5.06 ± 0.43	3.02 ± 0.28	8.96 ± 0.29	22.3 ± 0.57	31.9 ± 2.59	25.4
11	20.5 ± 1.47	9.79 ± 0.66	9.33 ± 0.74	5.29 ± 0.42	9.69 ± 0.53	19.3 ± 0.76	26.1 ± 2.43	24.9
12	28.8 ± 1.48	13.8 ± 0.31	8.35 ± 0.12	3.13 ± 0.21	9.4 ± 0.62	18.6 ± 0.64	17.8 ± 0.63	25.4

Notes: PL: Polar lipid, ST: sterol; TAG: triacylglycerol, MADAG: monoalkyldiacylglycerol, W: wax; FFA: free fatty acid; T: temperature; LPT: total lipid content.

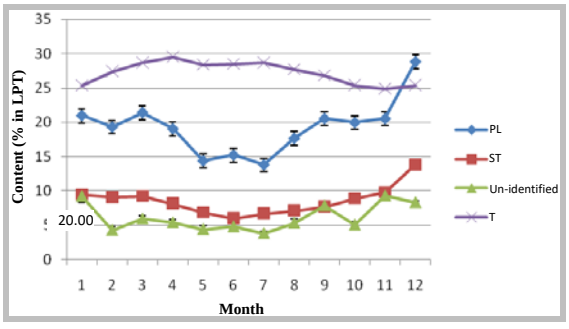

Figure 3. The content of sterol class (ST) and polar lipid (PL) and unidentified class in total lipid of *S. flexibilis* from January to December

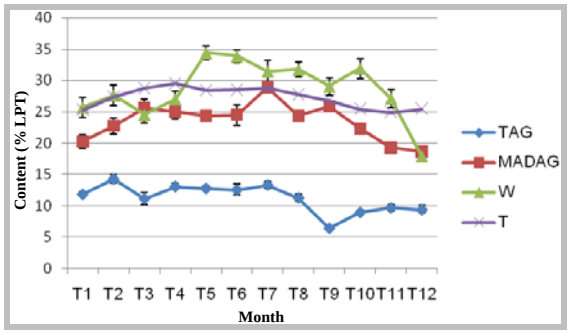

Figure 4. The content of triacylglycerol (TAG), monoalkyldiacylglycerol (MADAG) and wax (W) in total lipid of *S. flexibilis* from January to December

The W class possessed the highest level compared to the other classes of *S. flexibilis* for most time of the year except in December, of which the PL showed the highest level. The level of W class fluctuated in the range of 17.8 ± 0.63% to 34.4 ± 1.06% of total lipid; it was observed to reach a peak in May. This class level increased in April-May and significantly decreased in October-December.

Variations in the lipid classes composition of coral samples could be significantly influenced by the presence or decline of zooxanthellae microalgae. Thus, throughout the year, the lipid classes in the total lipid of *S. flexibilis* showed certain fluctuation. According to a study by Imbs et al. (2010), neutral lipids are the main components in host coral lipids, especially the MADAG class is almost absent from the lipid of zooxanthellae, but it is located entirely in the host coral lipid; at this time, the total lipid composition of zooxanthellae is rich in polar lipid classes (PL and ST) [11]. Therefore, the PL class is sensitive to rapid changes of environmental parameters because changes in ambient temperature always strongly affect the density of zooxanthellae in coral tissues. Another study (2010) also reported that when the sea temperature reaches above 32^{o}C, it will cause the loss of zooxanthellae symbiotic microorganisms and in consequence, the death of coral reefs. At that time, the level of PL will show a significant decrease [17]. Thus, when the sea temperature is high, the density of zooxanthellae in corals will decrease in the summertime, which is directly proportional to the low level of PL and ST classes (Classes that constitutes the main lipid component of zooxanthellae). The increased level of PL and ST in December could be related to the increase in density of zooxanthellae symbiotic microalgae. At this time, the environmental conditions of seawater (salinity, light, temperature, etc.) may be favorable for their development in symbiosis with corals.

In addition, *S. flexibilis* is a seasonally sexually reproducing coral. According to literature, the spawning period of this coral species happens from May to September in Barang Lompo island [18, 19]. There are no data on lipids of reproductive material in Sinularia species; however, several reports on the role of lipids in the reproduction of corals and other Cnidarian species indicate that their reproductive material is rich in storage lipids, most of which are W or TAG [20, 21]. Maturation and release of reproductive material are accompanied by energy expenditure and depletion of those storage lipids, especially W and TAG. In addition, the stability of the composition and number of phospholipid membranes in the PL class during the formation and subsequent regeneration of the population also have certain effects on the content of lipid classes. We assumed that the decreased level of TAG in September, W in October-December, as well as the increase of PL in November-December might be accompanied by coral maturation and reproduction of *S. flexibilis*.

CONCLUSION

Initially assessed the fluctuation in months year-round of total lipid content and lipid classes composition of soft coral *Sinularia flexibilis*. Obtained results indicate that the full lipid content reached the highest value in the period of March-May, declined in June-September, and bottomed in October-January. The total lipid content decreased significantly during May-June and September-October, increased in February-March, and remained unstable during October-February. The lipid class composition of *Sinularia flexibilis* contains PL, ST, TAG, MADAG, W and FFA. The FFA class accounted for a minor proportion; its content is stable throughout the year, less than 5% of total lipid. There is an unidentified class with small content, ranging from 3.82% (July) to 9.33% (November) of total lipid. The level of PL during summertime (April-August) is lower during wintertime (September-February). There was a significant increase in November-December, then declined during April-May and December-January. The ST level is higher in October-March than that in April-September. However, the difference is not significant. The other classes (W, MADAG, TAG) tend to increase in the summertime and decrease in the remaining months. The

fluctuation of lipid class composition during months year-round was closely related to the presence of symbiotic microalgae zooxanthellae in corals at different times of the year and is directly influenced by environmental conditions, especially sea temperature. In addition, this coral's maturation and reproductive processes are involved with the increase or release of reproductive materials.

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