

## Seasonal Changes in the Content and Composition of Lipids in *Acropora downingi*

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### Abstract

The lipid content and composition of the reef-building coral *Acropora downingi*, the largest coral reef of Hengam Island, were examined throughout a year. The results showed that the lipid content of *A. downingi* significantly differs in different seasons (t-test,  $P < 0.05$ ). Depending on the season and spawning times, the lipid content of *A. downingi* ranged from 16.5 to 37.7 % (dry weight). The total lipid content was the lowest in the cooler and the highest in the warmest seasons, when solar irradiance were at their highest. Gas chromatography-mass spectrometry (GC-MS) analysis of fatty acid composition of the lipid contents showed that palmitic acid (16:0) was the most abundant fatty acid component of these lipid classes, and other fatty acid concentrations were as follows: Stearidonic acid (18:3, n-6), Eicosapentaenoic acid (20:5, n-3) and Stearic acid (18:0), respectively.

Keywords: *Acropora downingi*, Hengam Island, Persian Gulf, Lipid, Fatty Acid

### 1. Introduction

Lipids are important sources of energy. They are mainly stored in the animal tissue as wax esters and triglycerides, or in the membranes as sterols and polyunsaturated fatty acids (PUFA). The lipid composition is often species specific and provides useful information about their autotrophic or heterotrophic origin (Treignier et al. 2008).

Tropical corals have often been regarded as lipid-rich organisms (Harland et al. 1993). Polar lipids and sterols are the structural basis of cell membranes in

corals and their symbionts, while triacylglycerols, wax, and sterol esters serve as storage lipids and determine the energy balance of corals (Imbs and Yakovleva. 2012). Lipid and FA compositions of a coral colony are related to the mode of nutrition and may be impacted by environmental conditions in their habitats (Oku et al. 2003). In corals, the photosynthetically fixed carbon is translocated from the endosymbiotic algae to the coral host. It provides daily metabolic energy requirements of the corals. Excess fixed carbon is stored in the host tissue as lipids, representing significant energy reserves. Investigation of the changes of total lipids content and

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fatty acid composition of corals gives insight on how they consume their lipid reserves (Grottoli et al., 2004).

The content of total lipids in corals varies with the season, the depth of habitat, illumination, and other environmental factors (Imbs, 2013). Light intensity affects biosynthesis of lipids in zooxanthellae (Crosslan, et al., 1980 and thus, lipid levels in tissues of coral vary with the intensity of the zooxanthellae activity and consumption of energy during coral breathing, cell replenishment, and release of reproductive material (Imbs, 2013).

Seasonal changes of fatty acids in reef-building corals have been reported for a number of Caribbean (Fitt et al. 2000; Harland et al. 1993), Red Sea (Harland et al. 1993), Japanese (Oku et al. 2003), and Hawaiian corals (Stimson 1987) habitats (Table 1). Despite such studies worldwide, lack of data and information about the largest coral reef at the Strait of Hormoz, the Hengam coral reef, occupied mostly with *A. downingi*, exists. This study examined the lipid content and composition of the reef-building

coral *A. downingi* throughout a year.

## 2. Materials and Methods

### 2.1. Sampling Sites

Samplings were performed in four seasons during March 2014 to January 2015 on Hengam Island, located in the north-eastern part of the Persian Gulf. The minimum, mean and maximum annual water temperatures at our sampling site were 20.2°C, 27.5°C and 34.2°C, respectively; and the mean daily water temperatures during the warm and cold seasons were 32.4 and 21.6 °C, respectively (Vajed Samiei et al. 2014). Experiments were performed on the 10-15 cm branches of *A. downingi*, collected from a depth of about 4 m and transported in seawater and low light condition to the laboratory. *A. downingi* experiences milder seasonal water temperatures compared to reefs situated more inward in the Persian Gulf (Vajed Samiei et al. 2014.)

Table 1: Lipid contents of reef-building corals of some locations in the world

Location	coral species	Total lipid (% dry wt)	Reference
Caribbean	<i>P. porites</i>	12.4	Harland et al. 1993
	<i>M. annularis</i>	31.8	
Red Sea	<i>P. verrucosa</i>	10.7	Harland et al. 1993
	<i>S. pistillata</i>	17.0	
	<i>G. retiformis</i>	10.8	
Okinawan	<i>G. aspera</i>	42	Oku et al. 2003
Hawaiian	<i>M. Verrucosa</i>	46.8	Stimson 1987
	<i>P. compressa</i>	33.0	
	<i>C. ocellina</i>	40.7	
	<i>P. meandrina</i>	32.3	
	<i>P. damicornis</i> (Y type)	41.3	
	<i>P. damicornis</i> (B type)	36.9	

## 2.2. Lipid Extraction

Coral fragments were decalcified in a solution of 10% formaldehyde and 10% acetic acid (Yamashiro et al. 1999) and thoroughly washed with tap water. In this study, no separation of symbiotic algae from the hosts was done. The samples were extracted with approximately 50 mL of dichloromethane-methanol (DM, 2:1 by volume) at room temperature. The lipid extracts were evaporated to dryness under N<sub>2</sub> at 40 °C. Total lipid in dried aliquot from dried tissue was determined gravimetrically using a Sartorius Supermicro balance and standardized to dry weight. Total lipids were measured on whole crashed coral samples (skeleton + animal tissue + zooxanthellae), normalized to the total ash-free dry weight of the organic fraction of the coral (animal tissue + zooxanthellae), and expressed as mg lipid per g dry weight (Grottoli et al., 2004).

## 2.3. Analysis of Fatty Acid Composition

The residue of the extract was re-dissolved in 1 mL methanol and then hydrolyzed with 1 mL of KOH methanolic solution (11g/L), at 90 °C, for 10 min. The free fatty acids originally present and those resulting from the alkaline hydrolysis were separated with 1 mL of BF<sub>3</sub> methanolic solution (10%), at 90 °C, for 10 min. Derivatization was stopped with addition of 3 mL water. Fatty acid methyl esters (FAMES) were extracted with 2×6 mL of n-hexane and anhydrous sodium sulphate was added to ensure the removal of water (Pereira et al. 2015). The resulting extract was evaporated to dryness under a stream of nitrogen and dissolved in 100 µL of n-hexane for injection to gas chromatograph. A gas chromatography-mass spectrometry (GC-MS) analysis of FAMES was carried out on an Agilent 6890 GC equipped with a 5973 M detector. A HP-5 (Agilent) capillary column (30 m × 0.32 mm inner diameter × 0.25 µm film thickness) was used at initial temperature of 50 °C

for 1 min, then increased at 20 °C min<sup>-1</sup> to 150 °C, 5 °C min<sup>-1</sup> to 285 °C and held for 5 min. The injector and interface temperatures were 250 °C and 300 °C, respectively. Helium (purity 99.999%) was used as the carrier gas at a constant flow of 1.2 mL min<sup>-1</sup>. FAMES were identified by Wiley 275 library.

## 3. Results and Discussion

### 3.1. Lipid Content

The lipid content of *A. downingi* showed a seasonal fluctuation (Fig. 1). It was found that although the lipid content was greater in March than in December it reduced from March to June, hiked July through September and reduced towards January. Lipid content of *A. downingi* was the lowest after spawning period (April and May) (Bauman, 2013) and in the cooler months (January). *A. downingi* is a hermaphroditic spawner with spawning during the period between Mar/Apr each year (Riegl and Purkis, 2012). Thus, lipid biosynthesis may be linked with oocyte development. Oocytes, like other coral tissues, showed increased the lipid content during spring, and hence spawning might have removed a fraction of the total lipid. The release of lipid-rich gametes during spawning reduces the lipid contents of the corals.

The lipid content of *A. downingi* varied from 37.7 % in September to 16.6 % in January on the basis of % dry tissue weight (Fig. 1). Higher lipid levels in warmer season (summer) could be due to greater photosynthetic production of lipids at higher summer light levels. Furthermore, spawning occurs in April and May, so growth over the summer may not be inhibited by the allocation of energy to gamete production during this period. Although the highest Photosynthetically Available Radiation (PAR) value occurred in June, spawning decreased the lipid levels in this month. On the other hand, the high lipid content in September in the studied shallow-water corals can be due to high photosynthetic activity and

temperature in this month. Although positive correlations were found between total lipids and temperature and PAR, but they were no significant ( $p \geq 0.05$ ).

As shown in Fig. 1, significant changes were not observed for salinity and pH during the sampling times. The temperature significantly affected on total lipid content compared to salinity. It may due to this fact that temperature can significantly effect on photosynthetic efficiency ( $p < 0.05$ ) compared to salinity (Kuanui et al. 2015).

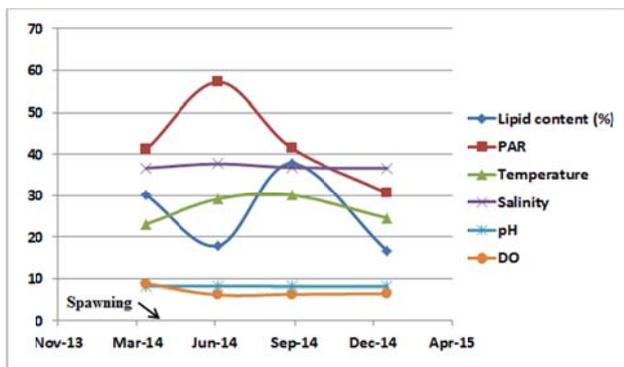


Fig. 1: Seasonal changes in total lipid content of *A. downingi*, monthly mean underwater temperature, salinity, pH, dissolved oxygen (DO) and surface PAR at the Hengam Island from Mar. 2014 to Jan. 2015.

### 3.2. Fatty Acid Composition

Lipid content and composition are useful indicators for studying the corals nutrition strategies and the variation of nutrient and food ingestion. Also, lipid reserves are good indicators of the resilience and the trophic plasticity of a coral (Seemann et al. 2013). Table 2 lists the fatty acid composition of the lipids and shows what they are each marker for. There was a broad spectrum of fatty acids of C10 to C22 with up to five double bonds. There were no branched chain fatty acids or more than 22 carbon atoms in the carbon chain in specimens. The lipids, therefore, were exclusively comprised of straight chain fatty acids. Odd numbered fatty acids were not detected or found only in trace amounts. Palmitic acid (16:0) was the largest

fatty acid component of this polar lipid class (Table 2) with no significant difference in winter and summer. Other major fatty acids were 18:4n-3, 20:5n-3, and 18:0.

The FAs 18:4n-3 and 22:6n-3 are markers of zooxanthellae and *Acropora downingi* is known to contain zooxanthellae. The FAs 16:1n-7 and 20:5n-3 indicates a diatom feeding (Dalsgaard et al. 2003), and are higher in summer than winter which indicates the increasing importance of herbivory in warm seasons for the studied coral. On the other hand, the higher percentage of C18 and C20 PUFA in summer compared to winter (43.5 vs. 34.4 %) indicates the increasing importance of herbivory for the corals.

In the total composition of saturated or PUFA, some diversity exists between the seasons. A predominance of PUFA was noted with the summer, concomitant with a decrease in the proportion of saturated fatty acids in winter. Major PUFA components were 18:4 (n-6), 20:5 (n-3), 20:4 (n-6) and 20:3 (n-7). The highest sum of PUFA was 43.87 % for summer. PUFA has been suggested as an indicator of external dietary source such as zooplankton or zooxanthellae photosynthates. It means the higher PUFA in summer than that in winter can be as a consequence of greater photosynthetic production. The bacteria and terrestrial matter are said to be positively coupled to an increase in nutrients (Bak et al. 1998). However, our findings suggest that bacterial feeding and terrestrial input, indicated from 18:1n-7 (Papina et al., 2003) and 18:2n-6 (Figueiredo et al., 2012), respectively, are higher in winter than those in summer, which was due to higher nutrient concentrations in winter than summer. The concentrations of nutrients (nitrate, nitrite, phosphate and silicate) in winter were significantly higher than those in summer (0.88  $\mu\text{M}$  vs. 0.1  $\mu\text{M}$  for nitrite, 0.8  $\mu\text{M}$  vs. 0.52  $\mu\text{M}$  for nitrate, 0.49  $\mu\text{M}$  vs. 0.25  $\mu\text{M}$  1.75  $\mu\text{M}$  vs. 2.33  $\mu\text{M}$  for silicate). Calcification rates of *A. downingi* is the highest in spring and the lowest

in winter, and intra-annual variation in calcification rate was significantly related to temperature and irradiance, but not aragonite saturation state (Vajed Samiei, et al. 2016).

Table 2: Fatty acid composition, PUFA and SFA in coral tissue

FA	Percent in	Percent in	Marker	Ref.
	Winter	Summer		
10:00	0.7±0.1	0.09±0.02	-	-
12:00	0.2±0.05	0.06±0.01	-	-
14:00	8.0±0.6	7.22±0.7	Copepods	Figueiredo et al. 2012
16:2n-6	0.7±0.1	0.40±0.02	-	-
16:1n-7	0.7±0.2	4.09±0.4	Diatoms	Dalsgaard et al. 2003
16:00	25.1±1.1	26.5±1.0	Zooxanthellae	Figueiredo et al. 2012
18:4n-3	15.2±0.9	12.0±1.2	Dinoflagellates	Papina et al. 2003
18:2n-6	2.3±0.5	2.0±0.4	-	-
18:1n-7	10.8±1.3	4.5±0.9	Green algae Terrestrial matter	Figueiredo et al. 2012; Dalsgaard et al. 2003
18:00	7.7±0.8	11.5±1.4	Bacteria	Papina et al. 2003; Imbs et al. 2007
19:00	0.2±0.1	0.2±0.3	-	-
20:4n-6	4.4±0.4	11.9±1.2	-	-
ARA				
20:5n-3	9.2±1.1	13.8±1.4	Diatoms	Dalsgaard et al. 2003
20:3n-7	2.2±0.8	2.7±0.5	-	-
20:3n-6	0.6±0.1	0.5±0.2	Zooxanthellae	Latyshev et al. 1991
20:2 n-7	0.4±0.05	0.5±0.02	-	-
20:1n-11	4.5±0.6	1.3±0.7	-	-
20:00	0.9±0.1	0.4±0.2	-	-
22:6n-3	0.07±0.2	0.07±0.03	Microalgae,Dino flagellates	Figueiredo et al., 2012
ΣPUFA	39.77	43.87	Zooxanthellae	Zhukova and Titlyanov, 2003
ΣSAFA	42.83	46.13	-	-

#### 4. Conclusion

The first screening of the FA composition of the lipids of reef-building coral *A. downingi* showed a predominance of the acids 16:0, 18:4, and 18:0; It was noted that the FA composition depends on the season. High contents of PUFA were found in summer than winter. Although, no significant correlation ( $p \geq 0.05$ ) was observed between the total lipids and ecological parameters, however, spawning, light and temperature are the parameters that could affect lipid production of the studied coral. High content of lipid was obtained in months with high underwater temperature.

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