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Variation in Levels and Location of Lipids in Corals Tissue of the ROPME Sea Area

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ABSTRACT. Histological examination was made and total lipid levels assessed in seven genera of corals from the ROPME Sea area at seven stations along the western coast. Lipids appear in histological sections as black particles 4-6 μm diameter in the gastrodermal layers, mainly in the lower half of the polyps, in mesoglea, in zooxanthellae and in eggs. The mean levels of stored lipids varied between 20.91 to 54.23% of dry tissue weight. There were significant differences among species and stations (2-way ANOVA). Lipid levels found in Arabian Gulf shallow water corals proved that oil pollution has no effect on lipid level and photosynthesis.

Introduction

Hermatypic corals possess symbiotic zooxanthellae which live inside vacuoles within the cells of the gastrodermis. These were originally grouped with the single species *Symbiodinium microadriaticum* (Freudenthal 1962). In symbiosis, zooxanthellae are single cells, spherical in shape and ca. 7-10 μm in diameter (Wilkerson *et al.*, 1988). The roles of zooxanthellae in corals are in determining the calcification rate (Goreau 1959), in recycling and conserving nutrients (Yonge and Nicholls 1931), and particularly in coral nutrition. Muscatine and Hand in 1958 revealed the first evidence of the translocation of photosynthetic products from the zooxanthelae to the host in the sea anemone, *Anthopleura elegantissima* and similar observations were made in corals (Muscatine 1967; Muscatine and Cernichiari 1969; Trench 1971a,b). The excess products of photosynthesis may be translocated to the host either as glycerol (Muscatine and Cernichiari 1969; Trench 1971a) or as lipid (Crossland *et al.*, 1980b; Patton *et al.*, 1977; Kellogg and Patton 1983). Within the host tissue, these products are further

metabolised and stored mainly as triglyceride and wax ester (Patton *et al.*, 1977; Blanquet *et al.*, 1979; Harland *et al.*, 1991). These studies also confirmed that corals could meet all their energy requirements from the translocated fixed carbon and the excess would pass to the surrounding water, probably as muco-lipids (Crossland *et al.*, 1980a; Edmunds and Davies 1986 and Sofyani 1991). Tropical corals contain about 30 to 46% of dry tissue weight as lipid (Patton *et al.*, 1977; Stimson 1987 and Sofyani 1991).

According to Knap (1987), the rate of photosynthesis of zooxanthellae of *Diploria strigosa* was reduced by 85% when it was exposed to Arabian crude oil and dispersed oil (Corexit 9527) at concentration of 19 ppm for eight hours. In the present study, experiments were therefore undertaken to assess the influence of the oil pollution on the levels of lipids stored in the Arabian corals tissue.

Materials and Methods

During the cruise of American ship (NOAA Mt. Mitchell s-222) leg V from 8 to 16 May 1992; seven sampling stations were investigated from the northern end of Qatar moving northward to Kuwait (Fig. 1). All sites were in shallow water, usually from 3 to 6 m deep.

From each station, coral samples were collected using chisel and hammer for the study of lipid content and histology. The collected materials were preserved in 7% formalin in seawater solution until its transportation to the laboratory in Jeddah.

For histological study, samples of *S. pistillata* were decalcified with a 7% solution of nitric acid. The decalcified tissues were post-fixed in potassium dichromate-osmium tetroxide solution (50 ml 2% osmium tetroxide and 50% potassium dichromate) for eight hours. Thereafter, the post-fixed samples were washed in running water for two hours and then preserved in 70% ethanol.

The preserved specimens were dehydrated in a series of strengths of ethyl alcohol, then cleared in xylene. The cleared specimens were infiltrated and embedded in paraffin wax.

Serial sections, 7 μm in thickness were cut, and then stained with Haematoxylin and Eosin.

For lipid content, the formalin fixed samples were washed in distilled water and dried at room temperature. Lipids from each branch were extracted with 15 ml chloroform : methanol (2:1 v/v) for 24 hours using a separating funnel. The solvent and lipids were then filtered through a coarse filter paper into a pre-weighed beaker. The branch and the filter paper were washed with an additional 10 ml solvent. Thereafter, the beaker contents were evaporated at 50°C in the oven overnight, whilst the nubbin was transferred to another container where a 10% solution of nitric acid was added to dissolve the coral skeleton. The decalcified tissue was washed with distilled water, dried at 60°C and weighed. Finally, the percentage of lipids in coral tissue was calculated by the following equation (Stimson 1987) :

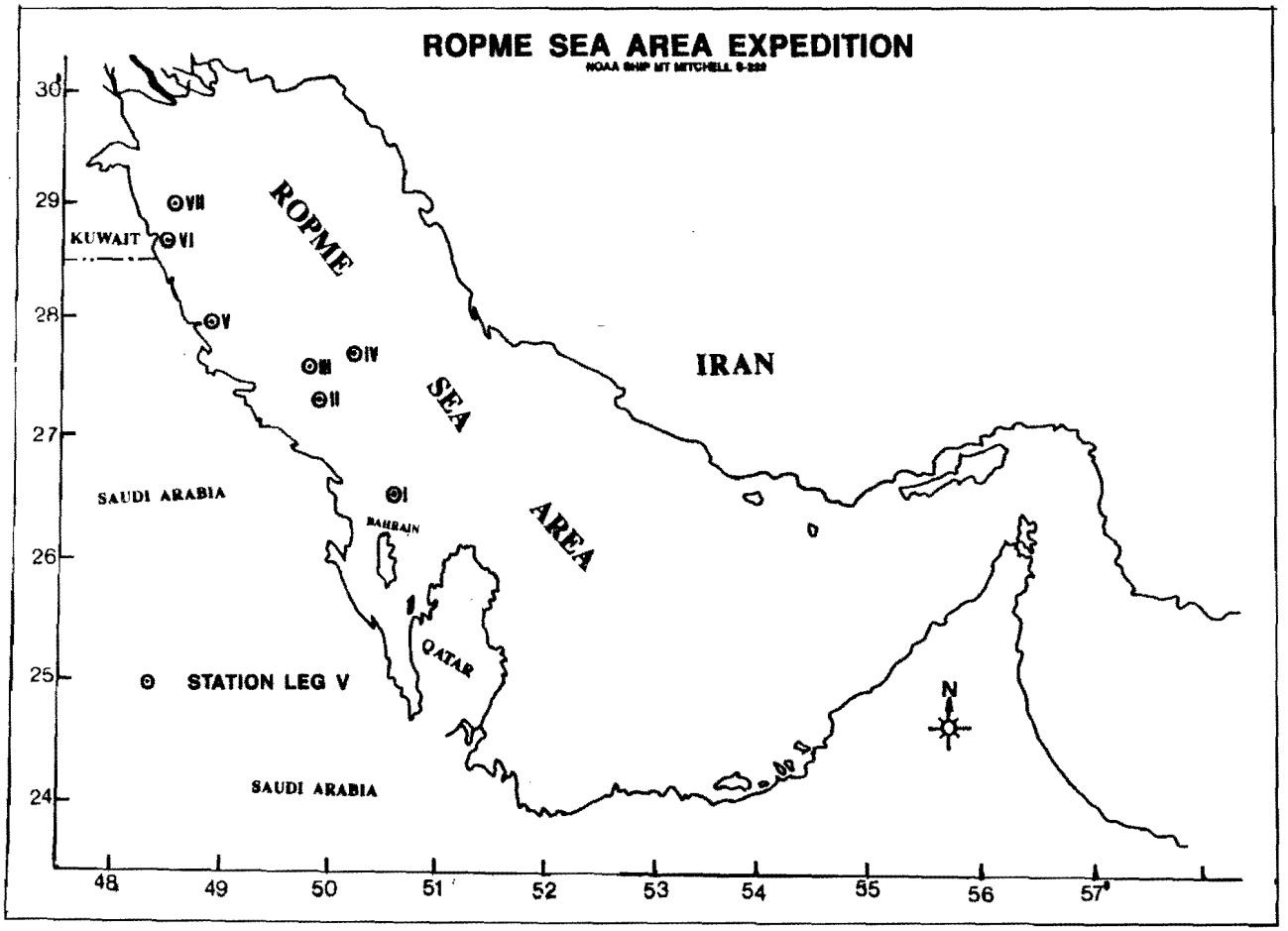


FIG. 1. ROPME Sea area and locations of study site (O), from north of Qatar to Kuwait.

$$\text{Lipid \%} = \frac{\text{Lipid weight}}{\text{Lipid wt + dry tissue wt}} \times 100$$

Results

Lipid was stained black in Osmium-Tetroxide-fixed tissue of *S. pistillata*. It was seen as black particles 4-6 μm diameter between the zooxanthellae (Fig. 2A), in the gastrodermis of the lower half of the polyp (Fig. 2B,D), in the mesoglea and in the eggs (Fig. 2C). Lipids were never observed in the epidermal layer.

Lipids as percentage of dry tissue weight, in seven species are shown in Table 1 and Fig. 3.

Species Variation

The mean lipid contents were highest in *Acropora* sp. at stations 2 and 4 and in *S. pistillata* at stations 2 and 3. They range from 51.103 ± 7.26 to 54.229 ± 1.62% and from 45.357 ± 7.94 to 49.947 ± 4.39% respectively. The lowest values were in *Platygyra lamellina* at station 4 and 7, and *Porites nodifera* at station 6, where they range from 20.907 ± 1.83 to 24.782 ± 2.24 and 27.826 ± 3.024% respectively. The other average lipid values of Arabian coral species at all stations were in the range of 31.171 ± 2.65 and 40.625 ± 4.21%. Comparing the average lipid contents between species and stations revealed very highly significant differences (Two-way ANOVA, $P < 0.0001$).

Stations Variation

Acropora sp.

The means of lipid contents were higher at station 2 and 4, they are 54.229 ± 1.62 and 51.103 ± 7.26%, whilst the lowest was at station 6(34.142 ± 3.954%). At station 3 and 6, the values were 34.953 ± 1.89 and 34.142 ± 3.954% respectively.

Favites sp.

The average lipid content was 33.28 ± 5.0% of dry tissue weight at station 7.

Platygyra lamellina

The mean values of lipids were higher at station 3 (38.756 ± 1.83%) and lower at station 4 and 7(24.748 ± 2.24 and 20.907 ± 1.83%) respectively. Other stations showed slight variation in the mean values of lipid which range between 31.171 ± 2.65 and 36.43 ± 1.03%.

Pocillopora damicornis

The mean lipid concentration in the colony tissue was in the range of 35.75 ± 4.58 to 43.124 ± 4.48%.

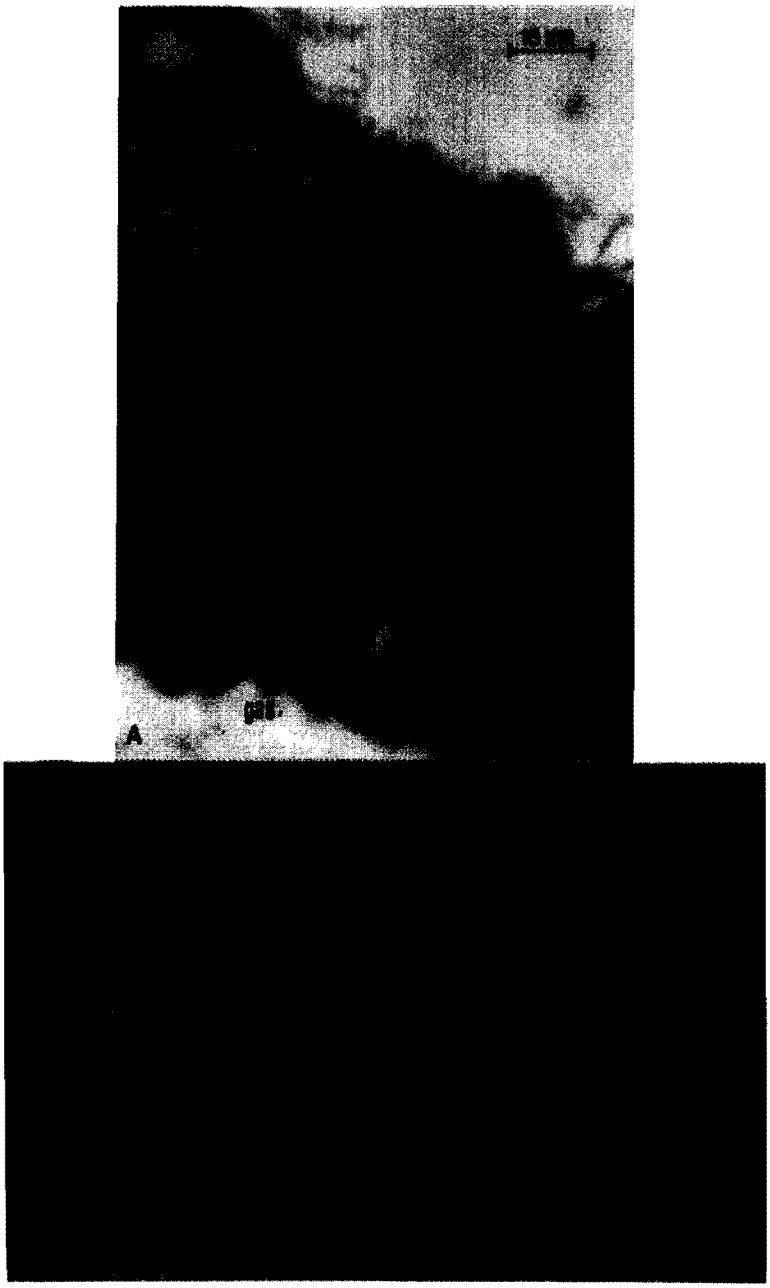
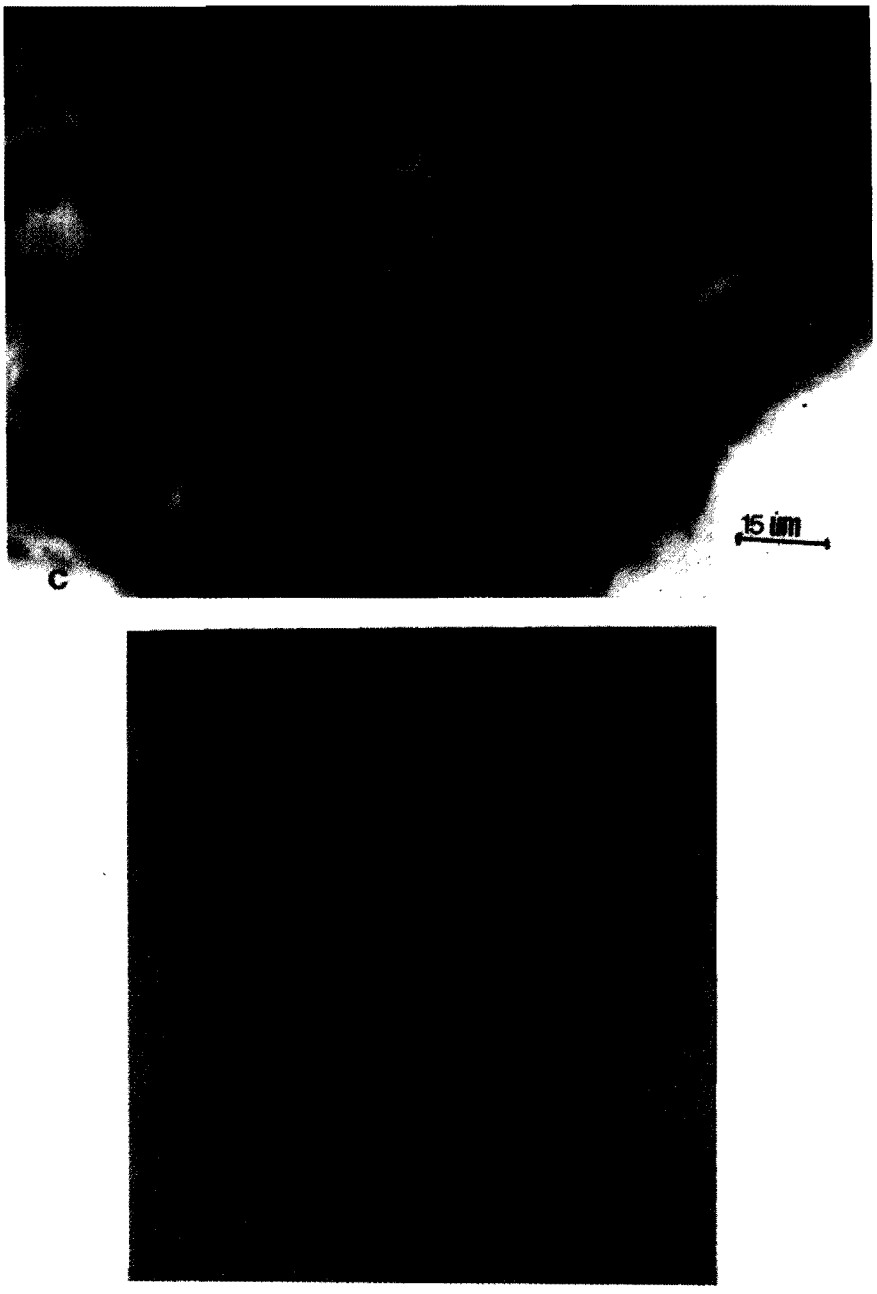


FIG. 2. Transverse and longitudinal section through the coenosarc (A), lower part of the polyp (B,D), Oocyte (C) of *S. pistillata*.



Note that in the coenosarc lipid appears as droplets in the gastrodermis, and the mesoglea, whilst there is no lipid evident in the epidermis.
Li., lipid droplet; epi., epidermis; gas., gastroderms; zox., zooxanthellae; mg., mesoglea; O, Oocyte.

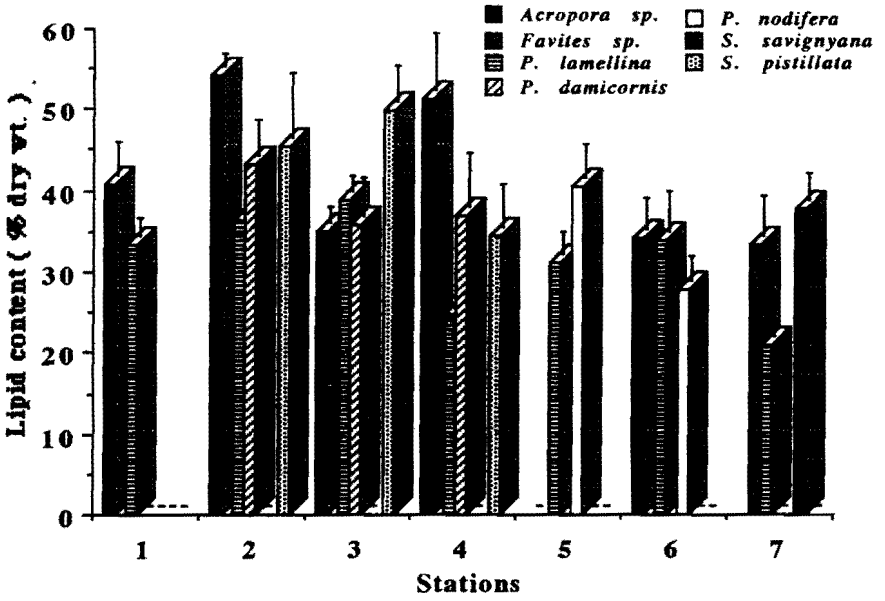


FIG. 3. Variation in lipid contents, expressed as % dry tissue weight at different stations along the western coast of ROPME sea area in seven coral species.

TABLE 1. Comparison of lipid levels (% of dry tissue weight) of corals collected from seven stations along the western coast of ROPME Sea area. The mean values of lipids were significantly different (Two-way ANOVA):

Between stations $F_{6,36} = 22.39$ $P < 0.0001$
Between species $F_{6,36} = 102.23$ $P < 0.0001$

Stations	1	2	3	4	5	6	7
Acropora sp.	40.625 ± 4.210(11)	54.229 ± 1.620(10)	34.953 ± 1.890(10)	51.103 ± 7.261(10)		34.142 ± 3.9549(10)	
Favites sp.							33.281 ± 5.000(10)
P. lamellina	33.360 ± 2.120(8)	36.431 ± 1.033(9)	38.756 ± 1.893(9)	24.748 ± 2.240(10)	31.171 ± 2.657(10)	33.805 ± 5.060(10)	20.907 ± 1.830(9)
P. damicornis		43.124 ± 4.480(10)	35.750 ± 4.582(10)	36.871 ± 6.554(9)			
P. nodifera					40.593 ± 4.088(10)	27.826 ± 3.024(10)	
S. pistillata		45.357 ± 7.940(10)	49.947 ± 4.390(10)	34.457 ± 5.280(10)			
S. savignyana							37.584 ± 3.456(10)

Porites nodifera

The average lipid contents were higher at station 5(40.953 ± 4.08%) and lower at station 6(27.826 ± 3.02%).

Stylophora pistillata

The mean lipid contents at stations 2, 3 ,4 ranged from 34.457 ± 5.28 to 45.357 ± 7.94%.

Siderastrea savignyana

The mean value of lipids was 37.584 ± 3.45% of dry tissue weight.

Discussion

The location of lipid in the eggs, the mesoglea, and the gastrodermal cells, especially those in the lower part of the polyp, is very similar to that showed for Hawaian corals (Stimson 1987) and Red Sea corals (Sofyani 1991). No lipids were observed in the epidermal layer in this study. Similar result was observed in Red Sea corals (Sofyani 1991). Crossland *et al.* (1980a) and Crossland (1987) showed that excess carbon fixed during photosynthesis of corals in daylight leaves the colony as muco-lipid. In the view of the absence of lipid from the epidermal layer, it seems likely that this would be released from the coelenteron.

In the present study, histological examination indicated no damage to the tissue and zooxanthellae of *S. pistillata*. Peters *et al.* (1981) observed that the tissue and the zooxanthellae of *Manicina areolata* were degenerated when it was exposed to petroleum hydrocarbons for three months. In addition, Loya and Rinkevich (1980) reported mortality to corals from the Gulf of Aqaba due to oil pollution.

The quantitative variations of lipids among ROPME Sea corals are similar to that showed in lipid concentrations between several coral species in Hawaii (Stimson 1987). In this study, the mean values of 20.91% to 54.23% lipid on dry tissue basis among ROPME Sea corals are similar to the values of 21.42% to 46% (Bergmann *et al.* 1956, Patton *et al.* 1977, Stimson 1987 and Sofyani 1991). The differences in lipid contents among species and station may be related to a lower irradiance level, a lower sea water temperature, and high sedimentation rate, which reduce photosynthesis production (Harland *et al.*, 1992; Davies 1991; Sofyani 1991; and Abdel-Salam and Porter 1988). In addition, planulation may reduce lipid reserves (Stimson 1987). In the light of the oil pollution after the Gulf war, it was shown that the photosynthesis of zooxanthellae of *Diploria strigosa* declined by 85%, when it was exposed to 19 ppm of Arabian light crude oil dispersed with Corexit 9527 (Knap 1987). However, it seems that ROPME Sea corals showed no reduction in the quantity of lipid which would be related to petroleum hydrocarbons. This study only shows results of eight days cruise in ROPME Sea area.

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اختـــلاف مستويات الدهـــون ومواقعها في أنسجـــة مراجـــين المنطقـــة البحريـــة للمنظمـــة الأقليمـــة لحمايـــة البيئـــة البحريـــة

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المستخلص . أظهرت الفحوص الهيستولوجية المعملية وقياسات مستويات الدهون الكلية لسبعة أجناس من مراجين المنطقة البحرية للمنظمة الاقليمة لحماية البيئة البحرية عند سبع محطات على طول منطقة الساحل الغربي للخليج العربي ، أن الدهون تظهر في القطاعات المجهرية كحسيبات صغيرة سوداء يتراوح قطرها من ٤ - ٦ ميكرون في مناطق الكاستروديرم خاصـة في النصف السفلي من الحيوان ، وفي الهلام المتوسط ، وفي الطحالب التكافلية (الزوكزنتيلي) ، وفي البيض .

بينما يتراوح متوسط مستوى الدهون المخزونة في أنسجتها مابين ٢٠,٩١ إلى ٥٤,٢٣٪ من الوزن الجاف .

كما أظهرت النتائج الإحصائية أن هناك اختلافات واضحة في كمية الدهون المخزونة بين الأنواع والمحطات (التحليل التبايني الثنائي) .

هذا وتشير النتائج إلى أن كميات الدهون المخزونة في أنسجة عينات المراجين المأخوذة من منطقة الخليج العربي ، في مستواها الطبيعي . وهذا يدل على أن التلوث البترولي لم يؤثر على مستوى هذه الـدهـون ولا على التمثيل الضـوئي للطحـالب . ويمكن القول بأن الحيوانات المرجانية لم تتأثر بحرب الخليج حتى الآن .