# Comparative Analysis of Lipids in a Stony Coral (*Pocillopora damicornis*) and a Sponge (*Acanthella carteri*), along Obhur Creek, Jeddah Coast, Kingdom of Saudi Arabia

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**KEYWORDS**

*Obhur Creek, lipid types, coral, Jeddah Coast, Spectroscopy.*

**ABSTRACT**

A comparative study of various types of lipids in a coral Pocillopora damicornis and a sponge Acanthella carteri was carried out. Samples were collected from the Obhur Creek of the Jeddah coast. The lipids were separated by column chromatography on silica and the individual class of lipids was estimated using standard procedures of spectroscopy. It was observed that phospholipids were the dominant in both the organisms (approximately 50% of the total lipids) followed by sterol lipids (9.50 mg g-1 in sponge and 2.70 mg g-1 in the coral. Triglycerides were found to be more in the sponge compared to coral (6.85 mg g-1 and 1.90 mg g-1, respectively). However glycolipids were higher in coral (1.30 mg g-1) compared to the sponge (0.45 mg g-1). The total lipids in the sponge were found to be higher compared to the coral.

# دراسه مقارنه لتحليل الدهون في المرجان الصخري *بوسيلوبورا داميكورنس* (*Pocillopora damicornis*) والإسفنج *أكانثيلا كارتيري* (*Acanthella carteri*) علي طول شرم أبحر ساحل جده، المملكة العربية السعودية

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**المُستلخص**

تم إجراء دراسة لمقارنه الدهون المختلفة في المرجان بوسيلوبورا داميكورنس (*Pocillopora damicornis*) والإسفنج أكانثيلا كارتيري (*Acanthella carteri*) . جمعت العينات من شرم أبحر علي ساحل جده. تم فصل الدهون بواسطة أعمدة الفصل الكروماتوجرافي علي السيلكا وتم تقدير الأنواع منفصله بواسطه طرق قياسيه للتحليل الطيفي. الدهون الفوسفاتية كانت هو الشكل الغالب والمسيطر في كلا الكائنين (تقريبا (50 %) من إجمالي الليبيدات الكلية). يليها الدهون الإسترواليه حيث كان تركيزها في الإسفنج 9.50 مجم/جم في حين كان التركيز في المرجان 2.70 مجم/جم. الجلستيريدات الثلاثية وجد أنها أكثر في الإسفنج مقارنة بالمرجان (6.85 مجم/جم) و (1.90 مجم/جم) علي التوالي. الدهون الجليكولية كانت أعلي في المرجان (1.30 مجم/جم) مقارنةً بالإسفنج (0.45 مجم/جم). إجمالي كمية الدهون الكلية في الإسفنج كانت أعلي من المرجان.

**الكلمات الدالة**

*شرم أبحر، أنواع الدهون، المرجان، الإسفنج، ساحل جده، الطرق الطيفية*

## Introduction

Lipids are an integral part of living organisms. They play a vital role in the biochemistry of any plant or animal. They are considered as the storer of energy. Their quantity and composition depends on marine ecological conditions (salinity, temperature, pH and pollution etc.). These lipids can change to improve the defensive functions of the cell membrane. Phospholipids are the components of the cell wall which encloses the cytoplasm and other contents of the cell and determine the permeability. Glycolipids are more in the photosynthetic zone and in cases of corals they are more in the zooxanthella (symbiont algae) than in the coral itself; they are more in the plant membrane particularly in the plants existing in the photosynthetic zone. Sterol lipids have their role as hormones and signaling molecule. In addition, these compounds have their own importance in cosmetics, food industries and in nanotechnology (Mashaghi, *et al*. 2013). They also play a vital role in the production of hormones and storage of vitamin ADEK (Michael, *et al*. 2009).

The coastal zone of Jeddah provide habitat for an array of marine organisms including sedentary organisms like sponges and corals. Very little work has been done in this part of the world on these types of organisms. Since lipids play an important role in the biochemical activities of the living system, it was desirable that a comparative study of various types of lipids in these two organisms be made. Two organisms; sponge *Acanthella carteri* and a coral *Pocillopora damicornis* were chosen for this purpose. Several groups of workers have studied the analysis of lipids in the marine organisms. The impact of change in temperature and seasonal variations on *Pocillopora damicornis* along the Hawaiian sea shore was investigated (Imbs, 2013). Similar types of studies were carried out on the same species near the Western Australian Coast (Ward, 1995). We considered this an interesting point to compare their results of lipid content with the same coral in this area of Jeddah sea shore.

## Materials and Methods

Samples of the coral *Pocillopora damicornis* and sponge *Acanthella carteri* were collected by Scuba Divers at the mouth of the Obhur Creek along the Jeddah coast, during November 2012. The samples were collected at a depth of 5 meters. They were brought in an ice box, thereafter they were kept in the laboratory in the freezer. These organisms were taxonomically identified by a marine taxonomist (Al-Sofyani, 2012), (figure 1). The salinity of sea water was 41%, the pH was 8.2 and the temperature was 29°C during the sample collection time. The samples were washed with seawater, ground into ~2 mm pieces. The specimen were dissolved in a mixture of MeOH:$CHCl_3$:$H_2O$ (8:4:1, v/v). It was stirred at room temperature for 24 hours and filtered (Whatmann Filter Paper No: 40). The filtrate was evaporated under vacuum in a rotary evaporator (temperature < 40°C) and finally under a stream of nitrogen. The crude organic gummy material of the coral and sponge were 5.89 and 11.50%, respectively, based on dry weight of the organisms.

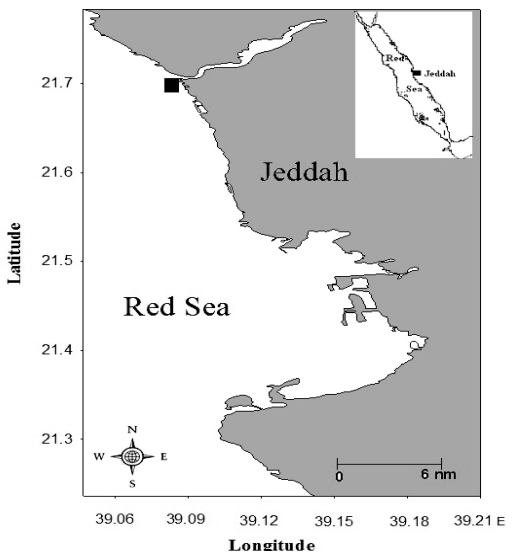


**Figure 1:** Coast of Jeddah, KSA (*Samples were collected from the Area Indicated by Black Square*).

**(1) Estimation of Lipids (Gravimetric Method)**

50g of the tissue was homogenized with methyl alcohol (100 ml) and chloroform (200 ml). It was shaken in a Warren Blender for 15 minutes. The mixture was filtered and the filtrate was mixed with 75 ml of 0.88% solution of KC1 in distilled water. It was shaken thoroughly and allowed to settle and then transferred to a separating funnel. When settled, two layers were distinctly visible. Upper layer constituted water and methanol (it

contains the non-lipid fractions). The lower layer constituted chloroform and methanol (it contains mainly lipids). The two layers were separated. The lower layer was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated under vacuum and the concentrate was further evaporated under a stream of nitrogen (Folch *et al.,* 1957) and weighed on an analytical balance.

**(2) Separation of Lipids**

Various types of lipids were separated by column chromatography on silica (100-200 mesh). Total lipids extract (10g) was applied to a chromatography column containing silica (100 g, 100-200 mesh). The column was eluted with increasing order of polarity, using hexane-chloroform, acetone and methanol (150 ml each). The hexane fraction contained pigments, hydrocarbons and terpenoids etc. The chloroform fraction contained triglyceride and sterol lipids. The acetone fraction contained glycolipids. The last fraction eluted with methanol contained phospholipids (Barnathan *et al.,* 2003).

**(3) Estimation of Phospholipids**

Phospholipids are estimated spectrophotometrically by making a complex with ammonium molybdate. This reagent was prepared by dissolving ammonium molybdate (4.4g) in water (0.40 l), and concentrated sulfuric acid (14ml) was added to it gradually to make up one liter water solution. Second reagent was a reducing reagent. It was prepared by dissolving sodium biosulfite (2.5g), sodium sulfite (0.5g) and 1-amino naphthol sulfuric acid (0.4g) and dissolved in distilled water (250ml). Standard solution for comparison was sodium dihydrogen phosphate. (0.12 g of the compound was dissolved in one litre of distilled water). Lipid sample (10mg) was added to perchloric acid (400 ~ 70%) and the contents were heated under reflux for 20 minutes. The solution was cooled and ammonium molybdate reagent (2.4ml) was added followed by reducing agent (2.4 ml). The solution was mixed thoroughly and heated on a boiling water bath for 10 minutes till a blue color appeared. On cooling the absorbance was recorded at 830 nm with sodium dihydrogen phosphate as a standard (Dimidris, 1970).

**(4) Estimation of Sterol Lipids**

Lipid sample (10mg) was dissolved in Bloor Reagent (ethanol: ether, 3:1) and evaporated to dryness on a water bath. Ferric chloride reagent (5ml) was added. This reagent was prepared by dissolving ferric chloride (1.5g) in one liter of acetic acid. It was mixed well on a shaker. Concentrated sulfuric acid (3 ml) was added to the vortex. Pink color was developed. Cholesterol standard was prepared by dissolving it in chloroform so that (1 ml = 1 mg), it was evaporated to dryness and the reagent was added the same way as above. After 10 minutes the absorbance was recorded at 560 nm (Zlatkis, 1953).

**(5) Estimation of Glycolipids**

Glycolipids were estimated by anthrone reagent method using glucose as a standard solution. Anthrone (0.2gm) was added to ethanol (8ml). Sulfuric acid, 80% solution (10ml) was added with cooling, so that the solution does not become hot. The rest of sulfuric acid (90ml) was gradually added. This reagent was added to the lipid sample (10mg dissolved in 1 ml dimethylformamide) and heated on a water bath for 10 minutes, till a blue color appeared. It was cooled and the absorbance was recorded at 625 nm (Jermyn, 1975) and compared against the absorbance of glucose standard solutions.

**(6) Estimation of Triglycerides**

The following reagents were prepared for triglyceride estimation for each sample:

(i) Heptane (2 ml), isopropanol (3.5 ml) was add to sulfuric acid (1 ml, 0.1N).

(ii) Potassium hydroxide solution (6 M) was prepared in distilled water.

(iii) Glacial acetic acid (5ml) was added to water (95 ml) and then sodium meta periodate (0.6 gm) was added.

(iv) Ammonium acetate solution (2 M) was prepared in distilled water and kept at -2$^{o}$C.

The lipid sample (10 mg) was taken up with: (i) reagent (5 ml) and mixed on the vortex for 10 minutes. The heptane layer was transferred to another tube. Reagents (ii), (iii) and (iv) were added to the tube (2 ml each). The contents were heated on boiling water bath for 10 minutes. A

yellow color appeared. It was cooled and the absorbance recorded at 425nm. Triolein was the standard solution. It was prepared by dissolving triolein (100 mg) in isopropanol (100 ml). It was further diluted to three, four and five times with isopropanol (Gottfried and Rosenberg, 1973).

## Results

Different types of lipids were extracted by ultra violet spectroscopy and the molybdate method for the estimation of phospholipids indicated the presence of this type of lipid in coral *Pocillopora damicorins* (7.16 mg $g^{-1}$; dry wt. of coral) and sponge *Acanthella carteri* (18.65 mg $g^{-1}$). The total lipids were found to be more in the sponge compared to the coral, the main reason being the bulk of the coral is a calcareous matter. Phospholipids were found to be the major component of the classes of lipids in both the organisms i.e. approximately half of the total lipids (table 1 and figure 2). Sterol lipids were determined by ferric chloride method. In coral, it was found to be 2.70 mg $g^{-1}$ (19.25% of the total lipids) and in sponge it was 9.50 mg $g^{-1}$ (26.79% of the total lipids,). Triglycerides showed a similar pattern (i.e. it was more in the sponge compared to the coral). *P. damicornis* showed the presence of triglycerides at 1.90 mg $g^{-1}$ (13.55 % of total lipids) while 6.85 mg $g^{-1}$ (19.32% of total) were found in *A. carteri* (Table 1 and Figure 2). Glycolipids showed higher values in the corals (1.30 mg $g^{-1}$; 9.27 % of the total lipids) and was less in the sponge (0.45 mg $g^{-1}$; 1.26 % of the total lipids).

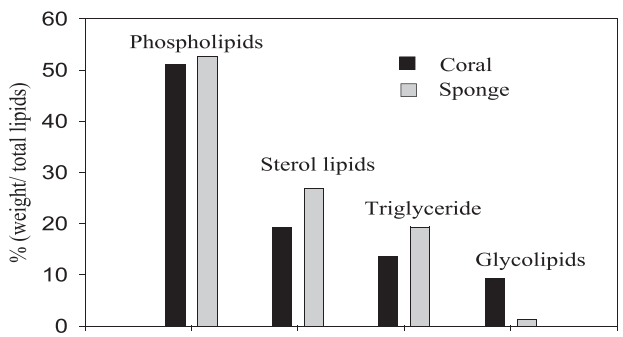


**Figure 2:** Percentage (weight/total lipids) of Various Types of Lipids in Coral *Pocillopora damicornis* and sponge *Acanthella carteri*.

**Table 1:** Lipid Composition in a Coral (*Pocillopora damicornis*) and Sponge (*Acanthella carteri*) (Dry Tissue wt.).

| Type of Lipid | *Pocillopora damicornis* (mg $g^{-1}$) | *Acanthella carteri* (mg $g^{-1}$) |
|---|---|---|
| Phospholipids | 7.16 | 18.65 |
| Sterol lipids | 2.70 | 9.50 |
| Triglycerides | 1.90 | 6.85 |
| Glycolipids | 1.30 | 0.45 |
| Total | 14.02 | 35.45 |

## Discussion

One can realize that there is symbiont algae with the corals and it occurs in a clear photosynthetic zone (euphotic zone), that explains the fact that it was more in coral compared to the sponge. Genin and co-workers (2008) studied the phospholipids in 22 different species of marine algae from various seas; although in most of the cases it was the major component but they also stated that no clear rule on phospholipids composition in marine sponges can be stated to date. Lipids have a variety of biological functions due to their diverse structure; the most important is that they are the biological components of all cell membranes (apart from their role as storer of energy). Glycolipids are involved in the signaling metabolic pathways. Sterol lipids are membrane reinforces. *Pocillopora damicormis* is a stony coral; the mass is predominantly a calcareous skeleton (sceleractinian) while *Acanthella carteri* is an orange color sponge. The crude extract of this sponge was found to be associated with antimicrobial activity (Ali *et al*., 2000). Estimation of the total lipids by Folch method indicated the amount of lipids to be 35 mg $g^{-1}$ in the sponge. While in the coral *Pocillopora damicormis* this amount was found to be 14.02 mg $g^{-1}$ This is plausible in the sense that the coral is mainly a calcium carbonate mass (Chauve *et al*., 1972) while in the sponge it is mainly organic tissue. Further the glycolipids were seven times higher compared to the sponge. One can anticipate this as there is a symbiotic algae in the coral and the marine organism is in the photosynthetic zone. When the symbiont alga (zooxanthellae) of this coral *Pocillopora damocornis* near the coast of Hawaii was subjected to lipid analysis, it

was found to contain 140 pg per cell in the coral (Achituv *et al*., 1994). Phospholipids constituted approximately half of the total lipids (both in the coral and the sponge). When eight species of sponges were examined along the Kenyan coast, the phospholipids were found to be dominant (Marsden, 1975). Glycolipids were found to be low in the organisms. A group of Bulgarian scientists studied the lipid content of the sponges near Canary Island (Nechev *et al*., 2004) and they extracted the lipids by solvent extraction and stated the total lipids to be 4.50% (total weight of the sponge) whereas our present investigation of *A.carteri* indicated the total lipids to be 3.55% (total weight of sponge). Some of the corals belonging to the genus *Millepora* were examined in the Russian coast and it was found that glycolipids were low compared to the other classes of lipids. It was observed that when *Pocillopor*a species were subjected to increase in temperature, it resulted in the exodus of the zooxanthellae thereby causing partial bleaching of the coral (Harley *et al*., 2006). Recently a study of the total lipid content of *Pocillopora damicornis* was investigated along the Hawaiian coast under different environmental conditions (Imbs, 2010). It was observed that the total lipid content ranged from 21 mg $g^{-1}$ to 38 mg $g^{-1}$ under different conditions whereas our present studies indicated reduced quantity of the lipids i.e. 14.02 mg $g^{-1}$. The low lipid content indicates the slower rate of metabolism of the organism, this can be due to higher temperature and reduced pH values. It has been noted that the temperature is gradually increasing in the Obhur Creek and after a couple of decades it may result in coral bleaching in this area. These corals act as hatcheries of the fish life so the problem is to be addressed carefully before it becomes difficult to be controlled. Less lipid content indicates more coral is more prone to bleaching.

## Conclusions

A comparative study of various types of lipids was carried out. A coral *Pocillopora damicornis* and a sponge *Acanthella carteri* were selected for this purpose. A sum of total lipids indicated that the sponge contained two and a half time more lipids than the coral. Greater lipid amount indicates more metabolic activities in the body of the marine organisms, and with cell membrane. Lipids are multifunctional in their activities in the biological systems. More glycolipids in the coral compared to the sponge can be due to the symbiont algae. However the percentage of phospholipids was maximum in both the organisms. Due to calcification process, one would expect less organic matter (consequently less lipids) in the corals compared to sponge (Young, 1973). Since Red Sea is experiencing higher temperature and salinity regimes the results will serve as baseline for understanding the metabolic activity of the corals and sponges .Coral bleaching is a phenomenon of global concern. The present study and a general survey of the area indicate that this process is also affecting the marine fauna. Lower lipid content would reduce the metabolic processes and this problem is to be addressed at an early stage.

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