Seasonal Variations in the Biochemical Components of Two Species of Hard Corals *Pocillopora damicornis* and *Pocillopora verrucosa* in the Red Sea

Yahya A. M. Floos
Yahya-floos2008@yahoo.com

Department of Marine Biology & Fisheries, Faculty of Marine Science & Environment, Hodiedah University, Republic of Yemen

Abstract

In this study two species of hard coral *Pocillopora damicor*nis and *Pocillopora verrucosa* were collected from the Ubhur Creek, coastal waters of Jeddah, Saudi Arabia to determine variation in the biochemical components (Protein, Carbohydrate, Lipid and Chlorophyll) between different species of hard coral and seasons that may reflect differences in their feeding strategy. This information is important in the investigation of their metabolic pathways and their mode of feeding. In *P. damicornis* the protein content was higher in winter than in summer, the same pattern was observed for *P. verrucosa*. In both species carbohydrate content was lower in summer than in winter. In *P. damicornis* the lipids content was 4 times higher in summer than in winter, whereas in *P. verrucosaat* it was approximately 5 times as high in summer compared to winter. In *P. damicornis* in winter there was lower variation in the chlorophyll "a" than in summer, the same pattern was observed for *P. verrucosa*. All these finding indicate that *P. damicornis* and *P. verrucosa* are predominantly dependant on autotrophic feeding **.**

Key words: coral reef , feeding , zooxanthellae, biochemical component

Introduction

Most reef-building corals contain photosynthetic algae, called zooxanthellae, that live intra cellular, residing in host derived vacuoles in the endodermal cells of the host that line the gastro vascular cavity (Barnes and Hughes, 1999; Farmer *et al*. 2001). In the relationship between scleractinian (hard) corals and zooxanthellae, a microscopic

dinoflagellates algae (*Symbiodinium microdriaticum*) have a mutualistic relationship, that are present at extremely high densities in the host (Muscatine and Porter 1977; Barnes, 1987; Lalli and Parsons, 1995; Barnes and Hughes, 1999). Most importantly, zooxanthellae supply the coral with glucose, glycerol, and amino acids, which are the products of photosynthesis. The coral uses these products to make proteins, fats, and carbohydrates, and produce calcium carbonate. In turn, the zooxanthellae receive vital inorganic nutrients from the coral host, which are passed along to the zooxanthellae as animal waste products. Some inorganic nutrients are also obtained from seawater. (Barnes 1987; Barnes and Hughes 1999; Lalli and Parsons 1995**).** Densities of symbionts may fluctuate over time in responseto seasonal variables such as irradiance, and temperature (Fagoonee *et al*. 1999 and Fitt *et al*. 2000). Internally, *Symbiodinium* cells possess a large nucleus containing permanently condensed chloroplast (s) with a spiked pyrenoid (housing enzymes for CO2 fixation), and pigments including chlorophylls *a* and *c*, peridinin and diadinoxanthin. Zooxanthellae reproduce asexually, by mitotic division when in the coccoid state only; the growing evidence also suggests that sexual recombination can occur (Stat *et al*. 2006).

The host relies on its dinoflagellate symbionts for survival; they release substantial amounts of photosynthetically fixed organic compounds (‘photosynthetic’), meeting up to 95% of the hosts energy requirements (Muscatine 1990; Wang and Douglas 1998). Heterotrophy contributes most to the material needs (nitrogen compounds), while photoautotrophy contributes to the energy and carbon demand of the coral (Piniak and Lipschultz, 2004; Houlbreque and Ferrier-Pages, 2008 and Sawall, *et al* 2011). Photosynthate consists largely of low molecular weight, energy rich compounds such as glycerol and glucose, almost all essential amino acids, organic acids, and, arguably, lipids (Markell and Trench 1993; Muscatine and Kaplan 1994). In return, the host provides carbon dioxide for photosynthesis and supplies metabolic waste products (containing nitrogen and other elements including phosphorus) to the symbiont. The symbionts recycle the nitrogen by incorporating it into amino acids, which are then, translocated back to the coral for using in respiration. These nutritional interactions promote the growth and development of coral reefs in nutrient-poor tropical seas (Muscatine and Porter 1977), with energy from the symbionts also assisting in

coral calcification and hence in the formation of the reef framework (Pearse and Muscatine 1971; Hoegh-Guldberg and Fine 2004).

The nature of the lipids in coral tissues is related to the mode of nutrition. Corals with predominant unsaturated fatty acids relied more on plankton capture, while corals with abundant saturated fatty acids relied more on the photosynthetic products translocated from their zooxanthellae (Meyers, 1979). Oku (2003) observed in coral *Montipora digitata* the low molecular weight compounds; sugars and amino acids once translocated from zooxanthellae to host cell were metabolized towards lipogensis as well as glycerol production. A Russian group of workers (Papina and vanWoesik 2005) studied it that when zooxanthellae were separated from the host animal and their fatty acids were determined separately by GC-MS. It was revealed that the zooxanthellae had a higher percentage of unsaturated fatty acids, so in short it is a point of controversy among the scientists that it is not always necessary that symbiotic algae would always reveal the formation of saturated fatty acids. A comparative light-dark feeding study of the coral *Galaxea fascicularis* showed that in dark the metabolic requirement of the zooxanthellae were in part met from the animal host through a heterotrophic mode of nutrition (Al-Moghrabi *et al*., 1995), so it is difficult to anticipate that the unsaturated compounds would be predominantly from the host cell, an element of uncertainty is observed.

Most of the coral's energy budget is made up of lipids from its symbionts (Falkowski *et al*., 1993). This is the reason that zooxanthellae corals can do so well in highly oligotrophic waters. The occasional prey that come into contact with coral host's tentacles supply sufficient protein for the corals to grow and reproduce. Factors that reduce the flow of lipids from the symbionts ultimately cause stress in the coral host. Stressed corals grow more slowly (Koop *et al.,* 2001).

The purpose of the present study is to determine variation in the biochemical components (Protein, Carbohydrate, Lipid and Chlorophyll) between different species of hard coral and seasons that may reflect differences in their feeding strategy.

Material and methods

The study area was selected within the Ubhur Creek, 35km north of Jeddah. The Creek runs in a SW-NE direction for about 9.3km and has an average width of about 500m. An area of reef was chosen for the study on the north side, just inside the

entrance of Creek (Figure 1). The reef edge is in about 1m of water and the reef front descends steeply to the sand at the base of the channel, bare rocks characterize the inshore zone occupying the first 15 m from the shoreline and dead coral fragments.

Corals were collected from the fringing reefs of Ubhur Creek during August 2009 and February 2010. Fresh samples were used each time and the top 2cm portion of the branch (nubbin) was involved in the analysis

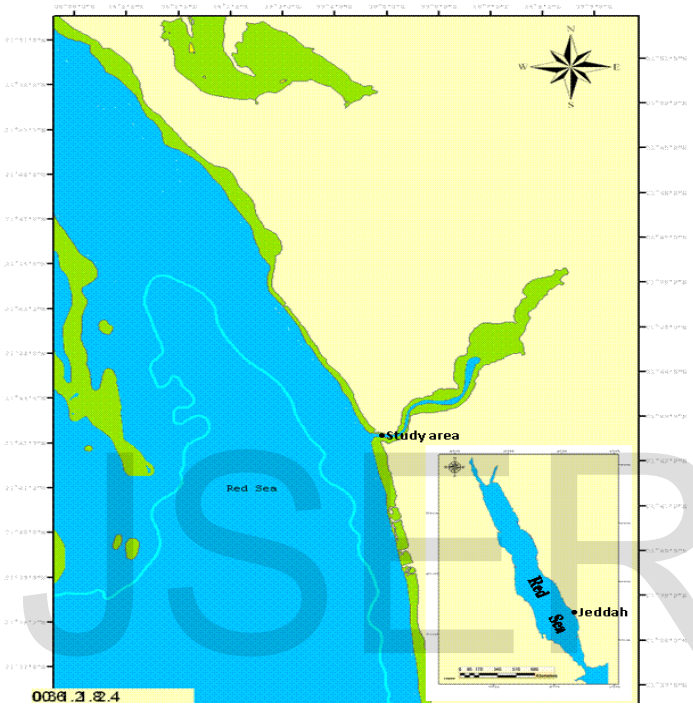

Figure. 1 Red Sea map and location of Ubhur Creek which lies in North of Jeddah City. The Study Site is Marked as (●) at the Entrance of Ubhur Creek.

Skeleton and Biomass Characteristics of the Two species

The skeleton density of *P. damicornis* and *P. verrucosa* were obtained by removing the tissue from the skeleton. A group of twenty nubbins from *P. damicornis* and *P. verrucosa* were left in 10% solution of chlorox and seawater for two days. The skeleton was then, cleaned with a water jet to remove any residual tissue. Their buoyant weights were recorded in seawater and its density was measured immediately before buoyant weighing the skeleton. Thereafter, the skeletons were rinsed in distilled water and dried to a constant weight at 60°C. The skeleton density was then calculated from the equation of Davies (1989). Skeletal weight and dry tissue weight were obtained by a group of 20 nubbins which were buoyant weighed and then fixed in 7% formalin, decalcified in 10% nitric acid and then dried the resultant tissue to constant weight at 60°C. The dry tissue weight was related to dry skeletal weight and expressed as mg dry

tissue wt/g skeletal wt. Skeletal weight and number of zooxanthellae were calculated by fixing, the nubbins in formalin, decalcifying in 10% nitric acid and then homogenizing the distilled water-washed resultan tissue with a hand-held potter homogenizer. After which the suspension was centrifuged and distilled water washed three times. They were resuspended in 1 mL of filtered distilled water and zooxanthellae concentrations within the cell were measured on a sub sample using a haemacytometer. The relationship between the zooxanthellae number and the dry tissue weight was estimated indirectly. Aluminum foil was fitted carefully on to the surface of each nubbin. The fitted foil was then weighed and a value for surface area derived, in order to obtain a relationship between surface area and skeletal weight; active coral reef building greatly diminishes below a depth of 3m (Levinton 1982). Chlorophyll was determined by dissolving it in 90% aqueous acetone and measuring the absorbance at 665 nm and 645 nm for 'a' type chlorophyll, respectively (Lichtenthaler *et al.*1987). Lipids were extracted using a modified Folch method (Folch *et al.* 1957). Estimation of the carbohydrates using the anthrone method (Hewitt 1958) was slightly modified, absorbance being recorded at 625nm. Standard solution of glucose were treated, and a calibration curve was drawn. Proteins were estimated by Lowry's method 1951 using egg albumin as a standard.

Results

Skeleton and Biomass Characteristics of *P. damicornis* and *P. verrucosa* are shown in (Table 1). The mean density of the skeleton of both species is identical at 2.78±0.018 (12) $g.cm^{-3}$ and 2.77 ± 0.026 (11) $g.cm^{-3}$ respectively. The mean surface area per g. skeleton is higher in *P. damicornis* 5.36±0.93 **(9)** cm^2 .g skeleton than *P. verrucosa* 3.76±0.66 (10)cm^2.g skeleton and is significantly different (t-test $P \leq 0.05$).The mean dry tissue weight per g. skeleton is higher in *P. damicornis* 28.1 ± 13.43(9) $mg.d.t.g^{-1}$ skeleton than *P. verrucosa* 24.92 ± 5.7 (10) $mg.d.t.g^{-1}$ skeleton but there is no a significant difference in relative biomass between the two species. The values remain significantly different when they are expressed on a surface area basis, i.e. 6.92±2.59 (10) $mg.d.t.cm^{-2}$ higher in *P. verrucosa* than *P. damicornis* 5.33 ± 2.74 (9) $mg.d.t.cm^{-2}$ (Table 1).

The contents of biochemical components in *P. damicornis* and *P. verrucosa* were determined (Table2) and (Fig.2 to 5) The difference can be seen in concentration among Protein, Carbohydrate, Lipids, Chlorophyll "*a"* in both summer and winter.

P. damicornis

The average rate of protein was higher 4.69±0.4 (3) mg g^{-1} in dry coral during winter than summer 1.5±0.5 (3) mg g^{-1}in dry coral. The difference was significant (t-test $p<0.001$) as well as the average rate of carbohydrates was higher 28.54±8.06 (3) mg g^{-1}in dry coral during winter than summer 11.22±1.08 (3) mg g^{-1} in dry coral. The difference was significant (t-test $p<0.021$) but the average rate of lipids was lower 3.2±1.1 (3) mg g^{-1}in dry coral during winter than summer 12.4±2.2 (3) mg g^{-1} in dry coral. The difference was significant (t-test $p<0.003$) and the average rate of Chlorophyll" a" was lower 1.46±0.4 (3) µg g^{-1} in dry coral during winter than summer 3.2±1.2 (3) µg g^{-1} in dry coral. The difference was not significant.

P. verrucosa

The average rate of protein was higher 4.37±0.43 (3) mg g^{-1} in dry coral during winter than summer 1.26±0.26 (3) mg g^{-1} in dry coral. The difference was significant (t-test $p<0.001$) shown in table (9) as well as the average rate of Carbohydrates was higher 29.64±9.36 (3) mg g^{-1} in dry coral during winter than summer 16.35±6.2 (3) mg g^{-1} in dry coral. The difference was not significant. but the average rate of Lipids was lower 2.1±0.1 (3) mg g^{-1} in dry coral during winter than summer 10.8±2.3 (3) mg g^{-1} in dry coral. The difference was significant (t-test $p<0.003$) and the average rate of Chlorophyll" a" was lower 1.7±0.8 (3) µg g^{-1} in dry coral during winter than summer 3.42±2.08 (3) µg g^{-1} in dry coral. The difference was not significant.

Table (1) The Mean Skeletal and Tissue Biomass Colony of *P. damicornis* and *P. verrucosa* at the Study Site Recorded at 3 m Depth .

Characteristic		*P.damicornis*		*P. verrucosa*		t-test	P-value
Skeleton							
Skeletal density							
($g.cm^{-3}$)		2.78±0.018	(12)	2.77 ± 0.026	(11)	1.5	0.148
Biomass Colony							
mg.d.t.g^{-1} skeleton		28.1±13.43	(9)	24.92 ± 5.7	(10)	0.69	0.50
cm^2 .g^{-1} skeleton	-	5.36±0.93 (9)		3.76 ± 0.66	(10)	4.42*	0.001
mg.d.t.cm^{-2}		5.33 ± 2.74	(9)	6.92 ± 2.59	(10)	1.30	0.21

Table (2) The Mean Biochemical Components of Two Species in *P. damicornis* and *P. verrucosa* of the Red Sea Coast with Standard Deviations (S.D ±), n=3

Components	*P. damicornis* Winter 2009	*P. damicornis* Summr 2010	t-test	p-value	*P. verrucosa* Winter 2009	*P. verrucosa* Summr 2010	t-test	p-value
Protein								
mg g^{-1} of dry coral	4.69	1.5			4.37	1.26		
S.D±	(0. 4)	(0.5)	8.6*	0.001	(0.43)	(0.26)	10.7*	0.001
Carbohydrate								
mg g^{-1} of dry coral	28.58	11.22			29.64	16.35		
S.D±	(8.06)	(1.08)	3.6*	0.021	(9.36)	(6. 2)	2.05*	0.111
Lipid								
mg g^{-1} of dry coral	3.2	12.4			2.1	10.8		
S.D±	(1. 1)	(2.2)	6.4*****	0.003	(0.1)	(0.1)	6.55*	0.03
Chlorophyll"*a"*								
µg g^{-1} of dry coral	1.46	3.2			1.7	3.42		
S.D±	(0.4)	(1.2)	2.3*****	0.076	(0. 8)	(2.08)	1.34	0.25

***significance (P≤ 0.05)**

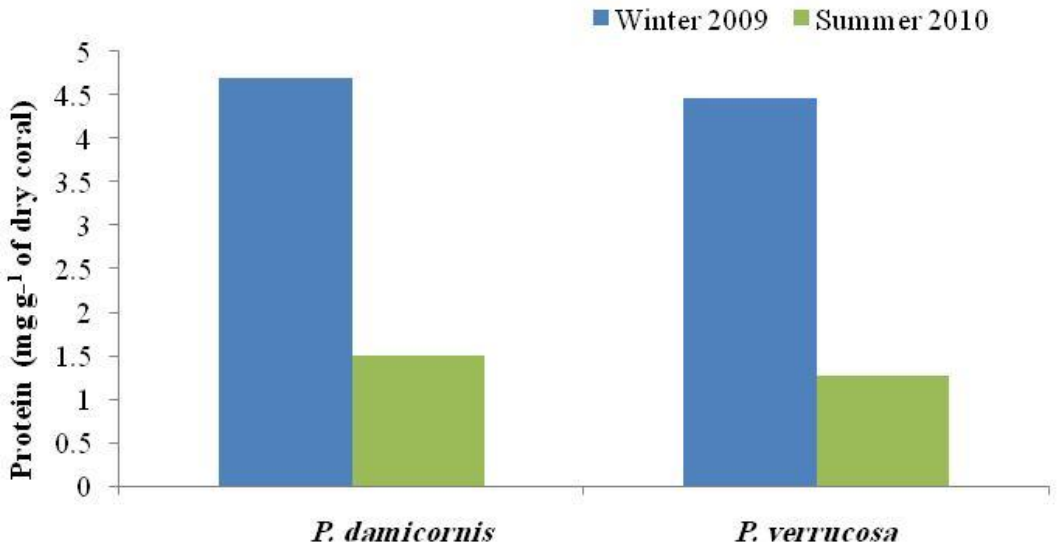

Figure 2 Comparison of the Mean Protein Rate between Two Species at 3m during Winter2009 and Summer 2010.

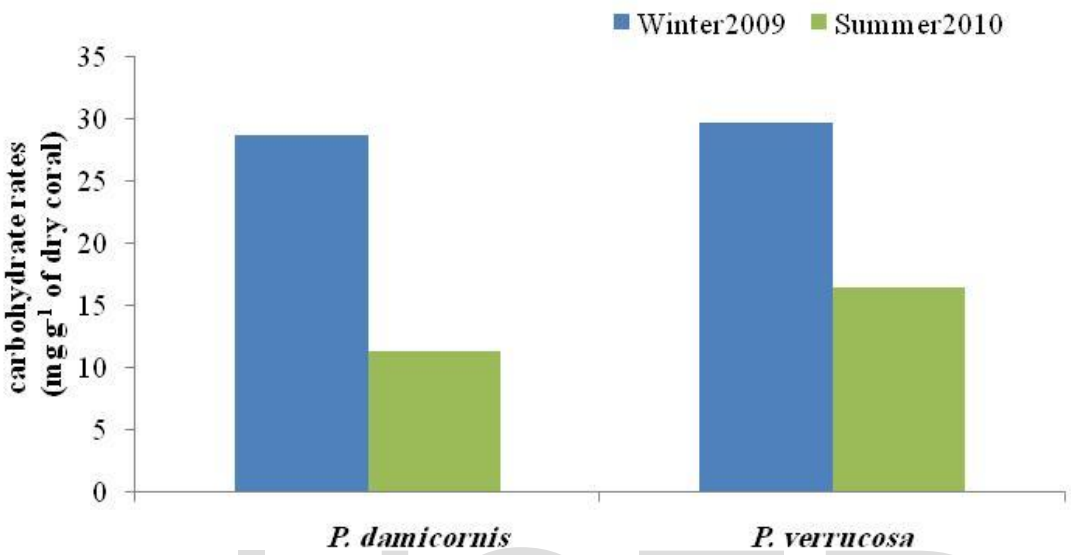

Figure 3 Comparison of the Mean Carbohydrate Rate between Two Species at 3m during Winter2009 and Summer 2010.

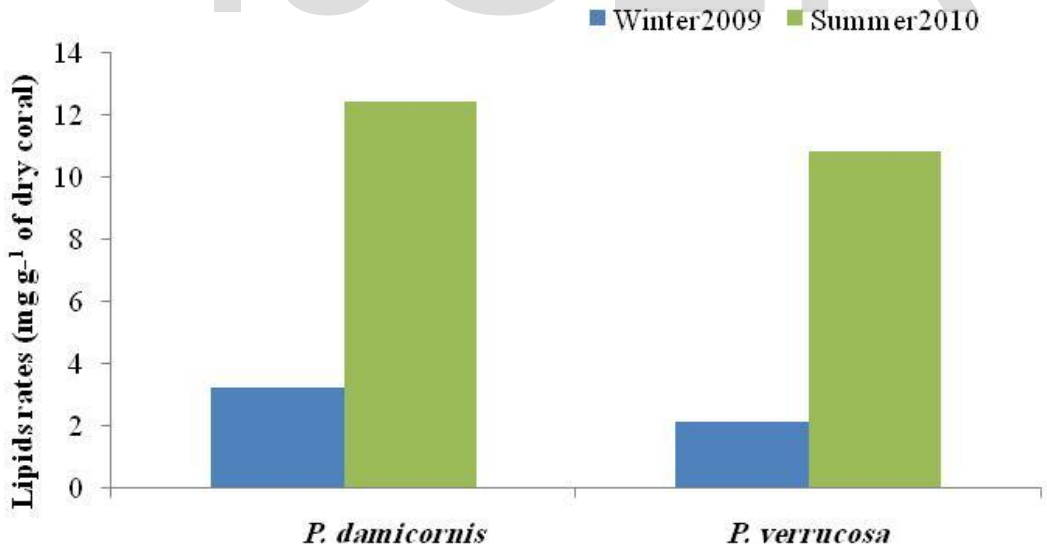

Figure 4 Comparison of the Mean Lipids Rate between Two Species at 3m during Winter2009 and Summer 2010.

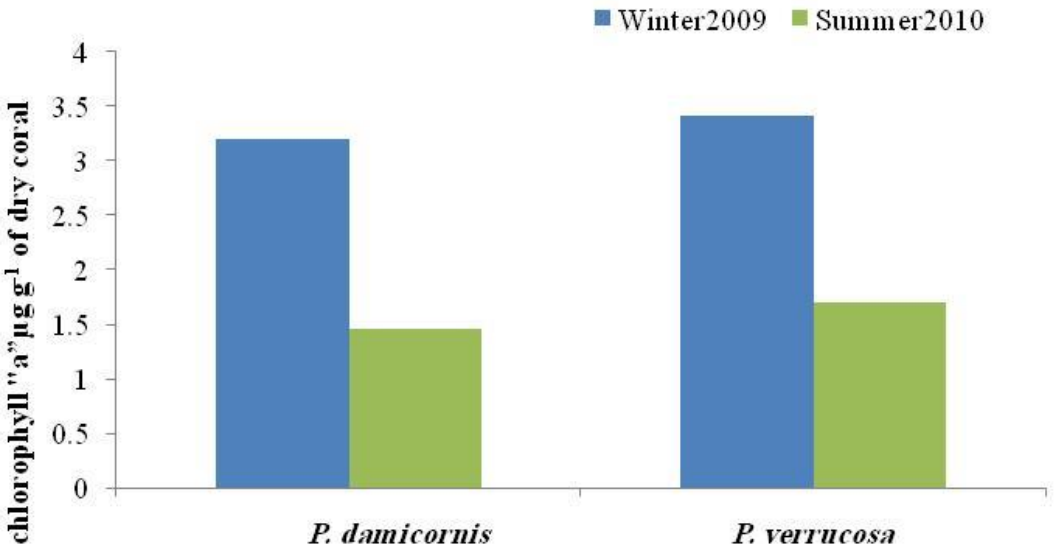

Figure 5 Comparison of the mean chlorophyll *"a"* between two species at 3m during winter2009 and summer 2010.

Discussion

Skeletal densities for both *P. damicornis* and *P. verrucosa* are similar 2.78 g.cm^{-3} and 2.77 g.cm^{-3} respectively that is comparable with 2.78 g.cm^{-3} recorded for both *Stylophora pistillata* and *Echinopora gemmacea* (AL-Sofyani, 1991), 2.783,g.cm^{-3} for *Pocillopora eydouxi* (Davies, 1984, 1989), 2.822 g.cm^{-3} for *Porites porites* (Edmunds and Davies, 1986) and lower than 2.95 g.cm^{-3} for *Fungia fungites* (Jan, 2001).

The variations in the skeletal density among the species may be due to the difference in amount of organic matrix in the skeleton (Davies, 1984, 1989). The similarity of the skeletal density of both species in the present study may reflect a similar percentage of the organic matrix in their skeleton.

P. verrucosa has a 11.32% lower biomass of tissue per mg dry tissue weight when compared with *P. damicornis*, contrary *P. damicornis* has a 22.98% lower biomass of tissue per surface area than *P. verrucosa* (Table 1). This may result from differences in the growth form of the two species or from the tissue location within the skeleton (Davies, 1991a and Al-Sofyani, 1991).

The tissue biomass, 28.1 mg.d.t.g^{-1}skel for *P. damicornis* and 24.92 mg.d.t.g^{-1}skel for *P. verrucosa* at depth of 3m is higher than the values of 4.73 mg.d.t.g^{-1}skel for *Pocillopora eydouxi* at depth of 5m and much lower than 44.29 and 45.02 mg.d.t.g^{-1}skel for *Montipora verrucosa* and *Porites lobata* at depth of 3m respectively (Davies, 1984 and Davies, 1991a). AL-Sofyani, (1991) reported 10.30 mg.d.t.g^{-1}skel and 12.86 mg.d.t.g^{-1}skel for *Stylophora pistillata* and *Echinopora gemmacea* from the Red Sea at depth of 3m respectively. Other study showed 2.2 mg.d.t.g^{-1}skel *for Fungia fungites* at depth of 2m (Jan, 2001). However, on a unit area basis (mg.d.t.cm^{-2}), the values of 5.33 mg.d.t cm^{-2} for *P. damicornis* and 6.92 mg.d.t cm^{-2} for *P. verrucosa* are close to the range of 5.56 mg.d.t cm^{-2} to 9.65 mg.d.t cm^{-2} for *Montastrea annularis* at depth of 2m and 10m respectively (Davies, 1980) and within the range of 2.8 to 12.5 mg.d.t cm^{-2} for six species at 2.5m from Barbados, West Indies (Lewis and Post, 1982). AL-Sofyani, (1991) reported lower values, 3.52 mg.d.t cm^{-2} for *Stylophora pistillata* and 4.91 mg.d.t cm^{-2} for *Echinopora gemmacea* at depth of 1m and 3m respectively, while Edmunds and Davies (1986) showed much higher value 18.59 mg.d.t cm^{-2} for *Porites porites* at depth of 10m. These differences of my result are due to the growth form or from the methods used for measuring surface area (Edmunds and Davies, 1986 and Al-Sofyani, 1991)

Biochemical Components of *Pocillopora* Species

The chemical composition of *Pocillopora* shows variation according to species and seasons. In present study statistically significant differences in (protein, Carbohydrate, Lipid and Chlorophyll) between different species and seasons.

In the present study, the Protein values of *P. damicornis* was ranging (4.69±0.4 to 1.5±0.5) mg g^{-1} of dry coral in winter and summer respectively. All of these values however, are higher than 4.37±0.43 mg g^{-1} to 1.26±0.26 mg g^{-1} of dry coral recorded for *P. verrucosa* during winter and summer respectively. which was similar description of some other Red Sea corals (Al-Sofyani and Niaz, 2007; Al-Sofyani and Niaz, 2006; Al-Otaibi, 2006; Al-Lihaibi *et al*, 1998; Sawall *et al*, 2011; Floos *et al*, 2015). Comparatively low Protein content and greater residual or calcareous material indicates its reliance on the primary products of photosynthesis.

Carbohydrate is the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes. The Carbohydrate content

of (28.58±8.06 to 11.2±1.08) mg g^{-1} of dry coral respectively in *P. damicornis* during winter and summer respectively was lower than (29.64±9.36 to 16.35±6.2) mg g^{-1} of dry coral respectively recorded for *P. verrucosa* during winter and summer respectively. Similar as described for Red Sea Corals (Al-Sofyani and Niaz, 2007; Al-Sofyani and Niaz, 2006; Al-Lihaibi *et al*, 1998; Sawall, *et al*, 2011; Floos *et al*, 2015).Carbohydrate were highest in *P. verrucosa* indicating great dependence on the primary products of photosynthesis. On the other hand, *P. damicornis* had lower carbohydrate content and a smaller quantity of plant pigments

In the present study, the Lipid values of (3.2±1.1 to 12.4±2.2) mg g^{-1} of dry coral respectively in *P. damicornis* during winter and summer respectively. All of these values however, are higher than (2.1±0.1 to 10.8±0.1) mg g^{-1} of dry coral recorded for *P. verrucosa* during winter and summer respectively. The Lipid concentration was significantly lower during winter than in summer for two species. The differences in lipid values between winter and summer may be related to timing of reproduction. The lower level in January coincides with the end of reproduction. On the other hand, a slightly higher lipid level in May coincide with the beginning of oogenesis when the Lipid is at its highest level. Richmond (1987) reported that about 5% of the energy content of *Pocillopora damicornis* is lost monthly for planulation

It was similar to that reported by Floos *et al*, 2015 found the Lipid value of *Seriatopora hystrix* and *Lobophyllia corymbosa* 6.3±0.19 and 3.6±0.24 mg g^{-1} at 5m depth than the summer 2.1±0.2 and 1.5±0.5 mg g^{-1} at 10 m depth respectively. While Al-Lihaibi *et al*, 1998 found the lipid value of *Stylophora pistillata*, *P. verrucosa*.and *Echinopora gemmacea* 1.9±0.27, 8.6±1.46 and 1.3±0.22 mg g^{-1} of dry coral respectively and Al-Sofyani, 1991 found the value of 32.94% to 40.49 % Lipid on dry tissue basis in *Stylophora pistillata* at 1m during winter and summer respectively and found the value of 22.55% to 27.48 % recorded in *Echinopora gemmacea* at 3m during winter and summer respectively. Stimson, 1987 found the value of 30-40 % lipid on dry tissue in Hawaian corals from shallow water. The difference in Lipid values between summer and winter, may be related to a lower irradiance level and a lower seawater temperature, which reduce photosynthetic production. it has been mention by other workers (Muscatine 1973 and Davies 1984) that the photosynthetic products, as result of autotrophism, are gradually passed on to the host cells (coral) and used as apart of their

nutrition. The difference in lipid values between winter and summer may by related to a lower irradiance level and a lower seawater temperature, which reduce photosynthetic production

The Chlorophyll“a“values of 1.46±0.4 to 3.2±1.2 µg g^{-1} of dry coral in *P. damicornis* during winter and summer respectively. All of these values however, are lower than 1.7±0.8 to 3.42±0.34 µg g^{-1} of dry coral recorded for *P. verrucosa* during winter and summer respectively, Was similar to that reported by Al-Lihaibi *et al*, 1998 found the Chlorophyll"*a"* value of *Stylophora pistillata, P. verrucosa.*and *Echinopora gemmacea* 0.9±0.44, 2.6±1.36 and 5.0±2.51 µg g^{-1} of dry coral. Dubinsky *et al* (1984) found that the external environmental factors also influence the growth and composition of the corals. For example, it was observed that when *Stylophora pistillata* was exposed to external nutrient resources and feeding on Artemia; it led to an increase in the aerial pigmentation in comparison with control colonies .and also similar to described for Red Sea Corals (Floos *et al*, 2015; Al-Sofyani and Niaz, 2007; Al-Sofyani and Niaz, 2006). Chlorophyll***"a"*** and Carbohydrates were highest in *P. damicornis* and *P. verrucosa* had indicating great dependence on the primary products of photosynthesis.

This information also provides clue to the mode of nutrition in the marine organism. Saturated fatty acids indicate more reliance towards autotrophic feeding; while unsaturated fatty acids indicate the dominance of heterotrophism. All these findings indicate that *P. damicornis* and *P. verrucosa*relies are predominantly dependant on autotrophic feeding .

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