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APPENDIX

APPENDIX A
Lugol's Iodine Solution

The Lugol's Iodine Solution is prepared mixing these chemicals

1. Potassium iodine	10	g.
2. Iodine crystal	5	g.
3. Acetic acid (gracial)	10	ml.
4. Distilled water	100	ml.

The solution is stable for at least one year in an amber bottle. For preservation, ratio of 1:100 (volume of the solution per sample volume) is used.

APPENDIX B

Protein Analysis

In this study the modified Lowry et al. method (Peterson, 1977) was used for soluble protein analysis. The scheme of procedure was briefly described as follows;

I. Stock Reagent

1. Copper-tartate-carbonate (CTC). A solution of about 20% sodium carbonate is added slowly while stirring to a solution of copper sulfate-tartate to give final concentration of 0.1% coper sulfate (pentahydrate), 0.2% potassium tartate, 10% soium carbonate. This solution is stable for at least 2 months at room temperature (20 ^{o}C)

2. 10% Sodium dodecyl sulfate (SDS)

3. 0.8 N Sodium hydroxide

4. Folin-Ciocalteu phenol reagent. Obtained from Koch-Light Ltd. as 2 N solution.

II. Working Solutions

1. Bovine serum albumin (BSA), 0.5 mg/ml. Fresh preparation is used.

2. Reagent A. Mix equal parts of stock CTC, NaOH, SDS, and H_2O. This keeps at least 2-3 weeks at room temperature (20 ^{o}C). The presence of a small amount of dark precipitate, or a white flocculate precipitate (SDS), dose not effect color development if shaken well before use. SDS will come out of solution more readily at colder temperature, but redisolves upon warming or dilution. Fresh Reagent A is prepared when the dark precipitate accummulate or when the viscosity becomes excessive.

3. Reagent B. One volume of Folin-Ciocalteu phenol reagent (2 N solution) is mixed with five volumes of distilled water. It is stable at room temperature (20 ^{o}C) in an amber bottle.

III. Assay Procedure

Bring 1.0 ml sample (coral's tissue homogenate or standard sample, BSA). Add 1.0 ml of Reagent A, mix and allow to stand for 10 minutes at room temperature. Add 0.5 ml of Reagent B and mix immediately. After 30 minutes, read absorbance at 750 nm. Read within 2 hours. Color loss is about 1 and 5% per houre at room temperature (20 ^{o}C) and 37 ^{o}C, respectively.

IV. Standard Curve

Eleven different standard concentrations (5-250 ug BSA) are prepared from 0.5 mg/ml BSA and conducting as procedure III. Five replicates of each concentrations are utilized to determine the standard curve. The linear regression is fitted by method of least-square analysis of the log-log data. The unknown protein is calculated from the following equation,

$$\text{Protein (ug)} = (I \times A750)^{S} \qquad \text{------------} \; [1]$$

where I is the reciprocal of the intercept and S is the reciprocal of the slope of the line.

From this study the results of analysis were presented in Table B-1 and Fig. B-1. The method of least-square analysis produced an estimation of constants of the equation [1] of 1.390 and 49.634 for S and I, respectively.

Table B-1. The results for analysis of protein statdard curve using standard protein: Bovine serum albumin (BSA).

BSA concentration (ug)	Absorbance (Abs) at 750 nm for each replicate sample					Average Abs. avg
	1	2	3	4	5	
5	0.048	0.05	0.058	0.084	0.078	0.0636
25	0.216	0.186	0.205	0.214	0.218	0.2078
50	0.321	0.32	0.323	0.317	0.357	0.3276
75	0.447	0.449	0.459	0.459	0.473	0.4574
100	0.57	0.574	0.548	0.56	0.557	0.5618
125	0.672	0.667	0.676	0.656	0.638	0.6618
150	0.763	0.777	0.766	0.642	0.68	0.7256
175	0.862	0.883	0.881	0.752	0.685	0.8126
200	0.936	0.931	0.933	0.843	0.802	0.889
225	0.919	0.988	1.023	1.035	1.036	1.0002
250	1.087	1.075	1.083	1.13	1.035	1.082
275	1.186	1.17	1.181	1.02	1.097	1.1308
300	1.222	1.242	1.23	1.161	1.122	1.1954
325	1.304	1.28	1.245	1.213	1.49	1.3064
350	1.338	1.324	1.34	1.323	1.171	1.2992
375	1.418	1.363	1.402	1.376	1.427	1.3972
400	1.424	1.351	1.434	1.438	1.42	1.4134

For 5-250 ug BSA

Regression Output:

Constant	-1.696
Std Err of Y Est	0.0085
R Squared	0.9995
No. of Observations	11
Degrees of Freedom	9
X Coefficient(s)	0.7192
Std Err of Coef.	0.0053

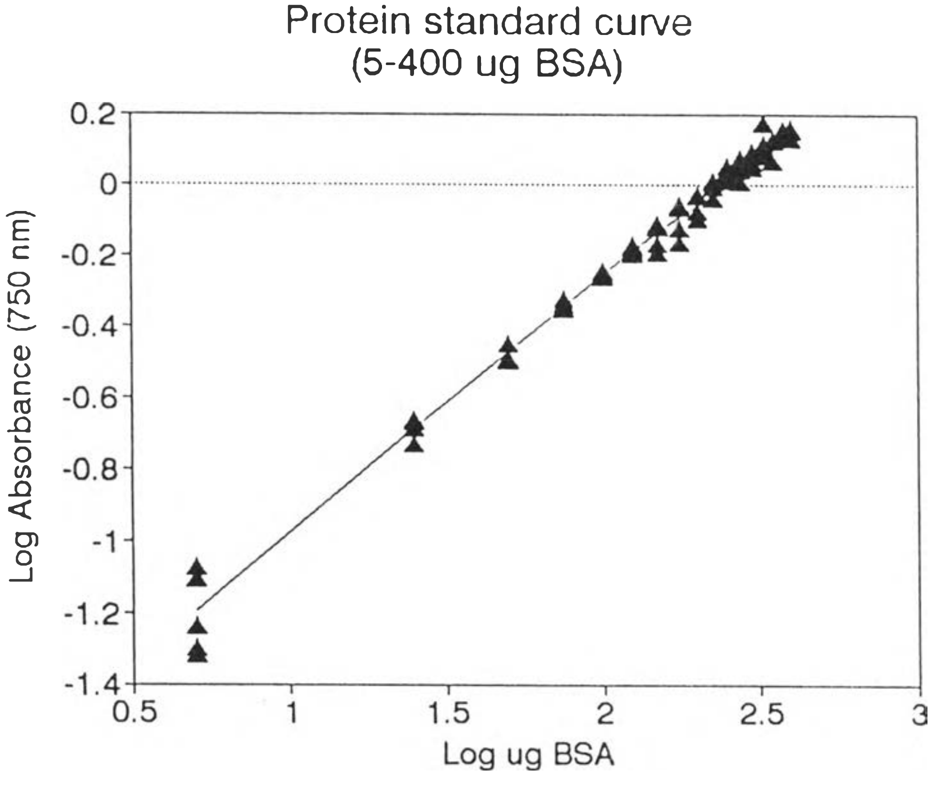


Fig. B-1. Regression line showing the correlation between BSA concentration and its absorbance values. (Log transformed values).

APPENDIX C

Surface Area Analysis

Weight measurements of rectangular cutting peices at 39 different sizes of aluminum foils were presented in Table C-1. The regression line (Fig. C-1) shows strong correlation (r^2 = 0.99) between foil weight and its surface area, and performed the relationship,

$$Y = 0.004575 (X) + 0.00537 \quad \text{---------------} \quad [2]$$

where Y is the weight (g) of any foil pieces and its surface area of X (cm^2). The equation 2 was used to calculate the surface area of coral sample by relating the weight of foil wrapped over a coral skeleton.

It is important to note that the foil used for determination of standard curve and wrapping over the coral skeleton should be the same roll. New standard curve should be made whenever the new roll of aluminum foil is used.

Table C-1. Summary of foil peices of known area and their weights.

Foil area (cm)	Weight (g) for each replicate					Average weight (g)
	1	2	3	4	5	
1	0.0061	0.0063	0.0064	0.0064	0.0066	0.00636
1.2	0.0065	0.0068	0.0066	0.0069	0.0064	0.00664
1.5	0.0100	0.0100	0.0101	0.0106	0.0103	0.01020
1.8	0.0103	0.0101	0.0104	0.0100	0.0112	0.01040
2	0.0132	0.0130	0.0131	0.0127	0.0128	0.01296
2.25	0.0144	0.0138	0.0143	0.0139	0.0146	0.01420
2.5	0.0130	0.0134	0.0136	0.0136	0.0138	0.01348
3	0.0192	0.0189	0.0188	0.0187	0.0193	0.01898
3.5	0.0198	0.0187	0.0188	0.0194	0.0187	0.01908
4	0.0260	0.0251	0.0254	0.0257	0.0254	0.02552
4.5	0.0249	0.0249	0.0244	0.0245	0.0246	0.02466
5	0.0316	0.0321	0.0322	0.0318	0.032	0.03194
5.5	0.0298	0.0302	0.0289	0.0291	0.0301	0.02962
6	0.0392	0.0393	0.0377	0.0387	0.0390	0.03878
6.25	0.0399	0.0385	0.0395	0.039	0.0398	0.03934
6.5	0.0346	0.0352	0.0357	0.0350	0.0359	0.03528
7	0.0446	0.0442	0.0440	0.0441	0.0442	0.04422
7.5	0.0483	0.0485	0.0478	0.0474	0.047	0.04780
8	0.0445	0.0430	0.0433	0.0436	0.0437	0.04362
9	0.0567	0.0570	0.0568	0.0573	0.0557	0.05670
10	0.0547	0.0547	0.0549	0.0543	0.0546	0.05464
10.5	0.0652	0.0649	0.0652	0.0656	0.0661	0.06540
11	0.0595	0.0591	0.0586	0.0583	0.0596	0.05902
12	0.0751	0.0766	0.0742	0.0762	0.0753	0.07548
12.25	0.0779	0.0776	0.0781	0.0778	0.0763	0.07754
14	0.0881	0.0885	0.0884	0.0885	0.0890	0.08850
15	0.0812	0.0808	0.0808	0.0813	0.0807	0.08096
16	0.1026	0.1014	0.1023	0.1019	0.1015	0.10194
18	0.1137	0.1142	0.1130	0.1140	0.1136	0.11370
20	0.1268	0.1269	0.1270	0.1264	0.1267	0.12676
20.25	0.1281	0.1281	0.1279	0.1286	0.1271	0.12796
22.5	0.1278	0.1277	0.1273	0.1281	0.1274	0.12765
25	0.1340	0.1344	0.1334	0.1337	0.1340	0.13390
28	0.1488	0.1486	0.1486	0.1488	0.1485	0.14866
30	0.1592	0.1628	0.1618	0.1615	0.1620	0.16146
32	0.1719	0.1720	0.1724	0.1723	0.1717	0.17206
35	0.1874	0.1876	0.1874	0.1870	0.1871	0.18730
38	0.2031	0.2053	0.2036	0.2036	0.204	0.20392
40	0.2144	0.2138	0.2130	0.2130	0.2137	0.21358

Regression Output:

Constant	0.004575
Std Err of Y Est	0.006059
R Squared	0.990082
No. of Observations	39
Degrees of Freedom	37
X Coefficient(s)	0.00537
Std Err of Coef.	8.84E-05

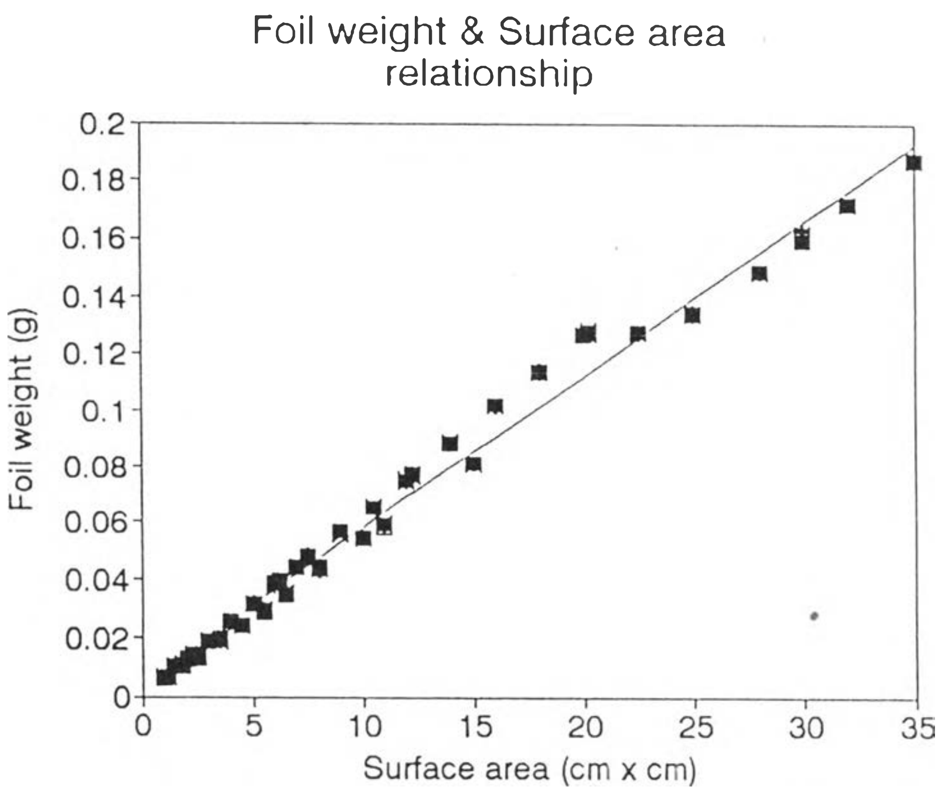


Fig. C-1. Regression line showing correlation between foil weight and its surface area.

APPENDIX D

Summary of Coral Tissue Biomass Analysis

Table D-1. Results on zooxanthellae densities of coral tissues. Thick vertical lines join mean values that are not significantly different (ANOVA; Multiple range test).

Coral species	Sampling period	Zooxanthellae density Cell x 10^6 / cm^2 Average	std.
L. edwardsi	1	0.469	0.120
	2	0.326	0.130
	3	2.863	0.231
	4	2.781	0.765
	5	3.400	0.727
	6	3.545	1.088
	7	4.302	0.995
	8	3.988	1.028
	9	4.937	1.834
M. ampliata	1	0.156	0.011
	2	0.273	0.179
	3	1.603	0.233
	4	2.219	0.830
	5	2.756	0.738
	6	2.657	0.289
	7	3.722	0.083
	8	3.118	0.696
	9	2.661	0.268
M. elephantotus	1	0.565	0.166
	2	3.382	1.285
	3	6.019	1.446
	4	5.988	0.565
	5	5.186	0.503
	6	4.905	1.662
	7	3.147	0.402
	8	3.499	0.940
	9	4.681	1.599
P. alcicornis	1	0.564	0.411
	2	0.395	0.477
	3	0.731	0.411
	4	1.102	0.578
	5	1.669	0.490
	6	1.424	0.235
	7	1.673	0.085
	8	1.740	0.063
	9	1.717	0.178
P. digitata	1	0.492	0.219
	2	1.417	0.462
	3	4.643	0.720
	4	4.021	0.541
	5	4.869	0.739
	6	4.107	0.714
	7	5.090	0.381
	8	3.877	0.260
	9	4.450	0.497

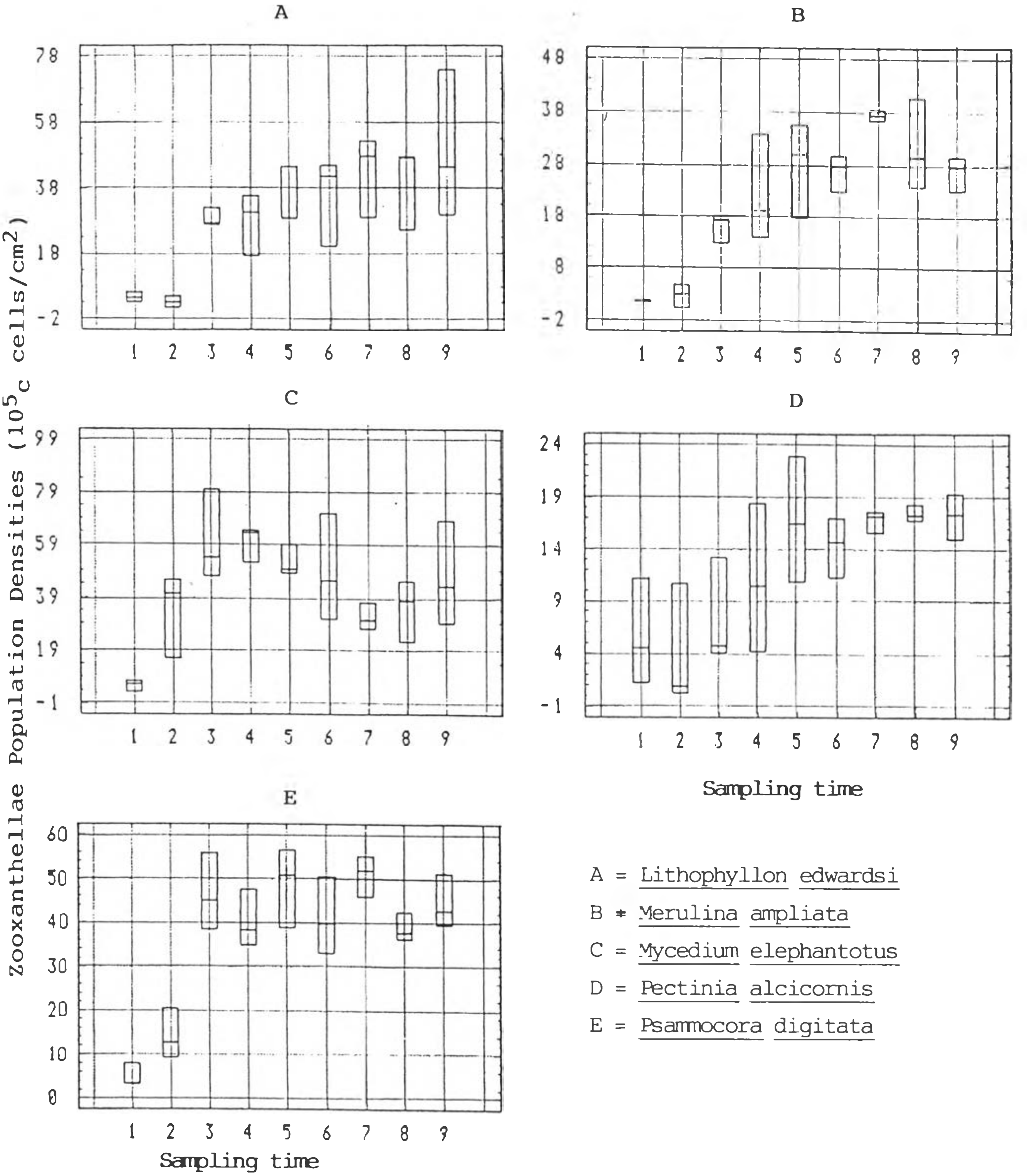


Fig. D-1. Multiple Box-and- Whisker plot showing range of data for zooxanthellae densities in time-matched samples of coral tissues.

Table D-2. Results on chlorophyll-a content (ug) per unit surface area (cm^2) of corals. Thick vertical lines join mean values that are not significantly different (ANOVA; Multiple range test)

Coral species	Sampling period	Chlorophyll-a content pg / cm ^ 2 Average	std.
L. edwardsi	1	0.996	0.291
	2	1.170	0.763
	3	8.213	1.068
	4	8.968	2.119
	5	10.525	2.066
	6	15.943	3.312
	7	21.047	3.031
	8	18.179	1.990
	9	19.107	9.198
M. ampliata	1	0.070	0.036
	2	0.304	0.129
	3	3.041	0.383
	4	5.506	1.897
	5	8.598	1.927
	6	7.685	0.741
	7	10.091	0.364
	8	10.658	2.455
	9	10.906	0.527
M. elephantotus	1	0.769	0.226
	2	9.203	2.969
	3	7.427	1.143
	4	8.200	0.353
	5	5.415	0.494
	6	4.870	1.095
	7	3.152	0.362
	8	6.534	1.189
	9	9.658	2.458
P. alcicornis	1	0.189	0.129
	2	0.971	1.372
	3	1.325	1.295
	4	2.557	1.288
	5	4.914	2.441
	6	2.993	0.300
	7	4.459	0.721
	8	4.653	0.199
	9	5.753	0.825
P. digitata	1	0.490	0.444
	2	4.222	1.103
	3	10.096	0.537
	4	9.196	2.940
	5	12.023	1.326
	6	12.358	1.333
	7	11.020	0.921
	8	11.647	0.765
	9	11.908	0.861

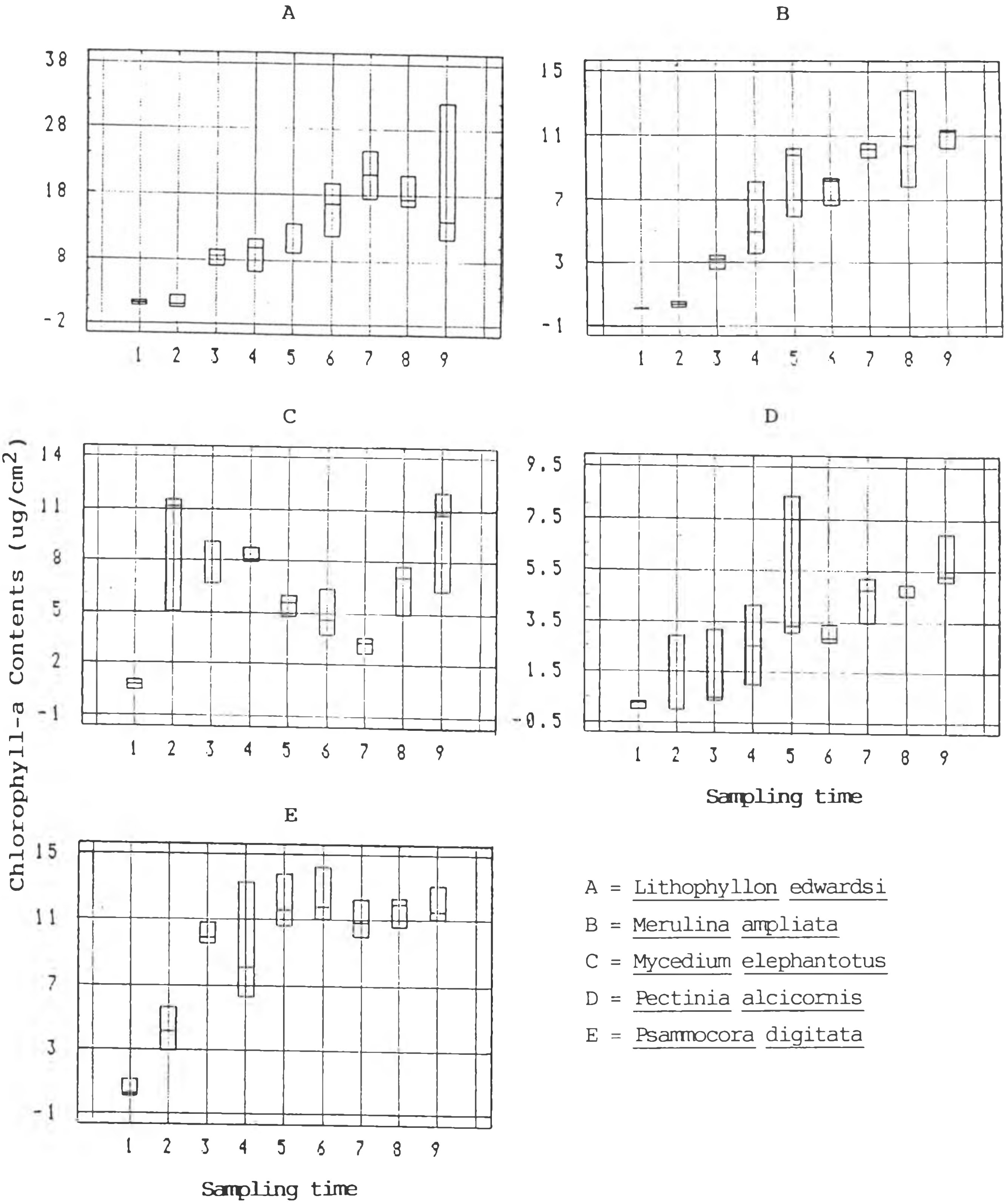


Fig. D-2. Multiple Box-and-Whisker plot showing range of data for chlorophyll-a contents in time-matched samples of coral tissues.

Table D-3. Results on chlorophyll-a content (pg) per zooxanthellae cell from coral tissue. Thick vertical lines join mean values that are not significantly different (ANOVA; multiple range test).

Coral species	Sampling period	Chlorophyll-a per Zooxanthellae pg / cell Average	std.
L. edwardsi	1	2.100	0.154
	2	3.235	0.909
	3	2.865	0.265
	4	3.292	0.406
	5	3.107	0.050
	6	4.715	0.755
	7	5.055	0.784
	8	4.849	1.168
	9	3.758	0.477
M. ampliata	1	0.438	0.193
	2	1.905	1.343
	3	1.904	0.051
	4	2.506	0.069
	5	3.171	0.293
	6	2.897	0.058
	7	2.711	0.039
	8	3.412	0.103
	9	4.151	0.558
M. elephantotus	1	1.366	0.062
	2	2.820	0.287
	3	1.260	0.121
	4	1.389	0.203
	5	1.046	0.057
	6	1.035	0.131
	7	1.004	0.042
	8	1.921	0.201
	9	2.128	0.297
P. alcicornis	1	1.006	1.056
	2	0.916	1.278
	3	1.417	0.706
	4	2.338	0.075
	5	2.866	0.754
	6	2.148	0.326
	7	2.684	0.527
	8	2.678	0.153
	9	3.437	0.866
P. digitata	1	0.810	0.434
	2	3.036	0.210
	3	2.209	0.225
	4	2.232	0.405
	5	2.533	0.474
	6	3.045	0.225
	7	2.180	0.256
	8	3.010	0.189
	9	2.687	0.104

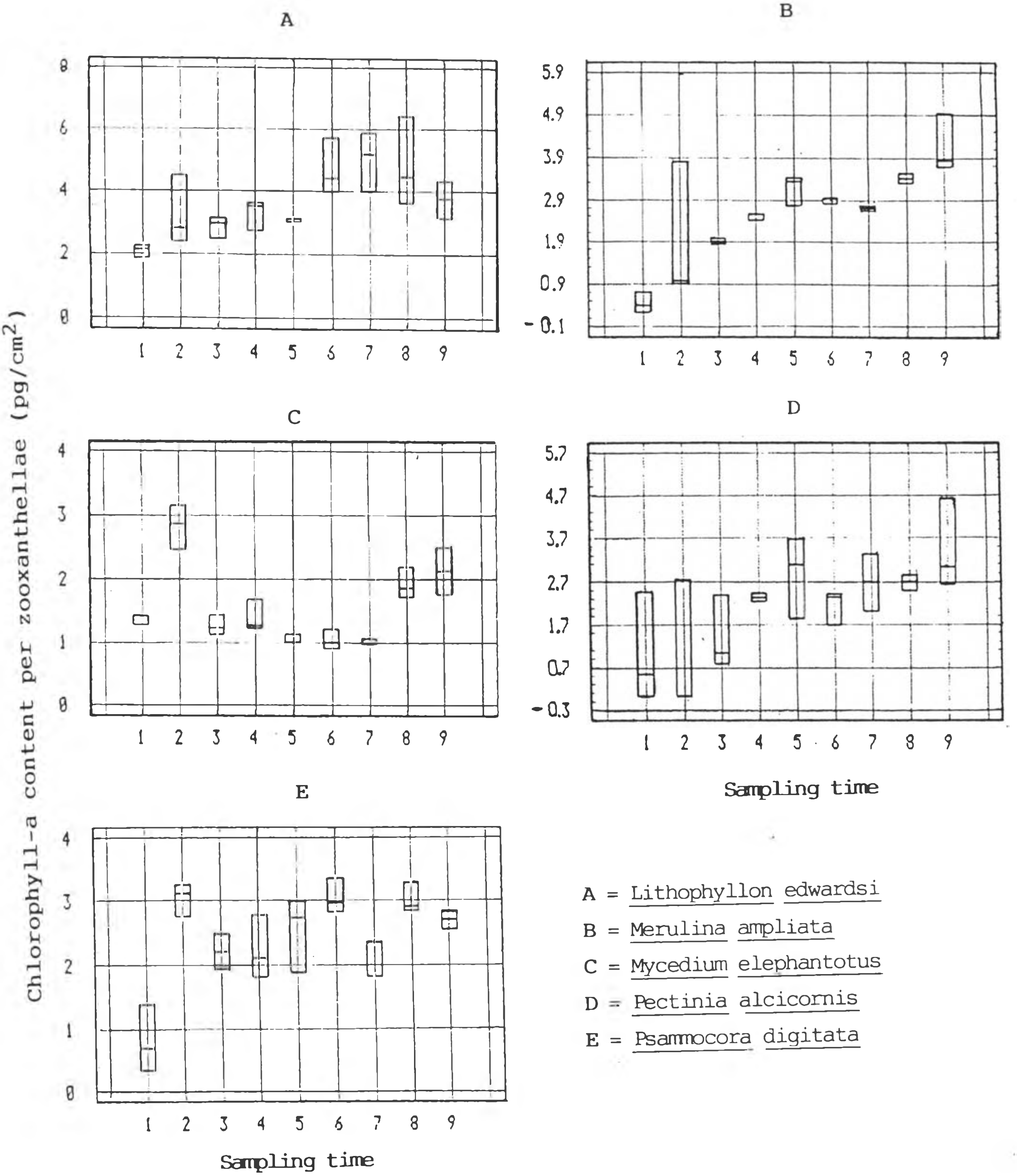
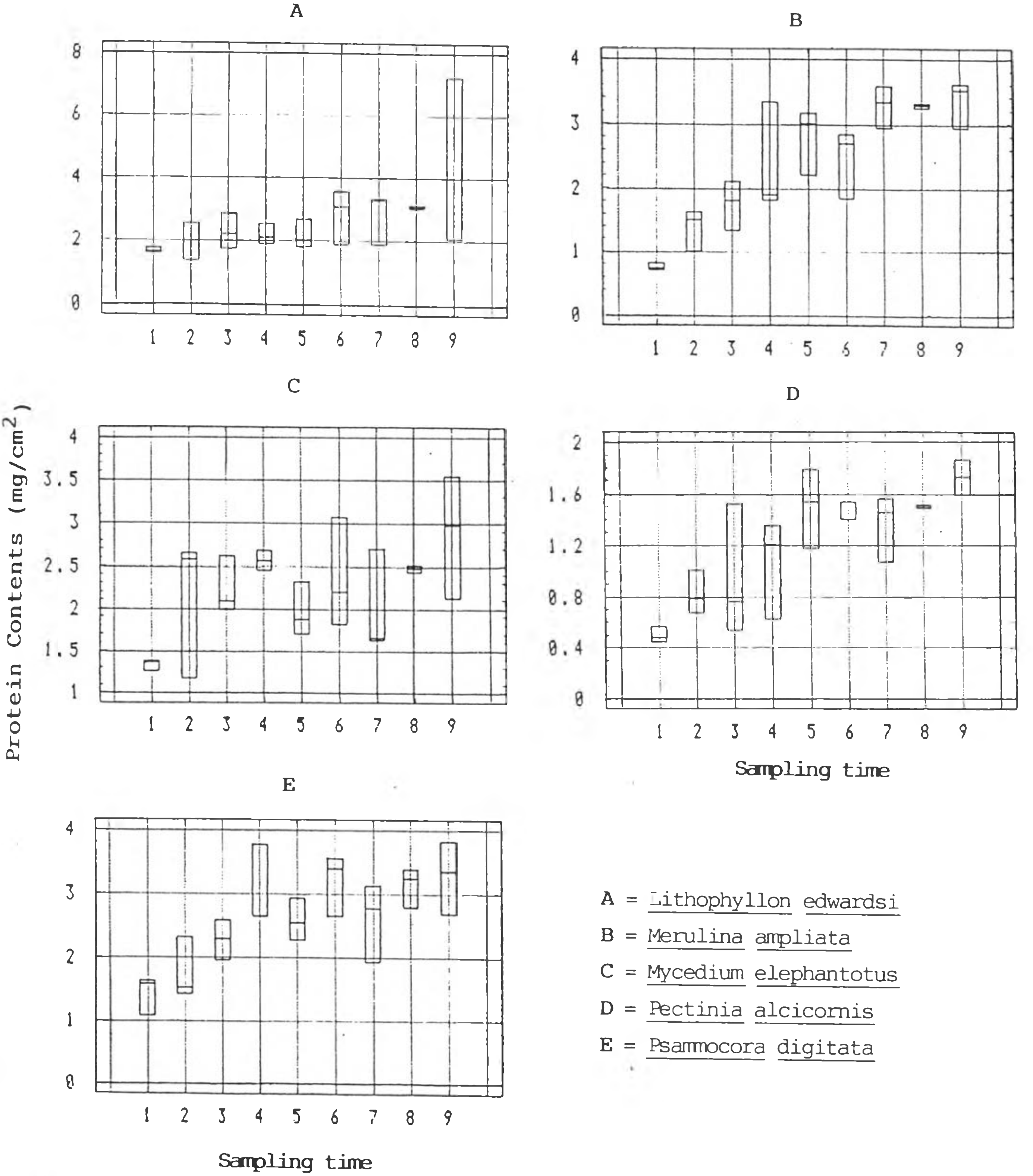


Fig. D-3. Multiple Box-and-Whisker plot showing range of data for zooxanthellae pigment (chlorophyll-a) in time-matched samples of coral tissues.

Table D-4. Results on protein content of coral tissue. Thick vertical lines join mean values that are not significantly different (ANOVA; Multiple range test).

Coral species	Sampling period	Protein content ug / cm^2 Average	std.
L. edwardsi	1	1.654	0.075
	2	1.746	0.546
	3	2.237	0.452
	4	2.171	0.269
	5	2.158	0.372
	6	2.839	0.706
	7	2.817	0.676
	8	3.057	0.032
	9	4.490	2.116
M. ampliata	1	0.756	0.049
	2	1.371	0.269
	3	1.742	0.318
	4	2.346	0.700
	5	2.788	0.420
	6	2.454	0.438
	7	3.280	0.267
	8	3.280	0.028
	9	3.355	0.299
M. elephantotus	1	1.330	0.057
	2	2.136	0.682
	3	2.227	0.281
	4	2.560	0.092
	5	1.958	0.255
	6	2.357	0.522
	7	1.997	0.496
	8	2.468	0.032
	9	2.883	0.581
P. alcicornis	1	0.499	0.051
	2	0.823	0.136
	3	0.941	0.418
	4	1.063	0.311
	5	1.497	0.252
	6	1.490	0.066
	7	1.363	0.209
	8	1.504	0.011
	9	1.733	0.112
P. digitata	1	1.428	0.248
	2	1.756	0.400
	3	2.274	0.259
	4	3.026	0.524
	5	2.582	0.274
	6	3.198	0.388
	7	2.612	0.505
	8	3.138	0.249
	9	3.287	0.461



A = Lithophyllon edwardsi

B = Merulina ampliata

C = Mycedium elephantotus

D = Pectinia alcicornis

E = Psammocora digitata

Fig. D-4. Multiple Box-and-Whisker plot showing range of data for protein contents in time-matched samples of coral tissues.

BIOGRAPHY

Mr. Ukkrit Satapoomin was born in Chonburi Province on February 19, 1967. He finished his high school education from Taweethapisek School, Bangkok, in 1984. He graduated with the degree of Bechelor of Science from the Department of Marine Scince, Chulalongkorn University in 1988, then he continued his study for a Master Degree in Marine Biology at the same institute. Recently he worked as a marine biologist at the Phuket Marine Biological Center, Deparment of Fisheries.