CHAPTER 3

MATERIALS

AND

METHODS

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3.1 Study Site

Cape Rachado is a fringing coral reef located at Port Dickson, Negeri Sembilan, west coast of Peninsular Malaysia, between latitudes 2°30'N and 102°E (Figure 4). The reef flats extend out for about 110 m and gently slope towards the reef edge to a depth of about 8 m (Goh & Sasekumar 1980). The reef flats are dominated by *Sargassum*, *Turbinaria* and *Padina* species. Other seasonal algae including *Caulerpa*, *Dictyota* and *Gracilaria salicornia*. Phang (1989) recorded 16 species of epiphytic algae from the macroalgal substrates (*S. binderi, S. oligocystum, S. ilicifolium* and *T. conoides*) at Cape Rachado. A list of algae which consisted of 69 species was recorded at this site by Phang (1995). Other studies were carried out and published by Phang (1985, 1988) and Mohamed and Badaruddin (1991).

Cape Rachado is a famous tourist destination. This study site chosen is at a bay which is surrounded by a rocky shore and is less disturbed compared with adjacent bays. The study area is an intertidal reef flat extending to a

maximum of about 110 m and about 500 m wide. This area is dominated by the *Sargassum-Turbinaria* beds. Sea cucumber (*Holothuria* sp.), crabs and polychaetes are also usually found among the plants.

The reef flats are completely exposed when the tide level is 0.3 m or less above sea level (Plate 1). It is completely exposed for more than three hours when the tide level is 0.1 m or less above sea level. The lowest tide level (0.1 m or less above sea level) during this study period is from February to May 1995 and January to May 1996. The reef flats were exposed for 145 times (daytime low tides of 0.3 m or less above sea level), for about 1-3 hours during the monitoring period (January 1995 to April 1996). Calculations were based on the Tide Tables Malaysia (1995, 1996) for Port Dickson, Negeri Sembilan Darul Khusus (Lat 02°31'N, Long 101°47'E).

3.2 Preliminary Study

3.2.1 Selection of Species

Two species of *Sargassum, S. baccularia* (Mertens) C. Agardh and *S. swartzii* (Turner) C. Agardh were chosen for this study. They are dominant at the study site all year round. Both species can be

differentiated easily in the field. The main morphological difference are the branches. The branches of *S. baccularia* plants are cylindrical with spines (Plate 2), whereas the branches of *S. swartzii* plants are smooth and flat (Plate 3).

Taxonomic identification of the species is based on "Taxonomy of Economic Seaweeds" by Abbott and Norris (eds.) (1985) and Abbott (ed.) (1988, 1992, 1994 & 1995).

3.2.2 Selection of Quadrat Size and Quadrat Number

A preliminary survey was carried out to determine the most appropriate size and number of quadrats for the biomass and growth studies.

A 100 m line transect was laid out perpendicular to the shore in January 1995. Quadrat size (Figure 5) was determined by Wiegert's Nested Quadrat Method (Wiegert 1962 in Coombs *et al.* 1985).

In this case, ten trial samples of different quadrat sizes (0.25 m, 0.5 m and 1.0 m) was collected. The time taken between quadrats and to sample the smallest quadrat size was recorded. The following are determined for each quadrat size :

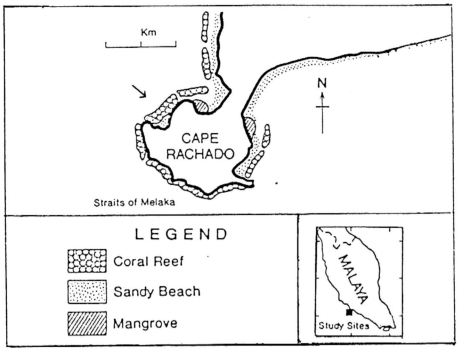

Figure 4. Location of the study site.

Plate 1. Low tide (0.3 m or less above sea level) at the reef flats of Cape Rachado.

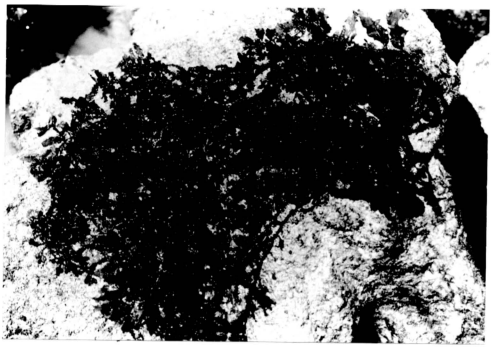

Plate 2. *Sargassum baccularia.*

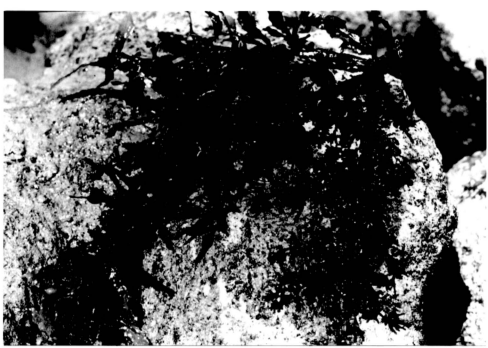

Plate 3. *Sargassum swartzii.*

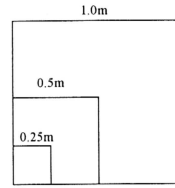

Figure 5. The arrangement of quadrats for determination of optimum quadrat area for sampling vegetation in order to estimate biomass using Wiegert's arrangement of nested quadrats in January 1995.

1. The mean biomass per m^2.
2. The variance of the mean biomass (V_m) per m^2.
3. The relative variance (V_r) obtained by dividing V_m for the quadrat area by V_m for the smallest quadrat area.
4. The relative cost (C_r),

$$C_r = \frac{C_f + x.C_v}{C_f + C_v}$$

Where, C_f = Fixed cost for each quadrat, i.e. the time spent walking between quadrats (in arbitrary units)

Cv = Time spent in sampling the smallest quadrat (in the same units)

x = area of quadrat

Then $V_r.C_r$ is plotted against quadrat area, and the lowest value of $V_r.C_r$ was taken as the optimum quadrat size.

Number of quadrats needed for a required degree of precision can be calculated by applying this formula :

$$n = \frac{(t.s)^2}{D.\overline{x}}$$

Where, n = Number of samples needed

t = Statistical function of Student's t test with (N-1) degrees of freedom (N = number of samples in trial)

s = Standard deviation of the trial samples

D = Confidence interval as a proportion of the mean (i.e. degree of precision required e.g. 20% or 0.2)

$\overline{x}$ = Mean of trial samples

3.3 15-Month Studies

3.3.1 Permanent Quadrats

Studies were conducted from January 1995 to March 1996. From the preliminary study, 0.25 m quadrat size was chosen for the growth studies in permanent quadrats and biomass studies for both *Sargassum* species.

Three permanent 100 m line transects with ten stations each 10 m apart were established. Plants found in the 0.0625 m^2 (0.25 m X 0.25 m) quadrats were monitored at times of daytime low tide biweekly or monthly intervals (Figure 6).

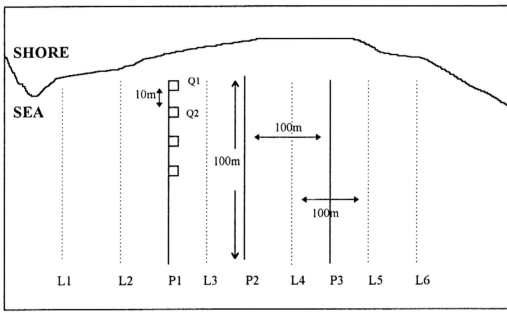

Figure 6. Map of the locations for the permanent line transects and destructive sampling line transects. (___ = Permanent line transects : P1, P2, P3; ----- = Destructive sampling line transects : L1 to L6; Q = Quadrat).

Twenty-eight plants of *S. baccularia* and twenty-nine plants of *S. swartzii* were tagged *in situ* on 20th January 1995. Self-locking cable ties with numbered tags (dymo-tape, 25 X 9 mm) were used to tag around the base of the plants (Plate 4).

Due to the high rate of lost of tags and plants, additional plants were tagged each month. It is assumed that there was no significant difference in the growth of plants for the same species tagged at different periods.

Observations were made using mask and snorkel when the plants were submerged even at low tide.

Plate 4. The Pole and tags in the field.

3.3.1.1 Growth Rate

Growth rate (mm day^{-1}) was determined by the net change in length divided by the number of days between sampling period. For this case study, only the positive growth rate was taken into account. Growth rate for all tagged plants at each sampling period was then averaged to give the mean growth rate.

3.3.1.2 Degenerative Rate

Degenerative rate (mm day^{-1}) was determined by the net change in length (decrease in length) divided by the number of days between sampling period. In other words, degenerative rate was the negative growth rate. Hence, only negative values were taken into consideration.

3.3.1.3 Variation in Thallus Length

Thallus length for each tagged plant was measured as the distance from the holdfast to the apex of the longest branch. The measured length of all tagged plants at each time interval was averaged to give the periodic mean plant length. This value was taken to represent the monthly average length of both *Sargassum* species in the community.

3.3.1.4 Length Classes

Percentage of individuals in various length classes for *S. baccularia* plants and *S. swartzii* plants was computed from the total thallus length recorded.

3.3.1.5 Reproductive State

Reproductive state of the tagged plants was recorded by the presence of receptacles on plants in each quadrat for each sampling period. Then, the percentage of plants having the reproductive structures was computed. A small number of receptacles from fertile tagged plants were collected each time in the field for detailed examination by cross-section of the receptacles (Appendix 1) under light microscope (Nikon Alphaphot YS) in the laboratory. This allowed the determination of male and female conceptacles.

3.4 Quarterly Monitoring of the *Sargassum* species

3.4.1 Destructive Sampling

Destructive sampling of *S. baccularia* and *S. swartzii* plants was performed quarterly between January 1995 to April 1996. Every three months, and during the lowest daytime spring tide, *S. baccularia* and *S. swartzii* plants were collected from 0.0625 m^2 (0.25 m X 0.25 m) quadrats using the line transect-quadrat method. Six line transects were established perpendicular to the shoreline, each 100 m apart. Ten quadrats samples were denuded at 10 m intervals along each line transect (Figure 6, page 42). Quadrats without either one of the *Sargassum* species or both were

also recorded in order to give the spatial pattern of the species. Thus, 22 to 58 biomass samples were processed each time to determine the seasonal variation of both *Sargassum* species.

Only *S. baccularia* and *S. swartzii* plants within each quadrat were harvested and placed in individual labeled plastic bags. The samples were stored in the ice-chest from sampling site to the laboratory; then they were kept in the freezer before processing. The plants were defrosted and rinsed in fresh water to remove sand, silt, epiphytes and other debris. The samples must be processed within a week before the plants began to rot.

3.4.1.1 Biomass Processing

Wet weight, dry weight and ash-free dry weight of the samples were obtained after washing and cleaning.

Wet weight of the samples was measured using a top pan balance (Ohaus portable advanced balance) to one decimal point after blotting the samples dry with paper towel. Wet weight values in term of g m^{-2}, were then calculated by multiplying the results obtained by 16, since the quadrats each covered an area of 0.0625 m^2.

Dry weight is the weight of tissue remaining after all water has been removed. Dry weight of the samples was obtained by drying the samples in the oven at 105°C for 48 hours. The samples were then weighed using the analytical balance (Mettler AJ100) to two decimal points. The values were converted to gram dry weight per meter square (g DW m^{-2}).

Ash-free dry weight gave the weight of the organic portion of the sample. The dried samples were placed in dry and clean ceramic crucibles. The crucibles were then combusted in the muffle furnace at 550°C for 6 hours. After combustion, the samples were weighed using the analytical balance (Mettler AJ100) to two decimal points and subtracted from the initial dry weight to give the ash-free dry weight. Subsampling was performed whenever entire samples combustion was impossible. These values were converted to gram ash-free dry weight per meter square (g AFDW m^{-2}).

3.4.1.2 Variation in Thallus Length

Thallus length of all the plants were measured before obtaining the wet weight. The methodology involved was mentioned in 3.3.1.3.

3.4.1.3 Length Classes

Percentage of individual studies samples in various length classes was computed. Comparison between each sampling period and between each line transect was performed.

3.4.1.4 Reproductive State

As mentioned in section 3.3.1.5.

3.5 Herbarium Specimens

S. baccularia and *S. swartzii* plants were collected for detailed morphological studies in the laboratory. Plants which looked alike were also collected.

Plants were collected preferably with holdfast, receptacles and vesicles. The samples were rinsed with fresh water in order to clean up sand, silt, epiphytes and small animals before pressing onto the herbarium paper. The samples were then dried in the oven at 60°C to 70°C.

Description and measurement of various parts of the plants were recorded. Sectioning (Appendix 1) of the receptacles was performed to distinguish male and female plants.

3.6 Environmental Parameters

Ambient temperature (minimum and maximum air temperatures in°C), mean number of hours of sunshine per day, mean solar radiation (MJm^{-2}) and total rainfall (mm) for Malacca (2°16'N, 102°15'E), were obtained from the Malaysian Meteorological Department.

The following environmental parameters were recorded every month using standard methods with calibrated equipment. About three to four measurements were taken every month before work on the permanent quadrats. Measurements were taken at sampling points randomly within the main sampling area. The results were averaged.

3.6.1 Salinity

Salinity was measured using an Atago hand refractometer. The unit for salinity is part per thousand (‰).

3.6.2 Water Temperature

Water temperature (°C) was measured using a salinity compensated dissolved oxygen meter (YSI Model 57) at the top 10 cm of the water.

3.6.3 Dissolved Oxygen

Dissolved oxygen in mg L^{-1} of the surface (top 10 cm) water was measured using a compensated dissolved oxygen meter (YSI Model 57).

3.6.4 pH

pH of the surface water was measured using an ATC pH meter (Piccolo 2). Water samples were collected periodically since August 1995 for pH determination using Mettler Toledo 320 pH meter in the laboratory.

3.6.5 Water Analysis

Polyethylene bottles (250 ml or 500 ml) were soaked in 10% Sulfuric acid (H_2SO_4) for 24 hours and then rinsed with deionised distilled water. These cleaned bottles were used to collect water samples for ammonia and nitrate analysis. Glass bottles (250 ml) soaked in 10% Hydrochloric acid

(HCl) for 24 hours and then rinsed with deionised distilled water. These cleaned bottles were used to collect water samples for phosphate analysis.

The water samples were collected randomly from the main sampling area and preserved by adding 0.8 ml or 2 ml of concentrated H_2SO_4 per liter water sample for ammonia or nitrate analysis respectively. All water samples were kept frozen (below 4°C) in the laboratory. Later, the samples were thawed and analyzed following the Standard Methods for the Examination of Water and Waste Water (1989). The procedure for the analysis of nutrients are shown in Appendix 2 (Ammonia), Appendix 3 (Nitrate) and Appendix 4 (Phosphate). Spectrophotometric readings for the nutrients were measured using Shimadzu UV-VIS recording spectrophotometer UV-160A.

3.7 Statistical Analysis

One way analysis of variance (ANOVA, $p<0.05$) and multiple range test (95% confidence level LSD) were used to establish whether there was any significant difference between the months for abiotic and biotic data and, between the line transects and station levels for biotic data.

The biotic data for 15-months monitoring in permanent quadrats (growth rate, degenerative rate, mean thallus length and number of plants bearing receptacles) and destructive sampling (wet weight, dry weight, ash-free dry weight, mean thallus length and number of plants bearing receptacles) were computed to monthly basis and correlated with the environmental parameters using simple correlation analysis and cross-correlation analysis. Cross-correlation analysis allows a refractory or lag period between environmental changes and plant responses to be incorporated into the calculations. Values above 0.5 were considered to be a strong correlation.

Simple correlation analysis was used to determine whether there was any significant difference among the biotic factors.

All the statistical analysis mentioned above was conducted using the computer package "Statgraphics Version 5.0".