CHAPTER 2

MATERIALS AND METHODS

Study sites

This study was carried out at two field sites in Phuket Island/Province (7°45' - 8°15'N 98°15' - 98°40'E) (Figure 5): Sirinart National Park (SNP) and Tang Khen Bay (TKB). SNP is located at the northwest side of Phuket and TKB on Cape Panwa on the southeast side. The sites are both in the intertidal zones. The environmental factors such as desiccation, light intensity, prevailing winds, amount of sedimentation and nutrient concentration that might influence the *Padina* population, were determined. In addition, during the monsoon the strong winds from the southwest hit the SNP site directly and are less forceful at TKB, on the more protected side of Phuket Island. The average rainfall showed the dry season in November-April (average rainfall 91.33±26.7 mm^3) and rainy season in May-October (average rainfall 325.93±22.77 mm^3) (http://www.tmd.go.th/province_stat.php?StationNumber=48565).

P. boryana is abundant at both sites attached to hard substrates such as dead coral and rocks (Figure 6). It occurs from 0-120 m from the shore at SNP and from 140-260 m at TKB. The exposure time during low tide of both populations is essentially the same.

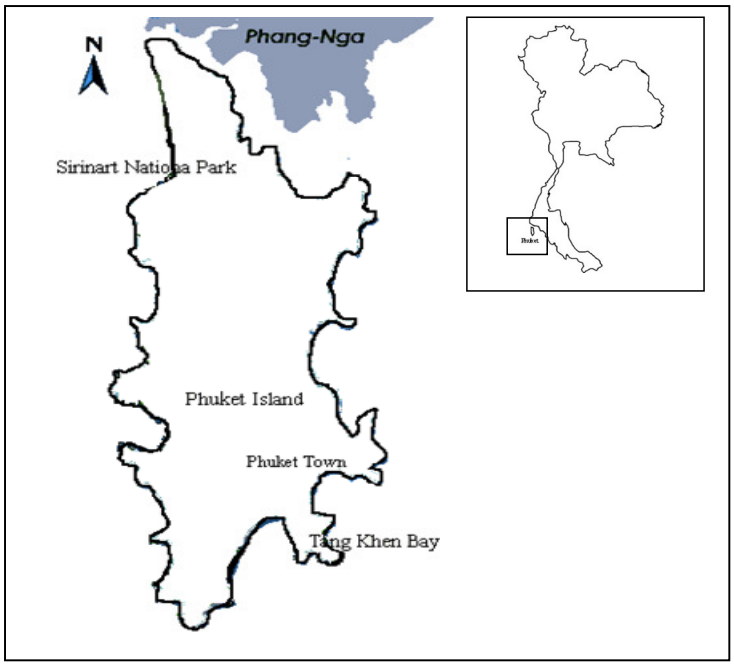

Figure 5. Map of Phuket showing the two study locations; Sirinart National Park and Tang Khen Bay.

Figure 6. Populations of *Padina boryana* Thivy from study sites; SNP (left) and TKB (right).

Materials and Methods

Growth, reproduction and recruitment were studied in the field. Thirty samples were collected randomly at 20 m. intervals to study the effect of location in the littoral zone on the population. Six intervals were established in the *Padina* distribution area (Figure 10). Samples were randomly collected throughout the year. Three permanent plots were also established at 20 m. intervals to study recruitment. The study began in September 2005 and was completed in August 2006. Salinity (by a salinity refractometer model XHO RHS-10ATC (Huake InstrumentCo. Ltd., Zhejiang, China) and water and air temperatures using thermometer were measured at both sites.

1. Growth study

The surface area of the fan-shaped thallus was calculated for the growth study. Samples were collected from the 6 shore levels. The radius (r) and length (l) were measured (Figure 7) for insertion in the following equation.

$$\text{Surface area} = \pi rl$$

To compare the population structure between two sites, all samples were grouped into size classes according to frequency of surface area. The histograms of the two study sites were compared.

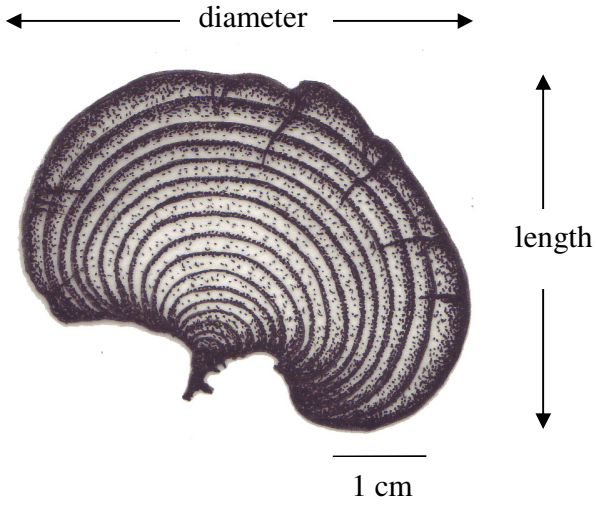

Figure 7. The whole thallus of *P. boryana* showing the length and diameter measurements.

2. Reproduction study

The samples were investigated separately at different shore levels as described above. Sporophytes, male and female gametophytes were determined. The portion of the sporangium sorus was 0.1-0.2 mm wide; the oogonial sorus was 0.8-0.13 mm wide and antheridial sorus was 0.8-0.1 mm wide on the blade. The sporophyte showed the sporangial size was bigger than the oogonial. They can be distinguished by their color and the reproductive line. In addition the oogonia are packed into a wave-like sorus on the blade while the sporangial sori are arranged more smoothly on the upper surface of the blade. The antheridia were difficult to detect by the naked eye. They are colorless and must be carefully observed under the compound microscope. The number of sporophytes, the number of reproductive rows, the quantity of spores and the quantity of released spores were monitored.

2.1 The development stage of *P. boryana*

Preliminary observations indicated that the specimens could be conveniently divided into 5 different phases according to their stages of development and reproduction.

Phase 1: the *Dictyerpa* stage*, a thin (1 mm) flattened, branched prostrate stage.

Phase 2: juvenile: thalli with small blades, no lobes, no reproductive sorus.

Phase 3: early mature adult: thalli with a single reproductive sorus (the sporangium size 0.04 mm).

Phase 4: mature adult: thalli with more than one reproductive sori (the sporangium size mostly 0.08-0.1 mm).

Phase 5: late mature thalli: the spores, eggs and sperm already released from the reproductive sori (the sporangium size mostly 0.08-0.1 mm).

*The *Dictyerpa* stage, considered to be a perennating thallus, and because of its finer, prostrate habit, avoids both the abiotic and biotic factors that would affect the characteristic fan-shaped thallus of *Padina*.

Each plant was observed under the microscope to identify and categorize it according to the above five phases and also as to whether it was a sporophyte, female gametophyte or male gametophyte. The percentages of maturity phase and life history stage were recorded. Percentage of maturity was calculated by the number of mature thalli multiplied by 100 and then divided into the total number of thalli as the following:

$$\% \text{ Maturity} = \frac{\text{Amount of thallus (phase 3-5)} \times 100}{\text{Total}}$$

2.2 Calculation of number of spore

Preliminary observations showed that each sporangial sorus has a different pattern as described below according to 5 types. The sori are basically uniform with very small variations in the number of sporagia per area. Therefore, the number of each type of reproductive row for each thallus could be calculated by counting the number of sporangia in a random sample of 1 cm of the sorus (Figure 9). The average number of sporangia for each reproductive row was calculated based on the 1 cm area for each of the 30 thalli (Table 1). This number was multiplied by the length of the entire sorus and that indicates the total number of spores in each row in any particular spore arrangement. The condition of sporangial sori was put into 5 categories (Figure 8):

Type 1: a loose arrangement of small sporangium size (0.04-0.07 mm).

Type 2: a dense arrangement of small sporangium size (0.04-0.07 mm).

Type 3: a dense arrangement of mixed sporangium size (0.04-0.1 mm).

Type 4: a loose arrangement of big sporangium size (0.08-0.1 mm).

Type 5: a dense arrangement of big sporangium size (0.08-0.1 mm).

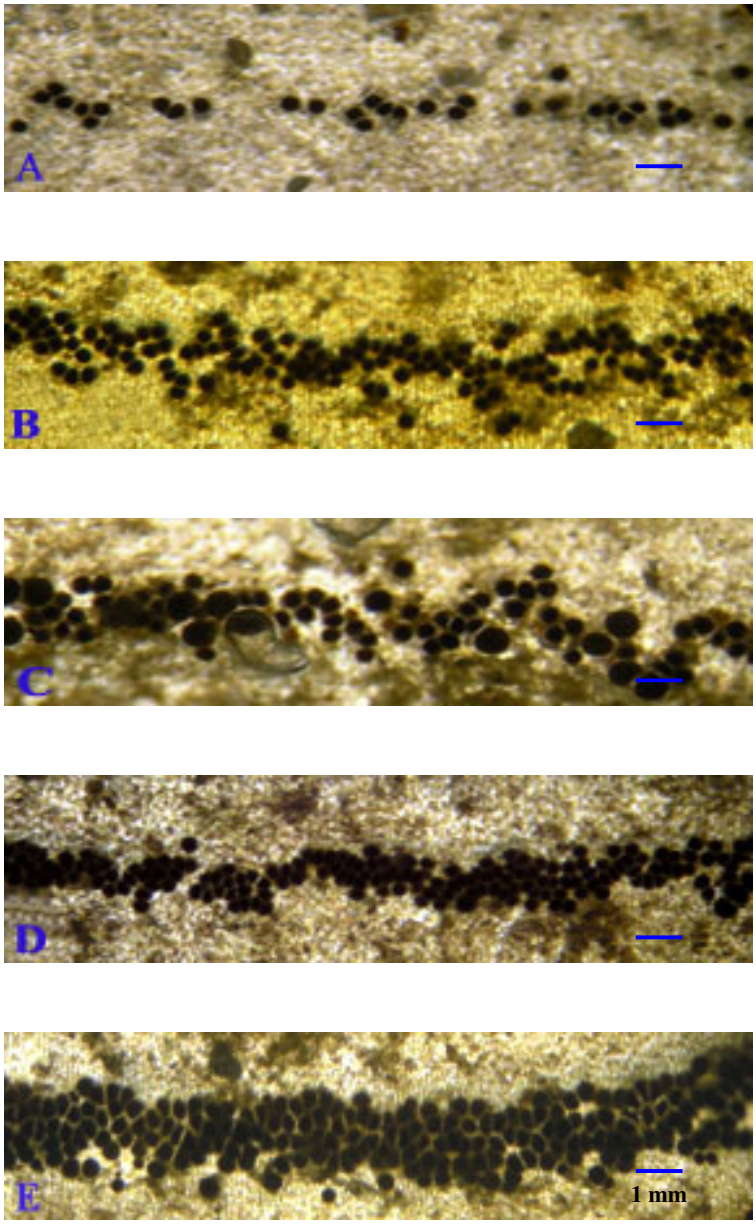

Figure 8. Sporangium arrangement on the surface of the blade (X40); A: type 1, B: type 2, C: type 3. D: type 4, E: type 5.

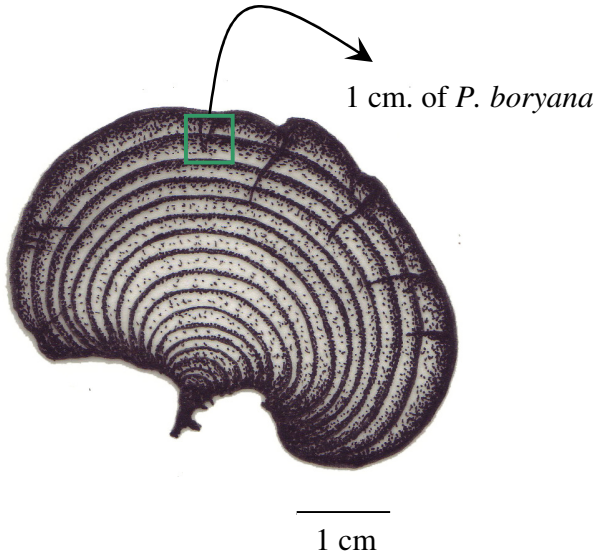

Figure 9. A one centimeter length of *P. boryana* reproductive line was randomly selected to investigate the number of sporangia/centimeter.

Table 1. The average number of each type of sporangium arrangement.

Type	Average number of sporangium/cm (mean±SE)
1	88±7
2	124±7
3	93±5
4	101±4
5	152±9

3. Recruitment study

Three 0.25 m^2 permanent plots were established randomly on hard substrata every 20 m in the *Padina* zone (Figure 10). All organisms in the permanent plots were removed and the substrate was scraped with a wire brush in the first month of study. Care was taken to remove the biota within a 1-2 cm border to avoid the bias of recruitment caused by "edge effects" (Foster and Sousa, 1985; Hutchinson and Williams, 2001). The substrate was also burned with a portable propane torch to sterilize it completely (Murray and Littler, 1978; Hutchinson and Williams, 2001). The number of new individuals was the basis for the data for this study. The percentage cover of *Padina* represented recruitment success by the quadrat method. The permanent plots were photographed to document the changes in the population.

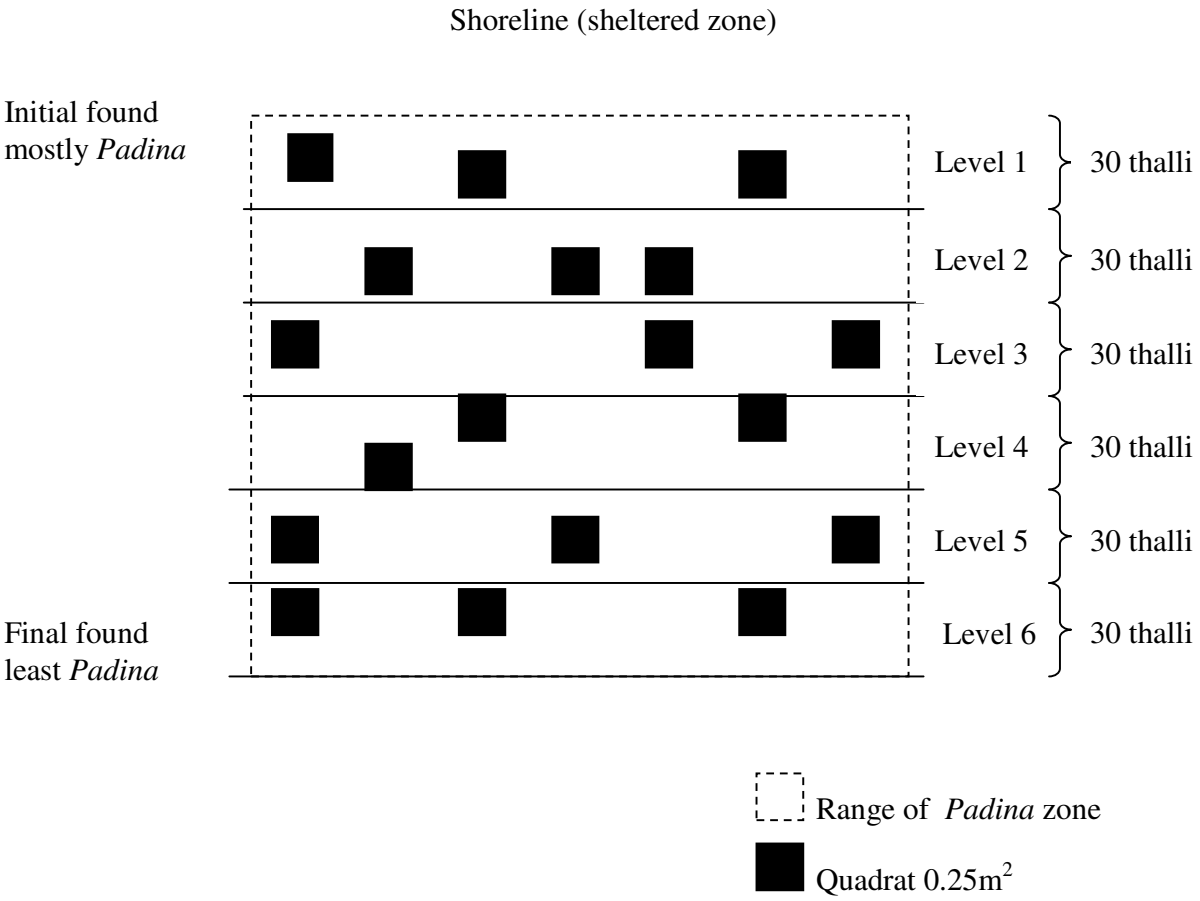

Figure 10. System of collection to assess the population distribution, reproduction and recruitment of *P. boryana*.

Statistical analyses

To compare the *Padina* population distribution at the 2 sites (TKB VS SNP), surface areas of 180 plants were measured and grouped into frequency size class distributions each month. The Kolmogonov-Smirnov test was employed to determine the differences of size distribution between sites and among months.

To compare the differences in average surface area, the number of produced and released spores, percentage of reproduction, percentage of sporophytes and percentage of gametophytes and percentage cover of *P. boryana* among shore levels (6 levels), among months (12 months) and between shores (2 shores). The data did not meet the assumptions of the parametric test even if they were transformed. Statistical results were presented based on non-parametric analyses (Stepwise Mann-Whitney U).

Spearman Rank Correlation Coefficient was employed to test the effects of physical factors such as air temperature, water temperature and salinity on average surface area, the number of produced and released spores, percentage of reproduction, percentage of maturity, percentage of sporophytes and gametophytes and percentage cover of *P. boryana*. Moreover, correlation between surface area and number of spores was also tested. All data were analyzed using SPSS for windows version 11.0. (Dytham, 1999; Monparwongsanon, 2003).