2. MATERIALS AND METHODS

Study site

The study site is located in the mid-intertidal zone, Koh Pling, Sirinat Marine National Park (8^o $05^'$N, 98^o $17^'$E), Phuket province, Southern Thailand (Figure 1 and 2). This national park covers an area of 90 square kilometers on the north-western tip of Phuket Island. It was established in 1992 and was previously known as Had Nai Yang national park. The climate of this area is under monsoonal influence. There are two dominant seasons, a rainy season dominated by southwest monsoon (May-October) and a dry season predominated by northeast monsoon (November to April). During this study, maximum water temperature overlying at this site ranged from 27°C in July 2004 to 37 °C in September 2004. In November 2004, maximum salinity was 35 PSU and the minimum salinity was 29.5 PSU in July 2004. The southwest monsoonal winds are extremely forceful from May to October, so the wind speed would be greater during this period. The winds may have a large effect on water movement and sediment in the shallow seaweed beds. This area has various types of marine habitats, such as rocky shores, coral reefs and seagrass beds. The reefs are located about 700 to 1,000 meters away from the shore and at the depth of 4 to 7 meters. It was previously reported that the area is rich in marine organisms (Prathep, 2005) and dominated especially by diverse algal groups such as red algae, brown algae, green algae and blue green algae. Observation found that herbivores in the study site are mainly damselfishes and parrotfishes (Figure 3). The dominant species of this site are *Abudefduf vaigiensis* and *Stegaster nigricans* and *Scarus niger*. Size of damselfish was varied between 2 to 5 cm in length. Damselfishes

(Demoiselles) are a large group of coral reef fishes including approximately 320 species worldwide and about 120 species from Australian seas. Most damselfishes are territorial and zealously defend their small plot against all intruders regardless of size. Damselfishes feed on a wide variety of plants and animals including seaweeds.

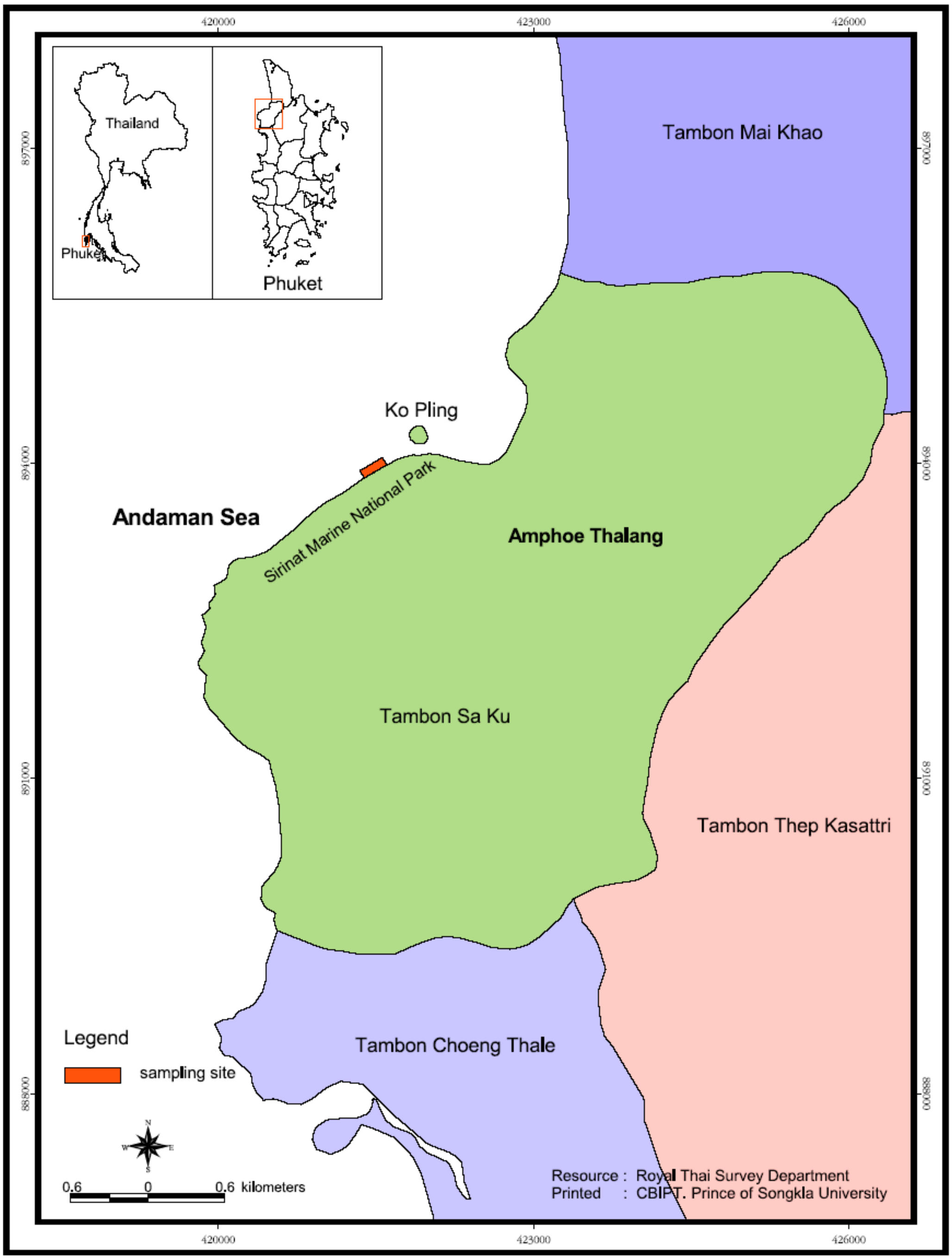

Figure 1. Map of the sampling site at Koh Pling, Phuket Province, Thailand.

Figure 2. Homogeneity substrate at the intertidal shore

Figure 3. Many herbivorous fishes are at the study site when the tide comes in.

Methods

The effects of herbivory and season of clearing on species composition and algal succession were experimentally tested in the mid-intertidal zone following the flow chart in figure 4. To determine the effect of season of clearing on algal succession or communities, dead coral patches were cleared by hand chiseling and then scraped with a wire brush and scalded with saturated sodium hydroxide (Padilla,1981, Gaines, 1982 see Littler and Littler, 1985 for references) in January 2004 and July 2004 to determine a season effect of clearing. This procedure cleared all organisms (including holdfasts, crustose algae, etc.) and sterilized the substrate. These clear patches were then used to quantify succession of algae. Ten experimental units (20 cm ×20 cm) were marked using thread and labeled, while another ten unmanipulated plots were marked and labeled as control. To determine the effect of herbivory on algal succession, cages were used to exclude herbivores. Herbivory were manipulated into two categories: five fully caged plots both experimental and control plots, and five uncaged plots both experimental and control plots. Cage size was 25 cm ×25 cm ×20 cm (larger than the experimental patches (20 cm×20 cm) to eliminate edge effects). Cages were made of a stainless steel frame covered with wire mesh (mesh size was 2cm×2 cm). All cages were fixed on the dead coral substrate with concrete nails and damaged cages were replaced occasionally (Figure 5). Light intensity and water motion inside and outside cage was measured using luxmeter model DIGICON LX-50 and a mini current meter model SD-4 (4A) (Sensordata a.s., Bergen, Norway). The herbivore exclusion cages used in this study decreased total light intensity by 5.14–14.66% compared to uncaged plots. However, total light intensity was much greater than the level needed for saturation of the photosynthetic rate of a broad variety of algae (Hata and Kato, 2003). The cages also decreased wave motion to 4.77±1.16% less than the uncaged plots. Therefore, we assumed that shading and wave motion decreasing by cages had a minimal influence or non influence to algal succession.

All patches were monitored every two months from January 2004 to July 2005. Monitoring was carried out during low tide when most of the patches were exposed to the air and direct sunlight. Data collection of the 1st set experiment was done from January 2004 (early dry season) to January 2005 and the 2nd set experiment was conducted from July 2004 (early rainy season) to July 2005. The two sets of experiments were designed to test for the effect of different clearing season on species composition and algal succession. Quadrates of the same size as the experimental plots, with 2 cm×2 cm subplots adapted from Kim and DeWreede (1996) marked off within the quadrate frame (100 subplots for 20 cm×20 cm) were used for measuring the abundance of organisms. Percent cover of each species was estimated by counting the number of subplots in which a species occupied. The accuracy and repeatability of this technique has been tested by Dethier *et al*., (1993) and they reported that this method is generally more accurate than the random-point-quadrate method. Algal covers included only those thalli whose holdfasts were in the plot.

All patches were photographed by using a digital camera. Specimens were collected and taken to the laboratory and identified using various taxonomical identification guides, *e.g.* Common Seaweeds and Seagrasses of Thailand (Lewmanomont and Ogawa, 1995), Seaweeds of Queensland (Cribb, 1996), Caribbean Reef Plants (Littler and Littler, 2000), Plant Resources of South-East Asia No 15 (1) and Cryptograms: Alga (Prud'homme van Reine and Trono, 2001). Algal samples were preserved with 4% formaldehyde and were photographed. Samples for sediment were collected bimonthly from March 2004 to May 2005. Samples were taken in three shore levels, upper, middle and lower shore level for comparing the amount of sediment between shore levels. Three samples were placed randomly at each shore level during the low tide and the patches were left 24 hours. After that the samples were placed in plastic bags and transferred to the laboratory and then the samples were dried at 60 °c. The dried sediment samples of same weight were then sieved using a shaker classified into seven size fractions: > 2.00 mm, 1.00-2.00 mm, 500 µm-1.00 mm, 250-500 µm, 125-250 µm, 63-125 µm and < 63 µm, and then each fraction was weighed.

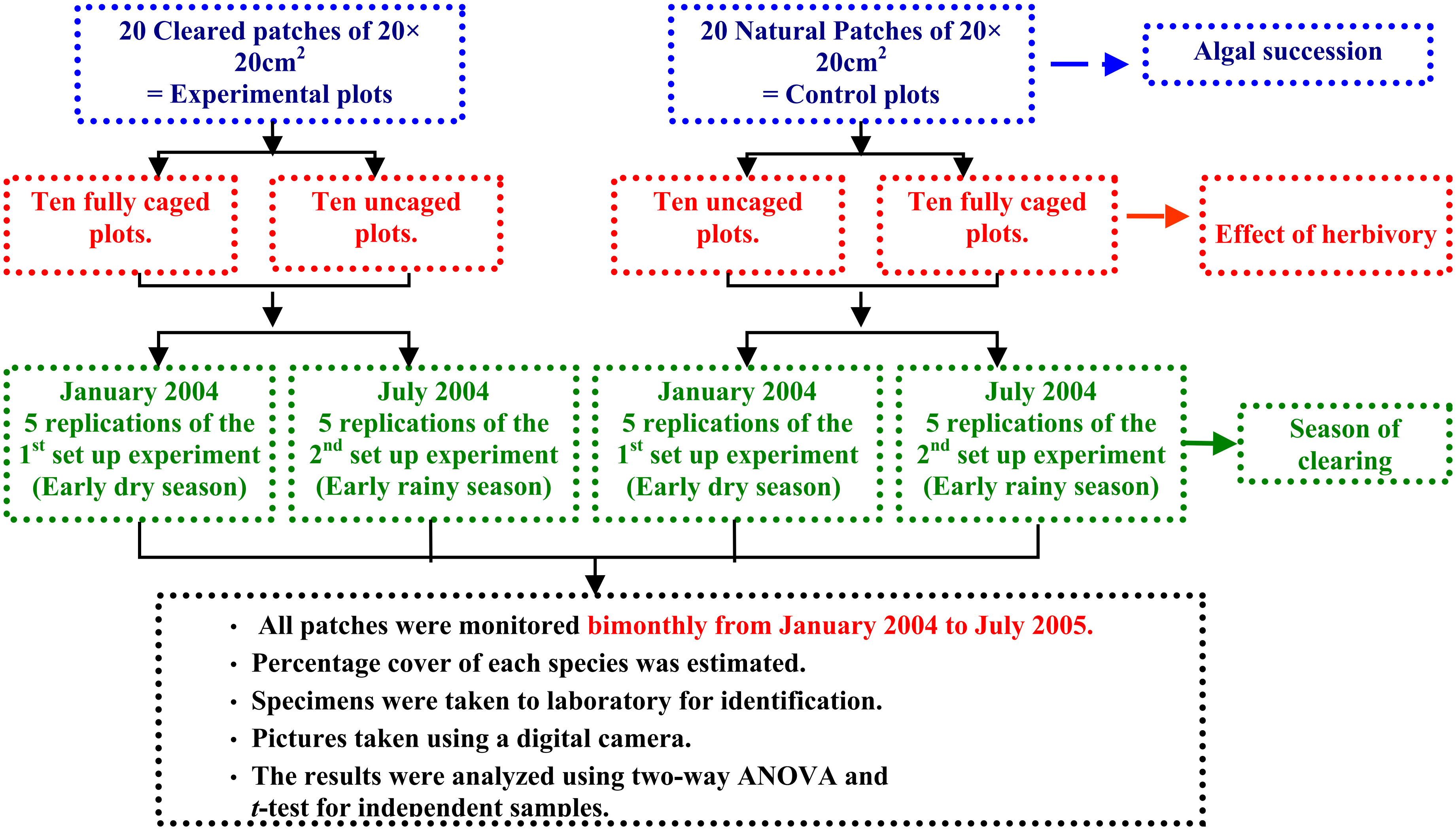

Figure 4. Flow chart of experimental design.

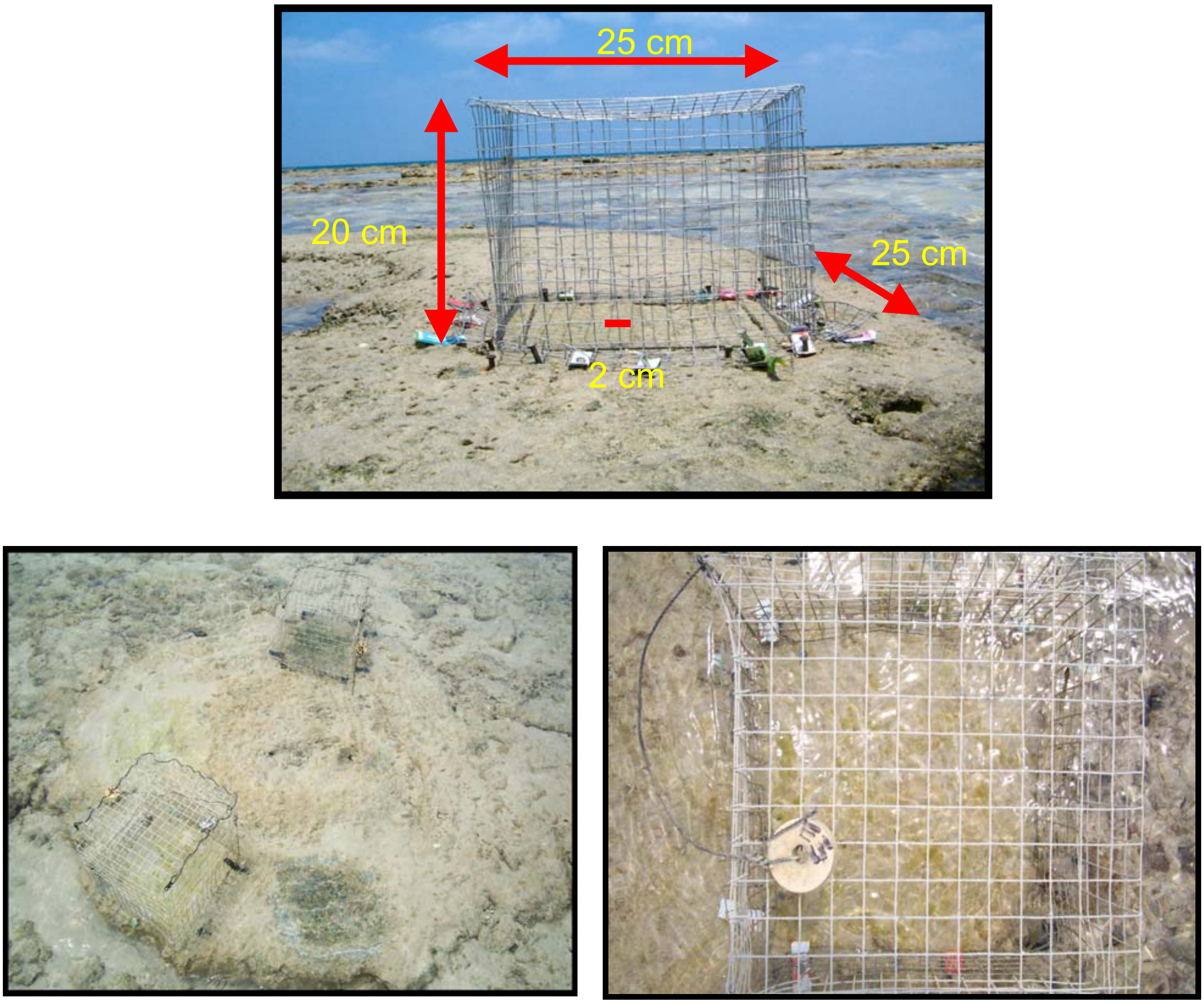

Figure 5. Cage was used to exclude herbivores.

Upper: Cage size was 25 cm ×25 cm ×20 cm and cage was covered with wire mesh (mesh size was 2 cm×2 cm).

Lower: Experiments during the tide came in.

Statistical analyses

The effects of herbivory, season of clearing and their interaction on change in percent cover of algae in manipulated plots were analyzed using two-way analysis of variance (ANOVA), with cage and season as fixed factors. Cochran's *C*-test was used to determine homogeneity of variances. The raw data in this study violated the assumption of homogeneity of variance and therefore appropriate transformations of the data were tests (Underwood, 1981). Log (x+1) was the most appropriate transformation. The percent cover of each algal species was log (x+1) transformed prior to analysis. Differences between mean effects of herbivore exclusion and season were tested with *t*-test for independent samples. Species diversity and species evenness can be calculated using the Shannon-Wiener index (Pielou, 1977) and Simpson index (Pielou, 1977) at different seasons and two densities of herbivory. All data were analyzed using the computer program SPSS for Windows version 10.0.