CHAPTER 2

MATERIALS AND METHODS

1. Study area

Thale-Noi, a shallow roundish lake, is located at the northernmost end of the overall Songkhla lake system in Phatthalung Province, Southern Thailand (Buapetch, 2002) between latitude 7° 45′ N to 7° 55′ N and longitude 100° 05′ E to 100° 15′ E (Pholpunthin, 1997). It covers an area of 30 km^2, has a shoreline of about 20 km, and contains about 32 M m^3 of water (Kuwabara, 1995). Thale-Noi is one of the few surviving intact freshwater wetland ecosystems in Thailand. It comprises several distinct topological areas: swamp forest, lake, moist evergreen forest and agricultural lands (Storer, 1977). All of these areas are important feeding sites for bird and wildlife species including aquatic animals, phytoplankton and zooplankton. Thale-Noi is an important waterfowl reserve in Southern Thailand (Leingpornpan and Leingpornpan, 2005). More than 187 species of waterfowl, including both migratory and indigenous birds, make their home at Thale-Noi. Thale-Noi has been named the first world wetland site in Thailand and there are aims to preserve the sustainable ecology of the area (Aiumnau *et al*., 2000). The principal inflow to the lake is the runoff from the steep forested slopes of the Banthad Mountains to the west. Outflow is via the Klong Nang Riam, Klong Ban Glang and Klong Yuan canals into Thale Luang, Lake Songkhla. The lake is rather shallow with a mean depth of 1.2m but water levels can fluctuate up to one meter, typically reaching their lowest level in

August. The lake is normally fresh to slightly saline (1.48 ppt). The salinity may rise during the driest months (to 3.5 ppt) when saline water from Lake Songkhla may intrude. The pH varies spatially and seasonally from 1.2 - 8.1 (average 4.4). The northern end (near the *Melaleuca* swamp forest) is more acidic than the south. Acidity increases during the rainy season from the leaching of acidic humus. The climate is tropical monsoon with an average annual rainfall of 2,208 mm, and the mean pan evaporation rate is 1,753 mm (Aiumnau *et al.,* 2000).

Twelve plankton sampling stations were selected for this study (Fig. 2 and 3). These stations were representative of four different habitats in Thale-Noi: a peat swamp zone (1, 2, 3), a small inlet zone (4, 5, 6), a resident zone (7, 8, 9) and a pelagic zone (10, 11, 12). The decision where to site the selection zones was based on a preliminary survey and information from previous studies (Angsupanich, 1985; Nookua, 2003). Data from the preliminary survey in the Thale-Noi area indicated diverse microhabitats of waterbodies, including swamp forest, *Melaleuca* forests, moist evergreen forest and agricultural lands. Therefore, twelve stations were designed to cover areas of all zooplankton sampling. Site locations were determined by using an electronic global positioning system (GPS).

2. Climate and monsoon system

Thale-Noi's climate is strongly influenced by the tropical monsoon system (Table 4); the northeast monsoon (November to April) and the southwest monsoon (May to October) (Colborm, 1975 cited by Suphakason, 1992). In addition, three principal seasons characterize the climatic periods: the light rainy period from late April to August, the rainy period from August to December, and the dry period

from January to April (Hembanthid, 2001). Monthly average precipitation levels recorded for the period 1991-1995 in the Thale-Noi area at Banpraw village, Parpayom District, ranged from 54.3 mm in January to 645.4 mm in November, with an overall monthly average of 193.3 mm (Thungwa *et al*., 1990 cited by Hembanthid, 2001). In the present study, decisions regarding the selection of sampling periods were determined by the precipitation pattern from ten years ago and the monsoon system. The data on the precipitation of Thale-Noi lake during the present study (2004-2005) was obtained from areas in the Khuan-Kanun District, Phatthalung Province (Fig. 4).

Table 4. The monsoon system pattern in the Thale-Noi area.

Period	**Monsoon system**
January – February	The northeast monsoon
March – April	The end of the northeast monsoon and a period of changing wind direction.
May – June	The beginning of the southwest monsoon
July – August	The southwest monsoon
September – October	The end of the southwest monsoon and a period of changing wind direction.
November – December	A period of changing wind direction and the beginning of the northeast monsoon

Source: Colborm (1975) cited by Suphakason (1992).

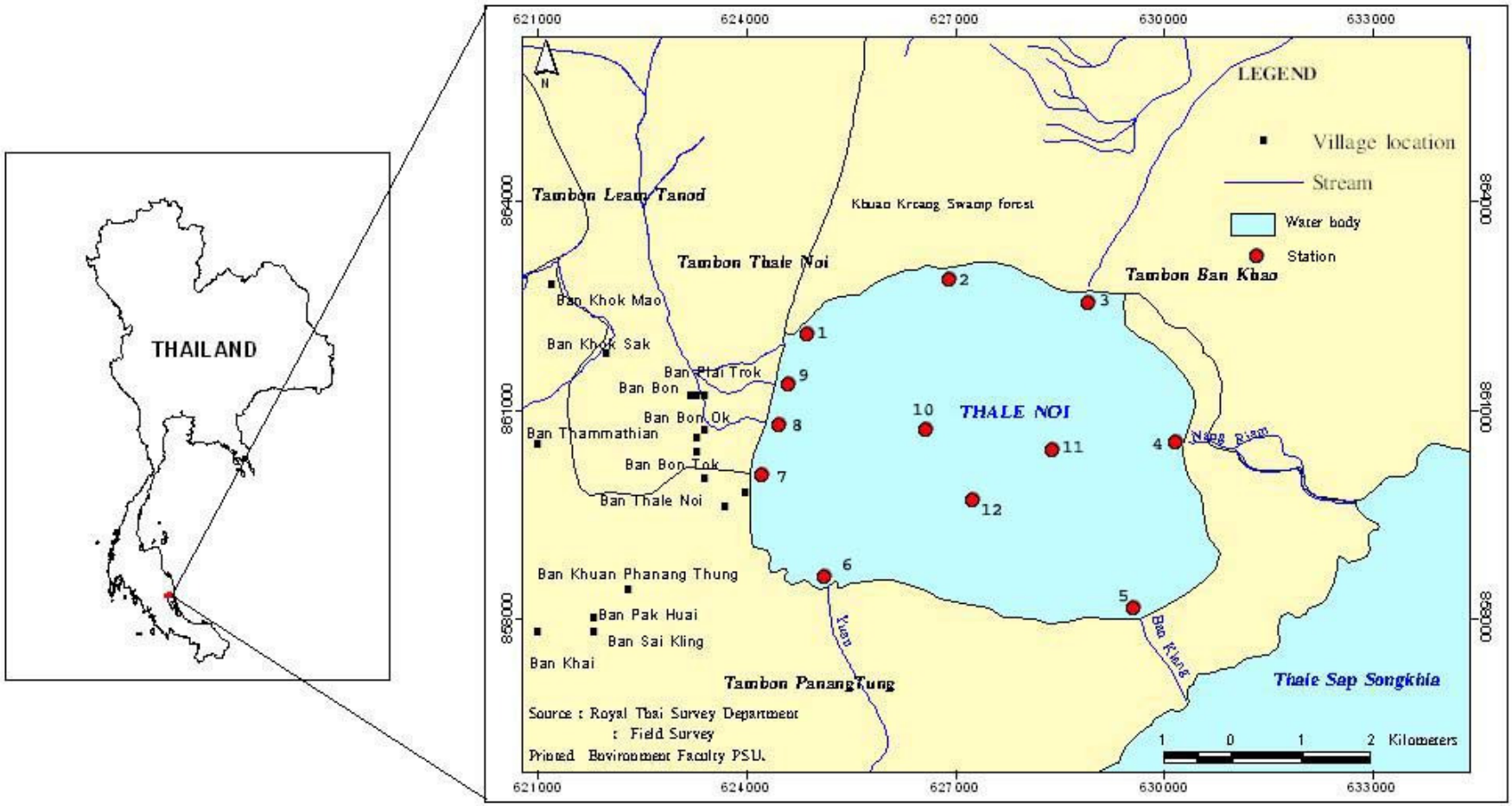

Figure 2. Study area and sampling stations in Thale-Noi, Phatthalung Province.

Specific information related to each zone

Peat swamp zone

Location: 07˚47.863'N - 07˚48.237'N, 100˚07.720'E - 100˚09.743'E

Stations: 1, 2, 3

The peat swamp zone is predominately situated at the north of the lake (next to Khuan Kreang swamp forest). This area is under water during times of flooding. The dominant types of vegetation are *Malaleuca* forests and grasses. The water is clear but the bottom has an accumulation of organic matter or peat. The water quality is highly acidic, lacking oxygen and nutrients. However, there are many lotus flowers and emergent plants in the water. This zone is rather shallow with a minimum depth of 0.4 m.

Small inlet zone

Location: 07˚45.894'N - 07˚47.133' N, 100˚07.974'E - 100˚10.569'E

Stations: 4, 5, 6

Thale-Noi has three outflows which are discharged into Songkhla Lake. They are the Nang Riam, Ban Glang and Yuan rivers. All of these small inlets were selected as sampling sites; stations 4, 5 and 6, respectively. The area of surface water and water quantity of this zone vary according to sea level. The water is quite turbid throughout the year. An exception is Nang Riam River, where the water is usually clear during the rainy season. Aquatic plants are less abundant as compared to the peat swamp zone. Water depth is relatively high, increasing to a maximum value of 3.0 m in the rainy season, especially in Nang Riam River.

Resident zone

Location: 07˚46.483'N - 07˚47.328'N, 100˚07.645'E - 100˚07.685'E

Stations: 7, 8, 9

The resident zone is located close to fish pens and the human resident area along the shore, situated on the western side of Thale-Noi. The dominant vegetation is submerged plants such as *Hydrilla verticillata*, *Ceratophyllum demersum* followed by floating plants such as lotuses. These act as food sources for fish and small aquatic animals. However, they can also adversely affect water quality by reducing the amount of dissolved oxygen concentration in the water.

Pelagic zone

Location: (07˚46.625'N- 07˚47.043'N, 100˚08.369'E- 100˚09.412'E)

Stations: 10, 11, 12

The pelagic zone is located in the middle of Thale-Noi where dense submerged vegetation covers the lake bottom. *Hydrilla verticillata* and *Utricularia flexussa* are the dominant species. The water color is brown and the bottom is covered with a thick detritus layer showing high biological production. The water transparency in this zone is increased when the submerged vegetation is dense. The amount of floating plants is very low; probably due to the effect of wind and wave actions. This zone is reliant on flood conditions.

Figure 3. Sampling stations in Thale-Noi lake: Stations 1-3, Peat swamp zone; Stations 4-6, Small inlet zone.

Figure 3. Continued. Stations 7-9, Resident zone; Stations 10-12, Pelagic zone.

3. Methodology

3.1 Zooplankton sampling and analysis

Quantitative zooplankton samples from each station were taken using two different sampling methods. The first was a horizontal towing using a 200 μm plankton net fitted with a flow meter towed by a low speed boat for three minutes. The second was a filtration of 20-50 liters of water through a 20 μm plankton net. The zooplankton samples were immediately preserved in a 5% formaldehyde solution and brought to the laboratory for further analysis. Zooplankton sampling was conducted twice a month in three bimonthly periods, comprising the moderate-water phase (light rainy period) in July and August 2004, the high-water phase (rainy period) in November and December 2004, and the low-water phase (dry period) in March and April 2005.

In the laboratory, the 20 μm net samples were separated into two nominal size fractions: 20-200 μm (microzooplankton), and > 200 μm (mesozooplankton) by filtering plankton samples through a 200 μm sieve. Between 50% and 100% of all specimens, from the two sampling methods, were counted and identified to genus or species levels using Olympus CH-2 Compound and Olympus SZ-40 Stereo microscopes. Zooplankton identification was based on information from the following experts: Koste (1978), Theodore *et al.* (1979), Idris (1982), Smirnov (1992), Korovchinsky (1992), Segers (1995-1996), Wongrat (2000), Sanoamuang (2002) and Maiphae (2005). Quantitative analysis of protozoans and rotifers was performed in a Sedgwick-Rafter Counting Cell, counting between three and five slides (depending on abundance) in order to determine the density and relative

abundance of all species. Crustacean zooplankton were counted in reticulated acrylic chambers in sub samples varying from 10 ml to the entire concentrated sample (30 ml), depending on the concentrations of organisms. Some species of rotifers were put on slides and mastex preparations with sodium-hypochlorite were made whenever necessary so that specimens could be examined under a compound microscope. Some specimens, such as cladocerans, ostracods and copepods, were placed on slides with a drop of glycerin, dissected using a stereo microscope, and examined under a compound microscope. The identification uses not only the outer morphological characteristics but also, in most cases, a more detailed examination of the inner characteristics. Thus, the dissection method for cladocerans, ostracods and copepods followed that described by Maiphae (2005), Wongrat (2000) and Sanoamuang (2002), respectively.

3.2 Water sampling and analysis

At each station, depth, transparency, conductivity, temperature, salinity and pH were measured *in situ*. Water depth was determined using a tape measure and transparency was determined using a Secchi disc. Additionally, temperature, conductivity and salinity were automatically measured using a YSI 30 model 30/10 FT, and pH was determined using a YSI60 model 60/10 FT. One liter samples of water were collected using polyethylene bottles. All samples were stored in ice boxes during transportation to the laboratory for chlorophyll *a*, total solid and dissolved oxygen (DO) concentration analysis.

Water samples were analyzed for total solids, dissolved oxygen and chlorophyll *a* in laboratory conditions following the Standard Method (APHA,

AWWA, and WEF, 1998). Size-fraction of the chlorophyll *a* was analyzed. 250 ml water samples were filtrated through 200 μm mesh nets to eliminate zooplankton. The filtrated water was then poured sequentially through 20 μm mesh nets. The residual on the 20 μm net was re-suspended in distilled water and analyzed for chlorophyll *a* fraction size of 20-200 μm. The samples that passed through the 20 μm mesh net were analyzed for chlorophyll *a* size fractions of < 20 μm. The two size-fractions of chlorophyll *a* were filtered through Whatman GF/C glass fiber filters and the filters were kept deep frozen for later analyses. Pigments were extracted in 90 % acetone. Absorbances of the extracts were measured at 750, 664, 647 and 630 nm with a spectrophotometer.

3.3 Data analysis

3.3.1 Calculation: The total number of zooplankton in the water sample

Density of organisms was calculated from the volume of water filtered and the size of each sub sample, and expressed as numbers of individuals per cubic meter. The total number of zooplankton present in a cubic meter of water sample from the Sedgwick-Rafter Counting Cell can be calculated using the following formula:

$$N = n \times v \frac{(1000)}{V}$$

where N : total number of zooplankters per cubic meter of water filtered;

n : average number of zooplankters in 1 ml of plankton sample;

v : volume of plankton concentrate (ml);

V : volume of total water filtered (l).

For the horizontal net (200 μm), the sample can be determined as follows:

$$T = N/V$$

Where T : total number of zooplankters per cubic meter of water filtered;

N : number of zooplankton in the concentrate plankton sample (individual);

V : total volume of water actually filtered by the net (m^3).

The volume of water filtered during a tow (V) can also be calculated using the following procedure:

$$V = a \times m \times n$$

Where a : the area (m^2) of the mouth of the net (the present study, πr^2 = 3.14x0.18x0.18 = 0.1 m);

m : the towing distance (m) per one flowmeter revolution in the calibration (the present study, m = 0.029 m);

n : the number of flowmeter revolutions.

3.3.2 Zooplankton community

Spatial and temporal variations of zooplankton density and environmental parameters were analyzed using an analysis of variance (ANOVA). Period of sampling and zone were treated as fixed factors. Abundance was transformed to log (x+1) to normalize the variance.

3.3.3 Environmental variables

Environmental data including temperature, pH, dissolved oxygen (DO), salinity, conductivity, total solid, depth, transparency, chlorophyll *a* fraction size of <20 μm and chlorophyll *a* fraction size of 20-200 μm (which were used without transformation) were analyzed using the multivariate test SPSS for Windows.

3.3.4 The influence of environmental variables on the zooplankton community

The correlation between the abundance of each zooplankton genus and environmental factors was investigated at each zone and during each period by Canonical Correspondence Analysis (CCA), incorporating the unimodal response of a species to environmental variables. Linear combinations of environmental variables were selected to provide maximum separation in species distribution. The CCA procedure produces an ordination diagram in which genera are represented by points and environmental variables by vectors. The statistical significance of the relationship between a set of environmental factors and genus composition was estimated using a Monte Carlo permutation test.

Zooplankton species data was converted to relative abundance values. To assess the homogeneity of variance, the relative abundance was transformed using log (x+1), which prevented the creation of undefined values due to having zeros in the data set. In addition, the zooplankton data was approved by distinguishing reference samples from impaired samples with downweight rare species, to be used in CCA.

Spearman rank correlations were also used to investigate the relationships between the two size fractions of chlorophyll *a* and zooplankton abundance (both zooplankton in each group and genus).

Statistical analysis details: CCA, using the PC-ORD program, version 3.2. ANOVA and Spearman correlation were employed in combination with the SPSS program, version 11.0 for Windows.