CHAPTER 3

METHODOLOGY

3.1 Study area

Geography

The selected sites are three mangrove canals located between latitude 12° 11′ 42″ to 12° 15′ N and longitude 102° 31′ 30″ to 102° 39′ 30″ E, Trat's Muang district, on the eastern coast of the Gulf of Thailand. The first, Bangphra Canal, 11 kilometres long, runs through 3 sub-districts of Wang Krajae, Nong Samed and Nong Khansong, and empties into Trat Bay. Along both sides of the canal covered with natural recovery mangrove after forbidding of woodcutting for charcoal, firewood and construction materials about ten years ago. Drainage of domestic sewage from Trat municipality also goes into this canal. The second, Thaprik Canal, 9 kilometres long, starts from Thaprik sub-district and passes through many parts of mangrove area, which were converted to shrimp farming, before opens to Trat Bay. The third, Thaleuan Canal, 8 kilometres long, its inner part is blocked by the water gate prior to running through the natural mangrove area of Takang and Chamrak sub-district, and then opens to Trat Bay (Figure 3.1). Mangrove forest in the study area comprised the common species of *Rhizophora apiculata, Rhizophora mucronata, Bruguiera gymnorrhiza, Ceriops tagal, Xylocarpus granatum, Excoecaria agallocha* and *Lumnitzera littorea* (Patanaponpaiboon, Rungsupa and Menasaveta, 1994).

Climate and rainfall

Trat province is classified by two monsoon seasons. The dry northeast monsoon covers four months from December to March and the wet southwest monsoon covers eight months from April to November. According to Trat provincial statistical office (1997), in 1996, Trat received 4,206.4 mm of rainfall with the highest rainfall began from May to October. The wettest month was in August with 1,018.9 mm of rainfall. The lowest rainfall started from December to February. The driest month was in December with 5.8 mm of rainfall. The annual average temperature varied between 21.2 °C to 33.6 °C. The lowest and highest temperature was 16.7 °C and 35.1 °C in January and November, respectively.

3.2 Study period

The period of research was divided into three phases. First, the fieldwork on sample collection was conducted in wet and dry season. In wet season, three months of sampling on fish, zooplankton, and water for biological, physical and chemical study were carried out in August, September and December 1997 while three months of collection in dry season started in December 1997, January and February 1998. Second, the laboratory work on water analysis, fish and zooplankton identification and the classification of land use were carried out as soon as possible after the finish of each field trip and they were completed in June 1998. The last phase, the data analysis and preparing of thesis proposal was conducted from July 1998 to March 1999.

3.3 Sampling methods and instruments

Species composition of fish

Fish collecting by using push net and drift gill net (Figure 3.2) was conducted in three mangrove canals, Bangphra, Thaprik and Thaleuan Canals, as following:

1. Fish collecting by push net was operated within the distance of 1 kilometre from each station, which was marked in each canal, the downstream (the mouth), the midstream and the upstream (Figure 3.1). Push net used in this study was 4 metres wide at the mouth, 4.5 metres long at the beam, 3 and 2.5 centimetres nylon mesh sizes of the mouth and the neck, respectively. The polyfilament drift gill net, with mesh size of 3.5 centimetres, 1.5 metres deep and 45 metres long, was used for collecting on pelagic fish. The fish sampling by drift gill net spent about half an hour per station.
2. The sampling in wet season was done in August, September and October 1997 while in dry season it was done in December, 1997, January and February 1998. The sampling by using both fishing gears was carried out twice a month, the one was during spring tide and the other was during neap tide. One day and one night of sampling by using each fishing gear was conducted in each tide. All fresh specimens of fish after catching were classified into groups, took photographs, measured the total length, weighted by One-kilogram Weighted Scales and counted the number of

individual. Then all specimens were preserved by 10 % neutral formalin before specific identifying in laboratory.

Biological parameters

1. Zooplankton was sampled once a month during spring tide in each station of three mangrove canals in each season.

1.1 Plankton net of 330 microns of mesh size, with 30 centimetres of mouth diameter was used for the collection of zooplankton. The flow metre was installed at the mouth of plankton net before the horizontal hauling for five minutes per each station.

1.2 Volume of zooplankton sample in each station was preserved by 5 % neutral formalin in 300-ml polyethylene bottle and then measured by 50-ml graduated cylinder prior to identification into various taxa in laboratory.

2. Chlorophyll a concentration was used as the indicator of the availability of phytoplankton (Strickland and Parsons, 1972). Chlorophyll a was measured once a month during spring tide in each station of three mangrove canals in each season.

2.1 Sampling of water was conducted at 0.5 metres deep from the surface of each station. Water sample was filtrated by the filtration instrument passing through the GF/C glass paper with diameter of 47 millimetres. In each station, the amount of water sample after filtration was 500-1,000 millilitres.

2.2 The GF/C glass paper which phytoplankton inside, was folded into a half circle and kept in a black plastic bottle, recorded about the sampling date, station and the place. Then it was frozen in icebox before the extraction of Chlorophyll a in laboratory as soon as possible.

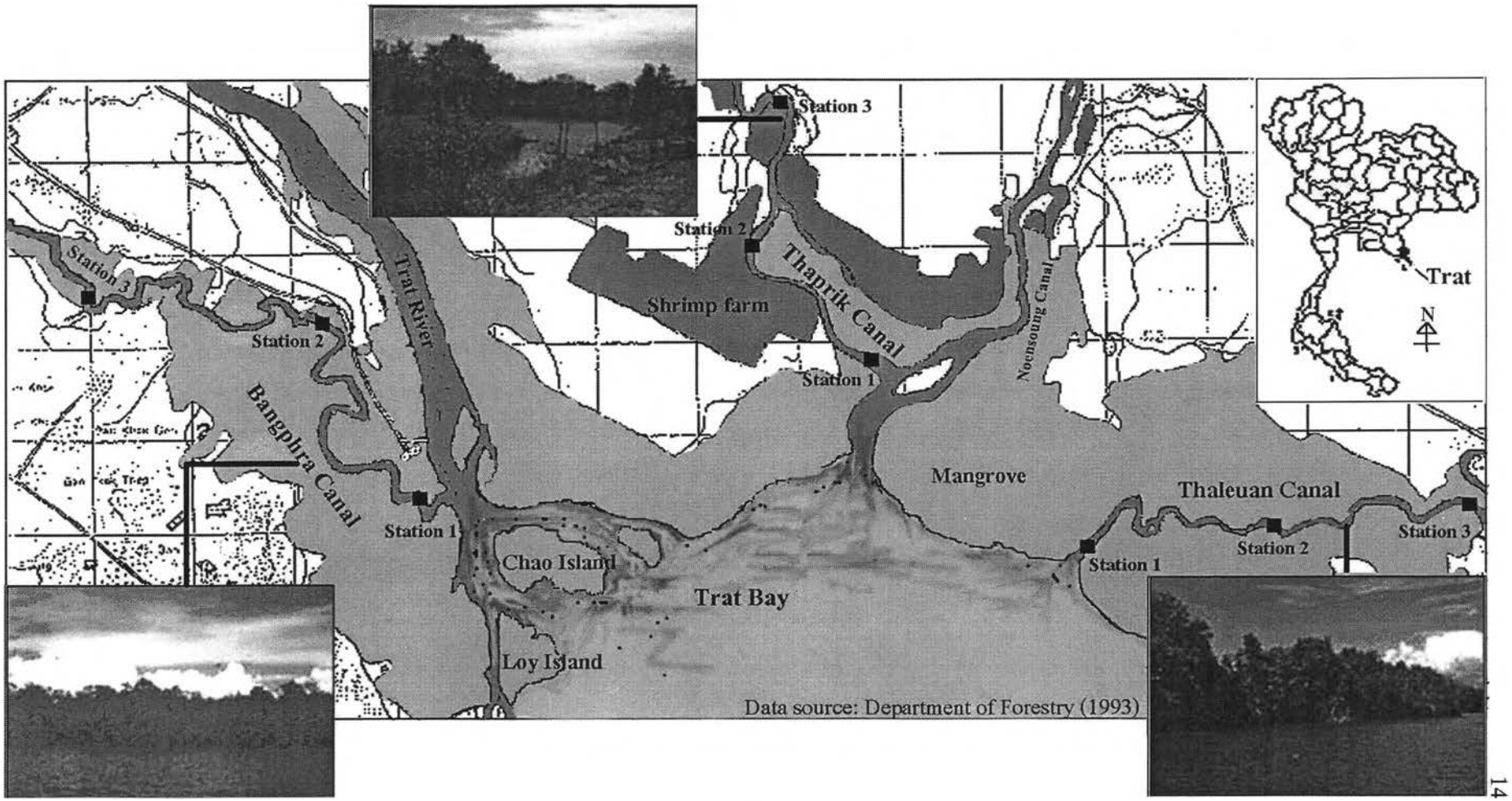
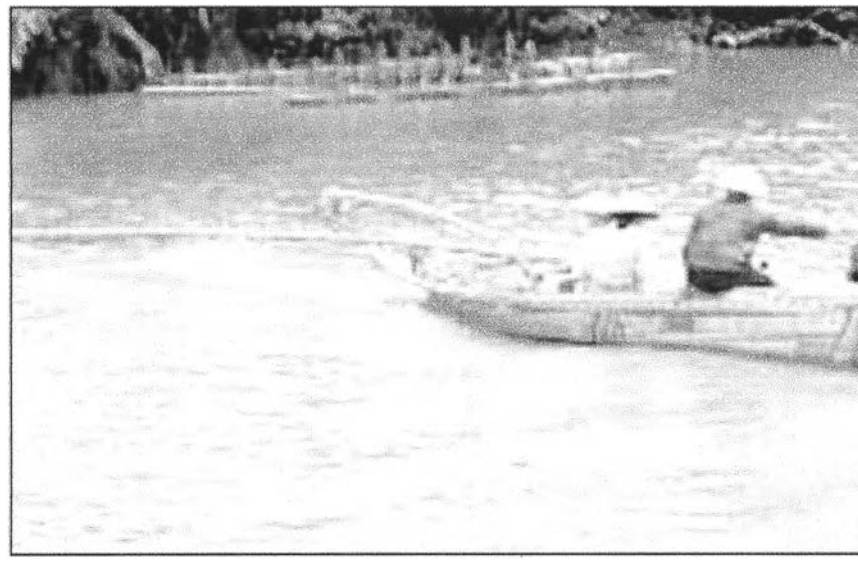
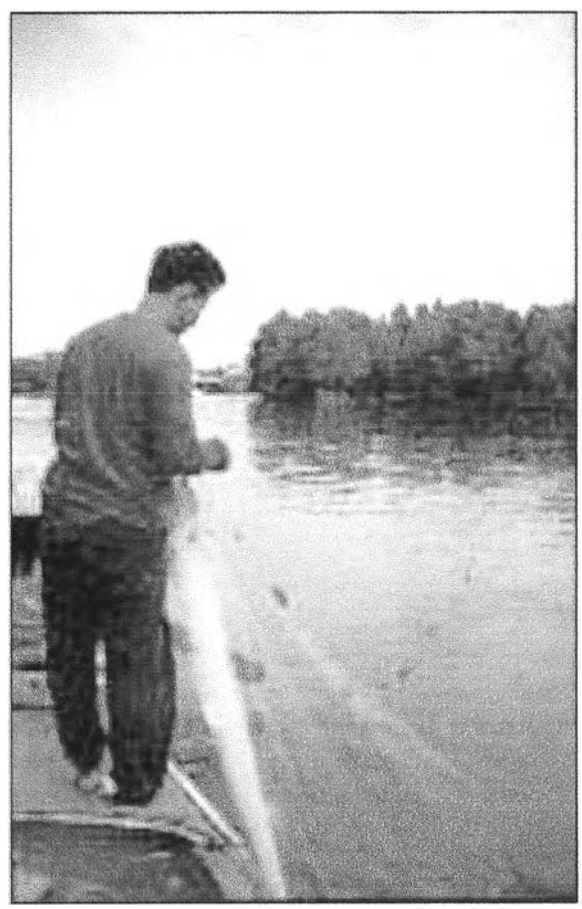


Figure 3.1: The study sites and sampling stations at Trat Bay



(A) Push net



(B) Drift gill net

Figure 3.2: Fishing gears used for fish specimen collecting

Physical parameters

The physical parameters of water in each station of three mangrove canals were studied once a month during spring tide in each season, including:

1. The width of each station was measure only one time in August 1997 while the depth of water in each station was measured once a month.
2. Turbidity of each station was measured in term of transparency by using secchi disc.
3. Temperature of water was recorded by using YSI model 57 dissolved oxygen meter once a month at two levels of each station. The first was at 0.5 metres deep from the surface and the latter was at 0.5 metres deep from the bottom of water.

Chemical parameters

The chemical parameters of water in each station of three mangrove canals were studied once a month during spring tide in each season, including:

1. pH of water was measured by pH meter at 0.5 metres deep from the surface of water.
2. Dissolved oxygen was measured by using YSI model 57 dissolved oxygen meter. The salinity of water was measured by using salinity refractometer. These two parameters were recorded at two levels of each station. The first was at 0.5 metres deep from the surface and the latter was at 0.5 metres deep from the bottom of water.
3. Phosphate and nitrate were measured as the representatives of nutrient as follow:
    - 3.1 Sampling of water was carried out at two levels from each station of each canal. First was at 0.5 metres deep from the surface and the second was at 0.5 metres deep from the bottom of water.
    - 3.2 About 1 litre of water sample from each sampling level of each station was filtrated by filtration instrument. Each water sample was kept in 1000-ml polyethylene bottle and frozen in icebox before the determination of phosphate and nitrate concentration by DR/3 spectrophotometer on the land in the same day.

Coastal land use

The study on coastal land use was divided into two parts, including:

1. Coastal land use of 7 coastal sub-districts of Muang district, Trat province, was classified by the visual interpretation of the image of LAND SAT-TM 1: 50,000 of Trat Bay in 1987, 1992 and 1997. The boundary of 7 sub-districts was overlaid on the LAND SAT-TM for land use estimation. Seven sub-districts were Wang Krajae, Nong Samed, Nong Khansong, Thaprik, Takang, Chamrak and Laemklad (Figure 3.3).
2. The interview on 91 coastal households (15%) from all 599 of coastal households of local small-scale fisherfolks in 7 sub-districts of Trat Bay was conducted (Figure 3.4).

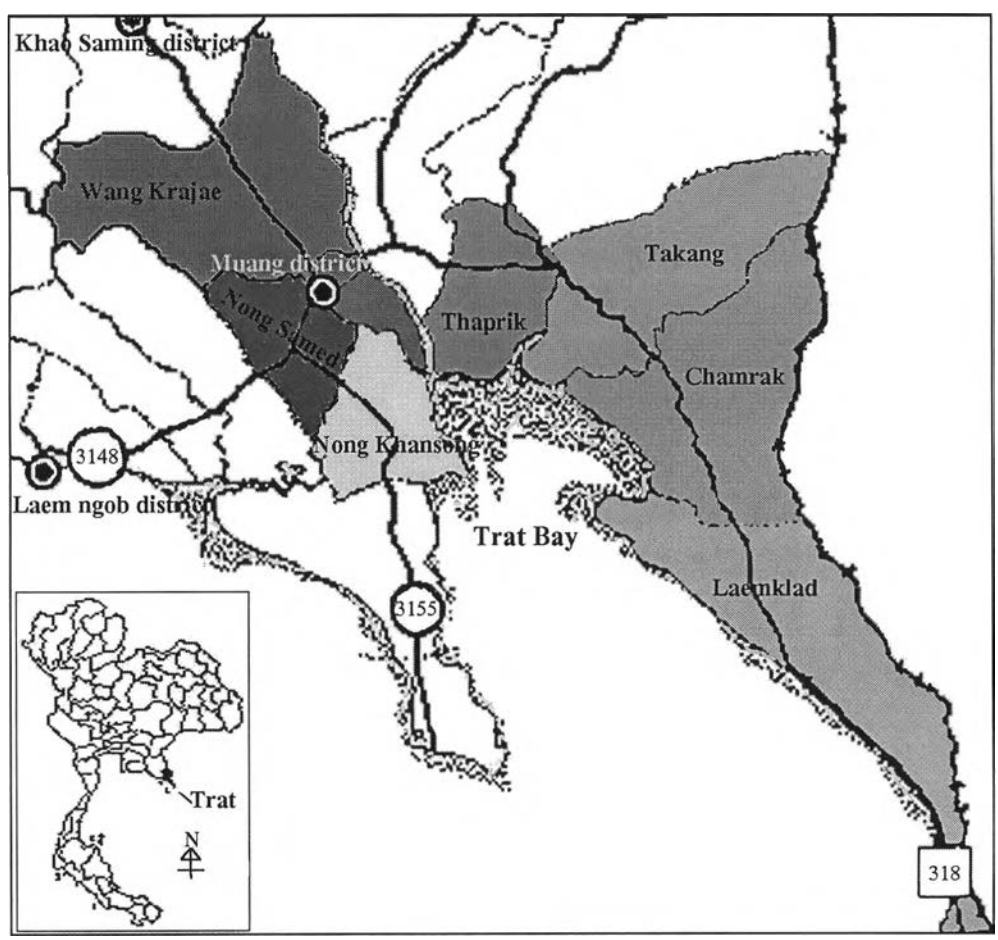


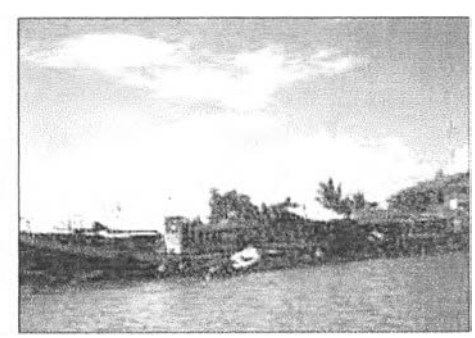
Figure 3.3: Seven sub-districts at Trat Bay, Wang Krajae, Nong Samed, Nong Khansong, Thaprik, Takang, Chamrak and Laemklad

(1) Bantaeng village

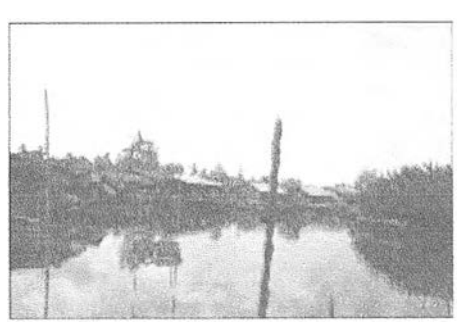
(4) Thaprik village

(5) Laemklad village

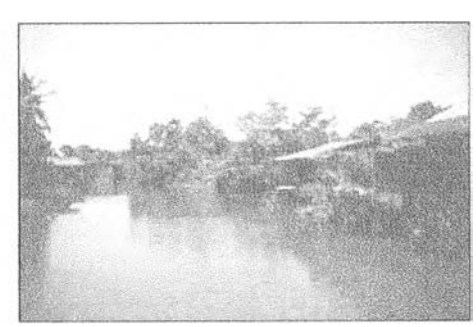
(2) Laembhin village



(6) Dankao villages



(3) Banna village



(7) Thalucan village

Figure 3.4: Villages of coastal fisherfolks in sub-district of (1) Chamrak, (2) Nong Khansong, (3) Nong Samed, (4) Thaprik, (5) Laemklad, (6) Wang Krajae and (7) Takang

3.4 Laboratory analysis

Species composition of fish

Fish collected from three mangrove canals were identified into species level.

Zooplankton

The zooplankton was identified to major taxa. The volume of zooplankton, by hauling of zooplankton net for five minutes in each station, was converted to zooplankton volume/m^3 of water. The water volume was calculated as follow:

$$\textbf{Water volume } (m^3) = \P r^2 d$$

When r = radius of the mouth of plankton net (0.3/2 m)

d = horizontal distance (m) converted from the round number of flow meter, which was installed at the mouth of plankton net and rotated through water during horizontal hauling for 5 minutes per station

Chlorophyll a determination

Each half circle of GF/C glass paper, which had phytoplankton inside, was extracted by 90 % AR grade acetone. The extraction and determination of chlorophyll a was carried out by the methods of Strickland and Parsons (1972), including:

1. Placed each half circle of GF/C glass paper, which had phytoplankton inside, in each 15-ml stopped graduated centrifuge tube.
2. Added approximately 10 ml of 90 % acetone, dispersed and disintegrated the paper by the stir and then stopped the tubes and shook the tubes vigorously.
3. Covered each tube by aluminium foil to avoid light and left all tubes in complete darkness

4. Placed all centrifuge tubes in the freeze shelf of a refrigerator. Shook the tubes vigorously an hour later. Then, placed all tubes in the refrigerator again and left them about 20 hours allowing the pigments to be extracted well.
5. Removed all tubes that covered with foil from the refrigerator and left them warm up in room temperature for a minute.
6. Added 90 % acetone to make the extracts from GF/C glass paper up to exactly 10 ml, stopped the tubes and shook vigorously again.
7. Centrifuged the content in the tubes at 3,000 round per minute for 2 minutes for packing most of paper to the bottom of the tubes, then stopped the centrifuge and removed the paper from the tubes. Returned all tubes to the centrifuge for 5 minutes.
8. Decanted the clear supernatant liquid into 1–cm cell spectrophotometer of which the extinction value of measuring multiplied by 10.
9. Measured the extinction of the absorption (A) of solution against the turbidity blank (a cell containing 90% acetone for standardisation) at the wavelength of 750, 665, 675 and 630 nm. The extinction of each solution was calculated for Chlorophyll a determination. The extinction value at 750 nm was minus from each value, which measured by other wavelength and might be affected from the colour and turbidity, to correct the extinction value.
10. Before measuring, adjusted the spectrophotometer at 100 % transmission of each wavelength. The Chlorophyll a content was calculated from the absorption extinction of the solution of each wavelength. The Chlorophyll a calculation was based on Parson & Strickland' s equation.

$$\textbf{Chlorophyll a (Chl.A)} = Ca*v/V \qquad \textbf{mg/m}^3$$

When $Ca = 11.6\ E_{6650} - 1.31\ E_{6450} - 0.14\ E_{6300}$

$E_{6650} = A_{665} - A_{750}$ $E_{6450} = A_{645} - A_{750}$ $E_{6300} = A_{630} - A_{750}$

A = the absorption value of solution at each wavelength

v = multiplying factor =10 when use 1–cm path-length spectrophotometer cell

V = water volume after filtration (litters)

Phosphate and nitrate

The analysis of phosphate and nitrate followed the water analysis handbook of HACH company (1982) of which include:

Phosphate determination

1. Filled a water sample into a clean sample cell to the 25 mark.
2. Added the content of one powder pillow of PhosVer® 3 Phosphate into water sample and stir to mix immediately. A blue colour will develop if phosphate is present. Waiting for minimum two minutes for full colour development but not more than 10 minutes before completing steps 3 through 6.
3. Filled another sample cell to the 25 mark with original water sample and placed it into the cell holder of the DR/3 spectrophotometer. Closed the light shield and inserted the Phosphate Meter Scale (Cat. No. 41786-00) into the meter and adjusted the Wavelength Dial to 700 nm.
4. Set the Mode Switch to LEFT SET and checked the left set adjustment, adjusted the LEFT SET control to align the meter needle with the extreme left mark.
5. Set the Mode Switch to NORM and adjusted the RIGHT SET controls for a meter reading of zero mg/l.
6. Placed the prepared sample in the cell holder, closed the light shield and read the concentration value of mg/l-PO_4^{3-}.

Nitrate determination

1. Filled a water sample into a clean 50-ml graduated cylinder to the 30-ml mark then poured the sample into a clean mixing bottle.
2. Added the contents of one powder pillow of NitraVer® 6 Nitrate into water sample and stirred for three minutes. Then allowed the sample to stand undisturbed for 30 seconds.
3. Carefully poured the prepared solution sample without cadmium particle into a clean sample cell to the 25 mark.
4. Added the content of one powder pillow of NitriVer® 3 Nitrite into the sample and stirred for 30 seconds. A pink colour will develop if nitrate is present. Waiting for minimum 10

minutes for proper colour development but not more than 20 minutes before completing steps 5 through 8.

5. Filled another sample cell to the 25 mark with original water sample and placed into the cell holder. Closed the light shield and inserted the Nitrogen, Nitrate Meter Scale (Cat No. 41788) into the meter. Adjusted the Wavelength Dial to 500 nm.
6. Set the Mode Switch to LEFT SET and checked the left set adjustment, adjusted the LEFT SET control to align the meter needle with the extreme left mark.
7. Set the Mode Switch to NORM and adjusted the RIGHT SET controls for a meter reading of zero mg/l.
7. Placed the prepared sample in the cell holder, closed the light shield and read the concentration value of mg/l-NO_3-N.

3.5 Data analysis

- The species diversity and similarity of fish found from each canal was estimated by using the index of diversity, dominance and similarity.
- Total weight of fish collected by each fishing gear were compared between canals, Bangphra:Thaprik, Banphra:Thaleuan and Thaprik:Thaleuan, by using Paired-Samples T-Test at 95 % confidential level.
- Species number of fish found and total weight of fish collected by each fishing gear were compared between seasons, tides, periods and among stations by using ANOVA at 95 % confidential level and Duncan's Multiple Range Test.
- The environmental condition (physical, chemical and biological parameter) of three study sites, among stations and between seasons was compared by using ANOVA at 95 % confidential level and Duncan's Multiple Range Test.
- The relationship between species number of fish and the environmental condition was analysed by using Regression and Correlation Coefficient Test.