Chapter 3

Modeling of phytoplankton and Polychaeta density

In this chapter contains the preliminary analysis for our two studies and two published articles. The first article is concerned with the statistical modeling of distribution and abundance of phytoplankton: influence of salinity and turbidity gradients in the Na Thap River, Songkhla province, Thailand and was published in the *Journal of Coastal Research* volume 27, No.3, 585-594, May 2011 issue.

The second article is concerned with modeling the Polychaeta organism density in Na Thap estuary and was published in the *International Journal of Biology* volume 3, No. 4, 30-35, October 2011 issue.

3.1 Preliminary Analysis

The first study

During the period of study, the phytoplankton community in the Na Thap River comprised 74 genera from seven divisions: Cyanophyta, Chlorophyta , Bacillariophyta, Chrysophyta, Pyrophyta, Euglenophyta and Cryptophyta, among which Bacillariophyta and Chlorophyta were the most diverse groups with a total of 29 and 27 genera, respectively. Of these, 31 dominant genera with more than 24% occurrence of all samples (n = 144) were selected for further study (Table 3.1).

Table 3.1: Percentages of phytoplankton occurrence of 31 phytoplankton genera in the Na Thap River from June 2005 to December 2007 (n = 144)

Division	Genus	Density ($\times 10^4$ cells m^{-3})	% occurrence
Cyanophyta	*Oscillatoria* spp. (Osc)	299.04	68.1
Cyanophyta	*Merismopedia* sp. (Mer)	7.73	27.8
Chlorophyta	*Closterium* spp. (Clo)	102.16	34.7
Chlorophyta	*Pandorina* sp. (Pan)	36.36	30.6
Chlorophyta	*Spirogyra* sp. (Spi)	203.87	29.9
Chlorophyta	*Pleurotaenium* spp. (PTa)	3.58	27.1
Chlorophyta	*Cosmarium* sp. (CMa)	6.51	26.4
Chlorophyta	*Hyalotheca* spp. (Hya)	255.13	25.7
Chlorophyta	*Micrasterias* spp. (Mic)	290.03	24.3
Chlorophyta	*Mougeotia* sp. (Mou)	110.80	24.3
Chlorophyta	*Staurastrum* sp. (Sta)	9.07	24.3
Bacillariophyta	*Coscinodiscus* spp. (CCi)	605.13	70.1
Bacillariophyta	*Pleurosigma* spp. (PSi)	431.84	50.0
Bacillariophyta	*Chaetoceros* spp. (Cha)	7,138.01	47.9
Bacillariophyta	*Bacillaria* spp. (Bac)	104.96	45.8
Bacillariophyta	*Thalassionema* spp. (Tha)	575.50	44.4
Bacillariophyta	*Serirella* sp. (Ser)	7.64	42.4
Bacillariophyta	*Nitzschia* sp. (Nit)	105.95	38.2
Bacillariophyta	*Rhizosolenia* spp. (Rhi)	2,284.98	38.2
Bacillariophyta	*Melosira* sp. (Mel)	1,584.18	37.5
Bacillariophyta	*Triceratium* sp. (Tri)	9.89	36.8
Bacillariophyta	*Navicula* sp. (Nav)	9.31	32.6
Bacillariophyta	*Synedra* sp. (Syn)	146.53	32.6
Bacillariophyta	*Bidduphia* spp. (Bid)	28.02	29.2
Bacillariophyta	*Bacteriastrum* sp. (Bte)	95.51	25.0
Bacillariophyta	*Guinardia* sp. (Gui)	203.37	24.3
Pyrrophyta	*Ceratium* spp. (Cer)	292.53	63.9
Pyrrophyta	*Protoperidinium* spp. (Pro)	315.65	45.8
Pyrrophyta	*Dinophysis* spp. (Din)	1,902.45	24.3
Euglenophyta	*Euglena* sp. (Eug)	129.56	61.1
Euglenophyta	*Phacus* sp. (Pha)	33.10	40.3

Spatio-temporal differences of phytoplankton density were marked at the basin. Cyanobacteria, euglenophytes and chlorophytes, dominated by *Closterium* spp., *Spirogyra* sp., and *Mougeotia* sp., were the main microplanktonic groups in the freshwater zone (Stations 1-5) while a diatom *Synedra* sp. was also present continually at relatively low density. *Euglena* sp. was the most common in this zone (mean: 22,781 cell l^{-1}) and Station 1 had the highest density (243,968 cell l^{-1}) in August 2007. At the stations further downstream, the phytoplankton community was dominated by an assemblage of diatoms and dinoflagellates. The most prevailing genera (> 80% of occurrence within the zone) in decreasing ranking were

Table 3.2: The Taxa, distribution (station), maximum densities and bimonthly occurrence of phytoplankton in the Na Thap River from June 2005 to December 2007. Data in bold indicates station and month of maximum density.

Taxa	Max density (cells l^{-3})	Distribution (station)	Occurrence
Division Cyanophyta			
Anabaena sp.	10,907	3 5 **6** 7 8 9 10	**Feb** Apr Jun Dec
Ankistrodesmus sp.	1,920	1 2 3 **4** 6 7 8 10	Feb Jun Aug Oct **Dec**
Chroococcus sp.	6,875	1 **2**	**Dec**
Lyngbya sp.	13,750	**1** 8	Jun Oct **Dec**
Merismopedia sp.	54,013	1 2 **3** 4 5 6 7 8 9 10	Feb Apr Jun **Aug** Oct Dec
Oscillatoria spp.	1,372,451	1 2 3 4 5 **6** 7 8 9 10	Feb Apr Jun Aug **Oct** Dec
Raphidiopsis sp.	7,628	**2** 4 8 9	Aug **Oct** Dec
Spirulina sp.	8,200	1 2 3 4 6 7 8 **9** 10	Feb Jun Aug Oct **Dec**
Division Chlorophyta			
Arthrodesmus sp.	81	**8**	**Dec**
Bambusina sp.	10,242	1 2 3 **4** 5 6 7 8 9 10	Oct **Dec**
Closterium spp.	291,906	1 2 3 4 5 6 **7** 8 9 10	Feb Apr Jun Aug Oct **Dec**
Cosmarium sp.	36,269	1 2 3 **4** 5 6 7 8 9 10	Feb Apr Jun Aug Oct **Dec**
Desmidium spp.	16,549	1 **2** 4 5 6 7 8 10	Feb Apr Jun Oct **Dec**
Euastrum sp.	2,292	1 2 3 **4** 5 6 7 10	Feb Apr Jun Aug Oct **Dec**
Eudorina sp.	2,925	**1** 2 3 4 5	**Feb** Oct
Hyalotheca spp.	2,395,940	1 **2** 3 4 5 6 7 8 9 10	Feb Jun Aug Oct **Dec**
Lagerheimia sp.	181	6 **8**	**Dec**
Micrasterias spp.	1,829,755	1 2 3 **4** 5 6 7 8 9 10	Feb Jun Aug Oct **Dec**
Mougeotia sp.	513,146	**1** 2 3 4 5 6 7 8 9 10	Feb **Apr** Jun Aug Oct Dec
Onychonema sp.	2,229	1 2 4 **5** 7	Jun Aug Oct **Dec**
Pandorina sp.	211,500	**1** 2 3 4 5 6 7 8 9 10	Feb Apr **Jun** Aug Oct Dec
Pediastrum sp.	4,583	1 2 4 5 6 7 **8** 9 10	Feb Apr Jun Aug Oct **Dec**
Phymatodocis sp.	13	**1**	**Dec**
Pleurotaenium spp.	14,012	1 2 3 4 5 6 7 **8** 9 10	Feb Jun Aug Oct **Dec**
Scenedesmus sp.	4,500	1 6 7 **8** 9 10	Feb Jun **Dec**
Selenastrum sp.	60	1 2 **7**	Aug **Dec**
Spirogyra sp.	788,467	1 **2** 3 4 5 6 7 8 9 10	**Feb** Apr Jun Aug Oct Dec
Spondylosium sp.	10,766	1 **2** 3 4 5 6 8	Apr Jun Aug Oct **Dec**
Staurastrum sp.	35,706	1 **2** 3 4 6 7 8 9 10	Feb Apr Jun Aug Oct **Dec**
Staurodesmus sp.	18	**1**	**Dec**
Triploceras sp.	4,665	1 2 3 4 7 **8** 10	Jun **Dec**
Ulotrix sp.	49,072,782	1 2 3 **4** 5 6 7 8 10	Feb Jun **Aug** Oct Dec
Volvox sp.	45,605	**1** 3 5 7 10	Apr Jun **Oct** Dec
Xanthidium sp.	39,185	1 **2** 3 4 5 6 7 8 9 10	Apr Jun Oct **Dec**
Zygnema sp.	651,514	**1** 2 3 4 5 7 8 9 10	**Oct** Dec

Table 3.2: (Continue)

Taxa	Max density (cells l^{-3})	Distribution (station)	Occurrence
Division Bacillariophyta			
Asterionella sp.	148,946	8 **9** 10	**Feb** Apr Dec
Bacillaria spp.	574,233	1 **2** 3 4 5 6 7 8 9 10	Feb **Apr** Jun Aug Oct Dec
Bacteriastrum sp.	498,857	4 6 7 8 **9** 10	Feb Apr Jun **Aug** Oct Dec
Bellerochea sp.	43,429	**3**	**Apr**
Biddulphia spp.	69,287	1 6 7 8 9 **10**	Feb Apr Jun Aug **Oct** Dec
Chaetoceros spp.	63,073,972	1 2 3 4 5 6 7 8 **9** 10	Feb Apr Jun **Aug** Oct Dec
Climacodium sp.	32,175	2 6 7 8 **9** 10	Feb Apr Jun **Oct**
Coscinodiscus spp.	2,094,610	1 **2** 3 4 5 6 7 8 9 10	Feb Apr Jun Aug **Oct** Dec
Dactyliosolen sp.	6,311	9 **10**	**Apr**
Dictyocha sp.	36,604	1 2 5 7 **8** 9 10	Feb Aug Oct **Dec**
Ditylum sp.	52,091	7 8 **9** 10	**Feb** Apr Aug Oct Dec
Guinardia sp.	437,413	7 8 9 **10**	Feb Apr Jun **Aug** Oct Dec
Gyrosigma sp.	7,881,566	1 2 3 4 5 6 7 8 **9** 10	Feb Apr Jun **Aug** Oct Dec
Hemiaulus sp.	108,658	1 7 8 **9** 10	Feb Apr Jun **Aug** Oct Dec
Hyalodiscus sp.	2,648	8 9 **10**	**Feb** Apr
Licmorpha sp.	52,417	1 2 4 6 7 8 9 **10**	Feb Apr **Jun** Aug Oct Dec
Melosira sp.	19,975,403	1 **2** 3 4 5 6 7 8 9 10	Feb **Apr** Jun Aug Oct Dec
Navicula sp.	39,824	1 2 3 4 5 6 7 8 9 **10**	Feb Apr Jun Aug **Oct** Dec
Nitzschia sp.	173,544	1 2 5 6 **7** 8 9 10	Feb Apr Jun **Aug** Oct Dec
Odontella sp.	10,357	3 8 **9** 10	**Feb** Apr Aug
Palmeria sp.	1,701	9 **10**	**Jun** Aug
Planktoniella sp.	2,396	**10**	**Apr** Dec
Pleurosigma spp.	2,209,131	1 2 3 4 5 6 **7** 8 9 10	Feb **Apr** Jun Aug Oct Dec
Rhizosolenia spp.	8,642,727	1 5 6 7 8 **9** 10	Feb Apr Jun **Aug** Oct Dec
Seriella sp.	13,251	1 2 3 4 5 **6** 7 8 9 10	**Feb** Apr Jun Aug Oct Dec
Skeletonema spp.	58,171	7 8 9 **10**	**Jun**
Synedra sp.	396,513	1 2 3 4 5 6 7 **8** 9 10	Feb Jun Aug Oct **Dec**
Thalassionema spp.	2,300,935	1 2 3 4 5 6 7 8 **9** 10	**Feb** Apr Jun Aug Oct Dec
Triceratium sp.	15,020	1 3 4 5 6 7 8 9 **10**	Feb Apr Jun Aug Oct **Dec**
Division Chrysophyta			
Dinobryon sp.	28,250	**1** 2 3 4 5 6 7 8	Feb Apr **Jun** Oct Dec
Division Pyrophyta			
Ceratium spp.	924,899	1 2 3 4 5 6 7 8 **9** 10	Feb Apr **Jun** Aug Oct Dec
Dinophysis spp.	22,543,407	1 **4** 5 6 7 8 9 10	Feb Apr Jun **Aug** Dec
Gymnodinium spp.	23,725,510	2 3 4 5 **6** 7 8 9 10	Feb Apr **Aug** Oct Dec
Noctiluca spp.	75,940	6 7 **8** 9 10	Feb Apr Jun Aug Oct **Dec**
Peridinium spp.	324,458,338	**1** 2 3 4 5 6 7 8 9 10	Feb Apr **Jun** Aug Oct Dec
Protoperidinium spp.	2,045,143	1 2 **3** 4 5 6 7 8 9 10	Feb **Apr** Jun Aug Oct Dec
Division Euglenophyta			
Euglena sp.	243,968	**1** 2 3 4 5 6 7 8 9 10	Feb Apr Jun **Aug** Oct Dec
Phacus sp.	100,960	**1** 2 3 4 5 6 7 8 9 10	Feb Apr **Jun** Aug Oct Dec
Division Cryptophyta			
Cryptonema sp.	2,236,925	6 7 **8**	**Oct**

Coscinodiscus spp., *Ceratium* spp., *Thalassionema* spp., *Protoperidinium* spp., *Rhizosolenia* spp., *Chaetoceros* spp., and *Pleurosigma* spp. The presences of these genera varied from stations to stations. *Coscinodiscus* spp. was the main group at Stations 7 and 8, although its density peak (915,542 cell l^{-1}) was observed in October 2007 at Station 10. The phytoplankton community at Station 9 and 10 was dominated by *Thalassionema* spp. during the period of February – May, and by *Rhizosolenia* spp. during June – October. Two dinoflagellates were also present across the sampling stations, with densities of *Ceratium* spp. generally higher than *Protoperidinium* spp.

The roles of variables are classified as determinants and outcome. These variables, their roles and data type as shown in Table 3.3. The outcome of interest is the densities of the thirty one genera of phytoplankton from ten sampling stations at sixteen bimonthly periods. These densities are of continuous data type. The predictor variables (determinants) consisted of the environmental factors and the unique variable derived from factor analysis.

Table 3.3: Variables, their roles and data type

Variable	Role	Type
Environmental factors (5)	Determinant	Continuous
Unique environmental parameter (4)	Determinant	Continuous
Density of 31 genera of phytoplankton	Outcomes	Continuous

The second study

For biological outcomes such as Polychaeta organism counts per unit area or volume, the normality assumption is usually invalid due to skewness in the distribution of the outcomes. In this case the skewness may be reduced substantially by taking logarithms of the outcomes, provided zero outcomes are handled in some way. Clark and Warwick (1994) suggested replacing y by the transformed variable log (1+y). However, for ease of interpretation, results need to be expressed in terms of the organism densities themselves rather than transformed outcomes, so it is necessary to correct the back-transformed means to ensure that the overall mean organism density is preserved.

Figures 3.1 and Figures 3.2 show water salinity in Na Thap River by sampling stations and observed-periods. The water salinity of Na Thap River was varied by stations and season. The salinity was high at station 1 (Pak Bang) which was located downstream where the river joins with the saltwater and decreasing all the way upstream to station 5 (Tha Klong Cha Nong) where there was freshwater. In the rainy season, the salinity was lower than in summer.

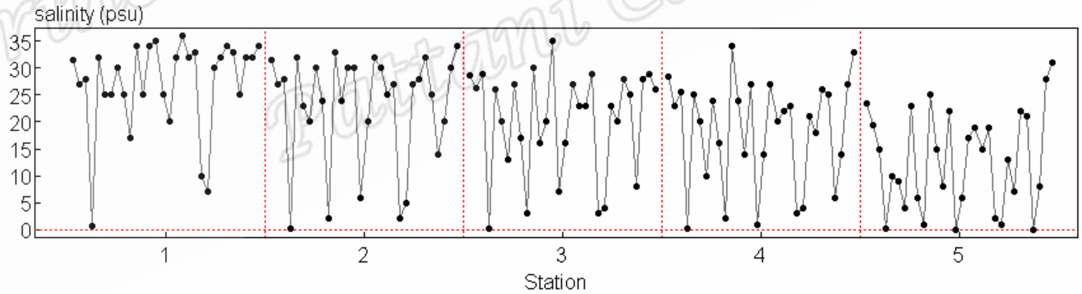

Figure 3.1: Water salinity in Na Thap River from June 2005 to May 2010 by station

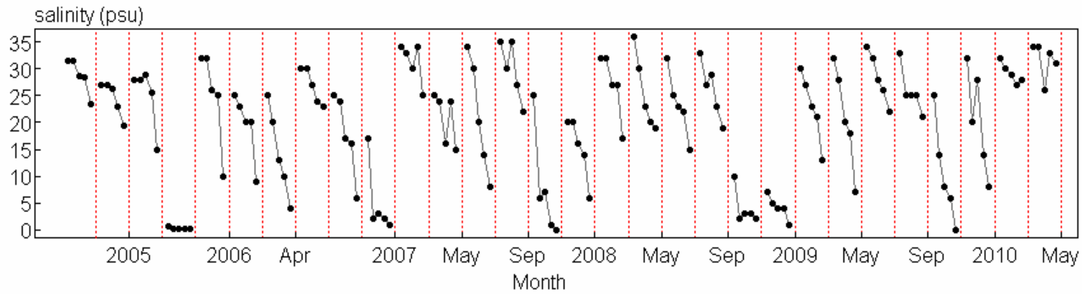

Figure 3.2: Water salinity in Na Thap River from June 2005 to May 2010 by month

Figures 3.3 and Figures 3.4 show Polychaeta organism densities in Na Thap River by sampling stations and observed-periods from June 2005 to May 2010. Polychaeta densities observed by months elapsed. The occasions when no Polychaeta were observed are as follows: at one site after 9, 32, 42 and 54 months, at two sites after 1, 3, 5 and 44 months, never at three sites, at four sites after 7 months, and at all five sites after 28 months.

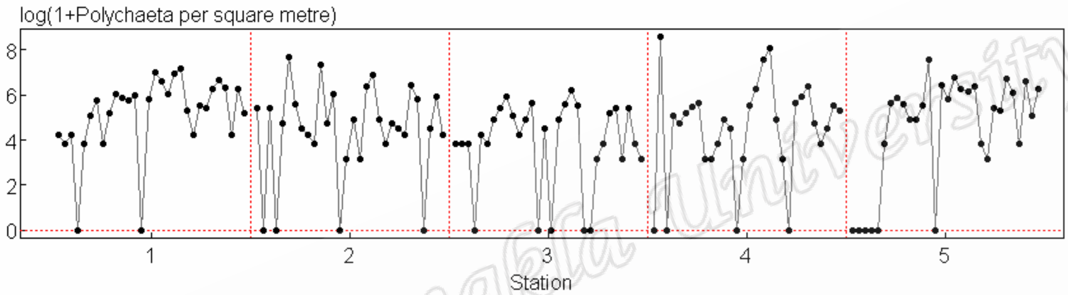

Figure 3.3: Natural logarithms of Polychaeta organisms per square meter in Na Thap River from June 2005 to May 2010 by station

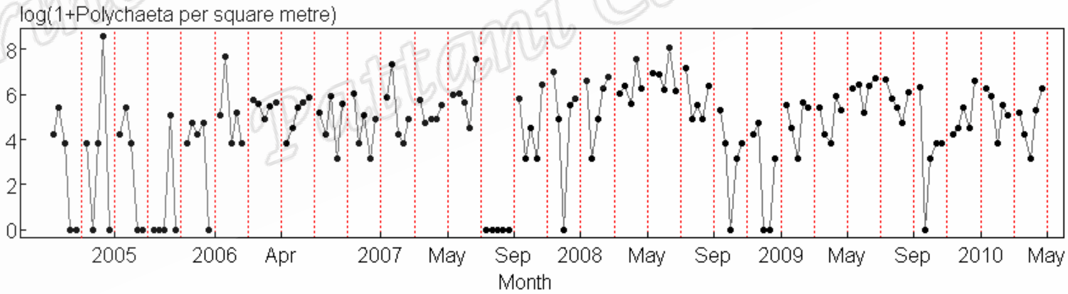

Figure 3.4: Natural logarithms of Polychaeta organisms per square meter in Na Thap River from June 2005 to May 2010 by month

Polychaeta is a class of phylum Annelida, which is a macrobenthic fauna, it was the most common in all stations and all month. Station 4 had the highest density (5355.56 organism m^{-2}) in August 2005. Polychaeta organism densities were varied by stations and season. The Polychaeta densities increased with distance from the station 5 (Tha Klong Cha Nong) to station 1 (Pak Bang). During Dry season, Polychaeta densities have higher than rainy season. Mean and maximum densities of Polychaetes in the Na Thap River from June 2005 to May 2010, during rainy (November–January) were 199.99 organism m^{-2} and 1066.67 organism m^{-2}, respectively, and dry (February–October) seasons were 426.53 organism m^{-2} and 5355.56 organism m^{-2}, respectively. Sampling stations 2, 4 and 5 are surrounded by communities, shrimp farms, fish cage farms, pig farms, seafood processing factories, and rubber industry (Sapaeing, 2007). Waste water containing organic matter from those areas may cause increasing density of polychaete. Polychaeta such as *Capitella* sp. and *Theora lubrica* in the family Capitellidae (Kikuchi, 1991; Ferraro et al., 1991) and *Polydora* sp. are biological indicators of organic pollutants. They are usually found in estuaries that contain hydrogen sulfide (H_2SO_4) and high organic mater (Chareonpanich et al., 1994).

3.2 The first article

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Distribution and Abundance of Phytoplankton: Influence of Salinity and Turbidity Gradients in the Na Thap River, Songkhla Province, Thailand

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ABSTRACT

LUEANGTHUWAPRANIT, C.; SAMPANTARAK, U., and WONGSAI, S., 2011. Distribution and abundance of phytoplankton: influence of salinity and turbidity gradients in the Na Thap River, Songkhla Province, Thailand. *Journal of Coastal Research*, 27(3), 585–594. West Palm Beach (Florida), ISSN 0749-0208.

Distributions of phytoplankton density and their relationships to physicochemical variables were investigated using multivariate analyses, based on data collected every two months from a tropical, inland, freshwater estuary in southern Thailand between June 2005 and December 2007. Results indicated 74 genera of phytoplankton in the samples. More than 75% of the genera were diatoms (30 genera; 40.5%) and chlorophytes (29 genera; 39.2%), and 20% were cyanobacteria (6 genera; 8.1%) and dinoflagellates (6 genera; 8.1%). Variations in phytoplankton density largely resulted from salinity and turbidity, which varied seasonally and geographically. Chlorophytes, cyanobacteria, and euglenophytes were the most common groups in the turbid freshwater habitat, whereas diatoms and dinoflagellates dominated along the salinity gradient of the clear estuarine environment. Our results suggest that the Na Thap River has been regulated mainly by the natural phenomena of marine and riverine influences, even though the river is situated on agricultural, aquacultural, and industrial land. Continued observations of phytoplankton density and composition are needed, emphasizing any unusual increases in density and/or the unexpected presence of harmful species. The long-term trends of phytoplankton provide an indication of the change in the trophic status of the basin, as well as a foundation for further studies of the distributions of upper-level aquatic species in freshwater estuarine ecosystems.

ADDITIONAL INDEX WORDS: *Spatiotemporal variation, multivariate regression analysis.*

INTRODUCTION

In estuaries, as in other aquatic systems, primary productivity is generated by phytoplankton. Phytoplankton are primary producers in the food chain, acting as a source of food or primary energy in the ecology of natural resources (Boney, 1975). The density of phytoplankton in estuaries not only depends on the availability of sunlight and nutrients but also relies on tides, salinity, turbidity, and river flows (Madhu *et al.*, 2007). Differences in geographical location, season, and pollutant substances from urban, industrial, and agricultural sources have an effect on declining water quality and, therefore, influence species richness and the density of phytoplankton in estuaries.

Many studies, such as those by Boyer, Christian, and Stanley (1993); Gasiunaite *et al.* (2005); Huang *et al.* (2004); Popovich *et al.* (2008); Trigueros and Orive (2001); and Wang *et al.* (2007), have investigated the effects of environmental determinants on phytoplankton abundance. However, most such studies have been carried out in temperate habitats, and there is less attention in the literature to similar studies in tropical ecosystems, although examples include studies in India by Nirmal Kumar *et al.* (2009); Pelleyi, Kar, and Panda (2008) and a study in Brazil by Costa, Huszar, and Ovalle (2009).

We used multivariate multiple regression to simultaneously analyze multiple species of phytoplankton data collected from the Na Thap River between June 2005 and December 2007, thus aiming to determine the relationships between their density and physicochemical variables. Such relationships are useful as basic knowledge for conservation planning that maintains sustainable ecosystems.

MATERIALS AND METHODS

Study Area

The Na Thap River is located in Chana district of Songkhla province of Thailand, with a watershed of approximately 232 km². It originates at the confluence of the Pho Ma and Luek canals and, after 26.5 km, enters the Gulf of Thailand.

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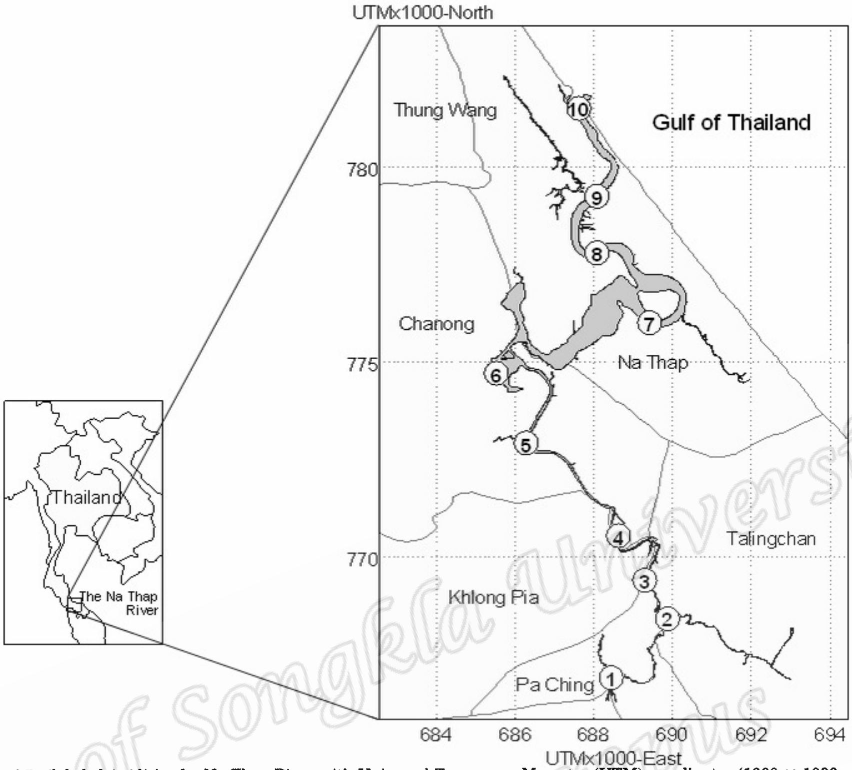

Figure 1. Sampling sites (labeled 1–10) in the Na Thap River with Universal Transverse Mercator (UTM) coordinates (1000 × 1000 m).

Therefore, its water body mixes freshwater and seawater and is subject to many influences, including tidal regimes, salinity influx, river flows, and surface runoff from the upland regions, which, in turn, have unique characteristics of both marine and freshwater.

The east coast of southern Thailand has a wet season from November to January and a dry season from February to October. This dry–wet seasonal pattern differs from the west coast, facing the Andaman Sea, and from the rest of Thailand because of the influences of two distinct monsoons. During the southwest monsoon, the Gulf coast experiences relatively low rainfall, compared with that of the Andaman coast, with its heavy rain and storms during the northeast monsoon.

The Na Thap River serves as a major source of water for more than 52,000 residents in the community and is used for irrigation, transportation, agriculture, aquaculture, fishing and recreation, and industrial and household purposes. Current land use in the basin involves cattle grazing, rice growing, rubber plantations, inland marine shrimp farms, and aquaculture production industries. Furthermore, the basin is surrounded by many types of wetlands, including melaleuca and mangrove swamps, providing habitats for a wide variety of plant and animal species.

Sampling

Water samples were collected every two months from 10 sampling sites on the Na Thap River between June 2005 and December 2007 (Figure 1). Five upstream sites (sites 1–5) were situated in the freshwater zone, with increasing salinity toward the river mouth (sites 6–10). The samples were obtained from eight sites (sites 1, 2, 4, 6–10) during the 2.5 years, with additional samples taken at sites 3 and 5 during the second half of the study period (October 2006–December 2007). Therefore, a total of 144 samples were taken during the period of study, with 13 and 51 samples taken during the rainy and dry seasons, respectively, in the freshwater zone, and 15 and 65 samples, respectively, taken in the estuarine zone.

At each site, samples were collected using a 1-L plastic bottle for analyses of physicochemical variables. The samples were measured for pH, salinity, total sulfate, transparency, turbidity, dissolved oxygen, nitrate–nitrogen, phosphate–phosphorus, ammonia–nitrogen, biological oxygen demand, oil and grease, total coliform bacteria, fecal coliform bacteria, total iron, cadmium, and copper. Analytical methods used were based on the standard method of the American Public Health Association, the American Water Works Association, and the

Water Environment Federation (1998). Spectrometric methods were used to measure total sulfate, nitrate–nitrogen, phosphate–phosphorus, and ammonia–nitrogen. Air–acetylene (or ethyne; C_2H_2) flame atomic absorption was used to measure total iron, cadmium, and copper. The tube fermentation technique was used to measure total coliform bacteria and fecal coliform bacteria. Other variables were measured using standard instruments.

Phytoplankton samples were collected by filtering 20 L of water through 20- and 69-μm mesh size nets. The samples were fixed with 5% formalin solution before transportation. In the laboratory, three subsamples from each sample were diluted with distilled water, and then, a 1-ml subsample was placed into a Sedgewick Rafter counting chamber for phytoplankton enumeration (Smith, 1950). Phytoplankton were counted and identified to at least genus level, and to species level where possible, under a microscope at a magnification of ×100 or ×400, expressed as cell concentrations *per* liter.

Statistical Methods

Multivariate analyses were used to investigate the relationships between phytoplankton density and physicochemical variables. Exploratory factor analysis with maximum likelihood estimation was used to identify underlying factors describing the correlations among the physicochemical variables. The appropriate number of factors was determined using a chi-squared test statistic. Any variable contributing relatively little information to the common factors, defined by a uniqueness of more than 0.75, was excluded from the factor analysis. To obtain more interpretable results, factors were rotated using the oblique *promax* rotation method. Multivariate multiple linear-regression analysis was then used to evaluate phytoplankton density in relation to the reduced set of physicochemical variables obtained from the factor analysis, as well as the individual variables that did not contribute to the factor analysis. Thirty-one dominant phytoplankton taxa, defined as those found in more than 25% of all samples, were selected as response variables and transformed using the transformation log(1 + *concentration*), where the concentration was scaled to satisfy the statistical normality assumption for residuals. Probability plots were used to assess this assumption.

RESULTS

Phytoplankton Composition and Distribution

During the period of study (June 2005–December 2007), the phytoplankton community in the Na Thap River comprised 74 genera from six divisions: Bacillariophyta (30 genera), Chlorophyta (29 genera), Cyanophyta (6 genera), Pyrrophyta (6 genera), Euglenophyta (2 genera), and Cryptophyta (1 genus). Of these, 31 dominant genera were selected for the further study (Table 1). At the freshwater zone, phytoplankton genera found on at least 50% of sampling occasions were euglenophytes (*Euglena* sp. and *Phacus* sp.), cyanobacteria (*Oscillatoria* spp.), and chlorophytes (*Closterium* spp., *Pandorina* spp., and *Cosmarium* sp.). Diatoms and dinoflagellates were the prevailing genera (>65% occurrence) within the estuarine zone. These genera were, in decreasing ranking, *Coscinodiscus* spp., *Ceratium* spp., *Pleurosigma* spp., *Chaetoceros* spp., *Thalassionema* spp., *Protoperidinium* spp., *Bacillaria* spp., and *Rhizosolenia* spp.

Substantial spatiotemporal differences in phytoplankton density were observed. In rainy seasons, chlorophytes, mainly *Closterium* spp., *Hyalotheca* spp., *Micrasterias* spp., and *Mougeotia* sp., and the diatom *Synedra* sp., were the main microplanktonic groups in the freshwater zone and were possibly carried by increasing current flows toward the lower sites downstream. At the estuarine zone, the phytoplankton community was dominated by an assemblage of diatoms (*Coscinodiscus* spp. and *Thalassionema* spp.) and dinoflagellates (*Ceratium* spp. and *Dinophysis* spp.). In dry seasons, mixed assemblages of phytoplankton were evident, mainly *Dinophysis* spp., *Melosira* sp., and *Protoperidinium* spp., in the freshwater zone, whereas diatoms comprising *Chaetoceros* spp., *Coscinodiscus* spp., *Guinardia* sp., *Nitzschia* sp., *Pleurosigma* spp., *Rhizosolenia* spp., and *Thalassionema* spp. occupied estuarine habitats.

Physicochemical Variables

Figures 2 and 3 show spatial and temporal patterns of the sixteen physicochemical variables studied. Salinity was zero at the site farthest upstream, gradually increasing downstream, and reaching more than 20 practical salinity units (psu) at the river mouth. A similar trend was found for total sulfate and water pH, with ranges of 0–4066 mg L^{-1} and 6.4–8.6, respectively. Salinity measured in December 2005 and 2007 dropped sharply at the lower sites, whereas salinity peaks were observed in April 2007 at the upstream sites.

Turbidity varied inversely with salinity and water transparency, decreasing from freshwater to estuarine sites, ranging from 0.57 to 70 Formazin turbidity units (FTU). Elevated levels of turbidity were observed in December months, especially at the lower reach of the basin. Site 3, 4, and 5 had transparency peaks in April 2007.

Phosphate–phosphorus, ammonia–nitrogen, and nitrate–nitrogen were fairly stable across all sampling sites. Phosphate–phosphorous levels were consistent from the early period of study to August 2006, and decreased during October 2006–August 2007, with the lowest level of 0.03 mg L^{-1} at site 7, and was increasing again toward the end of study. Ammonia–nitrogen varied from 0.01 to 1.45 mg L^{-1} and was considerably higher at sites 4, 5, and 6, with maximum levels of 1.45, 1.39, and 1.21 mg L^{-1}, respectively. Nitrate–nitrogen showed a temporal pattern, with peaks observed in two periods (August–December 2005 and December 2006–February 2007).

Concentrations of total iron were generally higher at the upstream sites, except for December 2005 and December 2007, when the concentrations increased throughout the entire basin. Concentrations of copper and cadmium exhibited trends that were opposite to that found for total iron. Copper and cadmium increased downstream, although neither metal was detectable on a few occasions, and cadmium was higher upstream in December 2005 and June–August 2007.

Total coliform bacteria and fecal coliform bacteria were much higher at the most upstream site and decreased sharply

Table 1. *Mean and maximum densities of 31 dominant phytoplankton genera in the upstream freshwater area (sites 1–5) and the downstream estuarine area (sites 6–10), during rainy (November–January) and dry (February–October) seasons in the Na Thap River from June 2005 to December 2007. The minimum densities of the dominant genera were zero, except for* Euglena *sp., which had 447 cells L^{-1} and 82 cells L^{-1} during rainy seasons in the freshwater zone and in the estuarine zone, respectively. Sample sizes of genera were 13 and 51 for the rainy and the dry seasons, respectively, in the freshwater zone, and 15 and 65, respectively, in the estuarine zone.*

	Mean Density (cells L^{-1})				Maximum Density (cells L^{-1})			
	Freshwater		Estuarine		Freshwater		Estuarine	
Taxa	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
Bacillariophyta								
Bacillaria spp. (Bac)	40	13,468	792	12,494	519	574,233	4669	117,113
Bacteriastrum sp. (Bte)	0	4	379	21,068	0	194	4583	498,857
Biddulphia spp. (Bid)	0	7	7250	4529	0	333	39,696	69,287
Chaetoceros spp. (Cha)	3861	14,709	4038	1,568,100	24,100	322,409	24,627	63,073,972
Coscinodiscus spp. (CCi)	998	67,848	38,075	71,838	4970	2,094,610	162,799	915,542
Guinardia sp. (Gui)	0	0	1498	44,708	0	0	16,573	437,413
Melosira sp. (Mel)	1715	440,921	370	4575	15,820	19,975,403	3750	188,966
Navicula sp. (Nav)	216	85	1546	1595	2292	1833	14,554	39,824
Nitzschia sp. (Nit)	34	1088	4224	21,637	438	45,974	36,169	173,544
Pleurosigma spp. (PSi)	1372	251	4954	94,054	12,391	3917	34,732	2,209,131
Rhizosolenia spp. (Rhi)	0	81	1576	505,784	0	2187	19,301	8,642,727
Serirella sp. (Ser)	1621	406	675	894	7415	3667	4938	13,251
Synedra sp. (Syn)	72,329	5549	45,992	3028	380,913	72,289	396,513	112,428
Thalassionema spp. (Tha)	309	45	69,498	111,359	2275	875	407,341	2,300,935
Triceratium sp. (Tri)	107	8	3110	1447	875	417	15,020	10,449
Chlorophyta								
Closterium spp. (Clo)	43,835	2273	52,318	10	126,085	22,303	291,906	472
Cosmarium sp. (CMa)	4305	521	741	0	36,269	7130	9167	0
Hyalotheca spp. (Hya)	222,569	3019	28,397	3086	2,395,940	43,313	175,092	147,521
Micrasterias spp. (Mic)	251,350	1209	56,400	19	1,829,755	41,280	403,583	667
Mougeotia sp. (Mou)	27,823	22,319	6369	0	100,029	513,146	52,106	0
Pandorina sp. (Pan)	7507	7578	2603	6	35,893	211,500	15,676	417
Pleurotaenium spp. (PTa)	1775	118	1411	20	6568	1031	14,012	875
Spirogyra sp. (Spi)	10,929	38,612	6568	11,168	44,062	788,467	56,208	271,892
Staurastrum sp. (Sta)	5979	604	1427	10	35,706	5258	10,056	667
Cyanophyta								
Merismopedia sp. (Mer)	141	1576	355	365	1750	54,013	2342	10,181
Oscillatoria spp. (Osc)	10,803	11,381	48,005	44,081	76,954	149,306	440,570	1,372,451
Euglenophyta								
Euglena sp. (Eug)	8708	17,675	8775	11,069	19,510	243,968	85,131	131,098
Phacus sp. (Pha)	1984	7332	1345	873	9333	100,960	4710	44,917
Pyrrophyta								
Ceratium spp. (Cer)	434	1343	80,360	45,122	2417	19,583	668,751	924,899
Dinophysis spp. (Din)	35	519,709	21,005	8840	429	22,543,407	121,702	204,972
Protoperidinium spp. (Pro)	0	71,866	1456	13,205	0	2,045,143	9149	297,198

downstream, with several peaks observed in August 2006. Oil and grease varied from 0 to 1 mg L^{-1} and were detected intermittently throughout the study period, particularly at the inland sites.

Factor Analysis of Physicochemical Variables

Table 2 shows the factor loadings, with magnitude values less than 0.20 suppressed. If only loadings with magnitudes greater than 0.45 are considered, the five factors do not contain any overlapping variables, with the exception of water pH, which shared a similar amount of the common variability for both factors 1 and 5. The model provided a reasonable fit using five factors ($\chi^2 = 28.6$; df = 16; $p = 0.027$).

The proportion of total variability explained by the five factors was 63%. Factor 1 is indicative of salty river discharge, containing positive loadings for salinity, water pH, and total sulfate. Factor 2 is characterized by heavy metal, comprising positive loadings for cadmium and copper. Factor 3 represents water clarity, containing positive loadings for turbidity and total iron and a negative loading for transparency. Factor 4 characterizes the concentrations of bacteria in the water column, with positive loadings of total coliform bacteria and fecal coliform bacteria. Finally, factor 5 encapsulates the concentration of dissolved oxygen in the basin, consisting of positive loadings for dissolved oxygen and water pH and a negative loading for nitrate–nitrogen. Oil and grease, biochemical oxygen demand, phosphate–phosphorus, and ammonia–

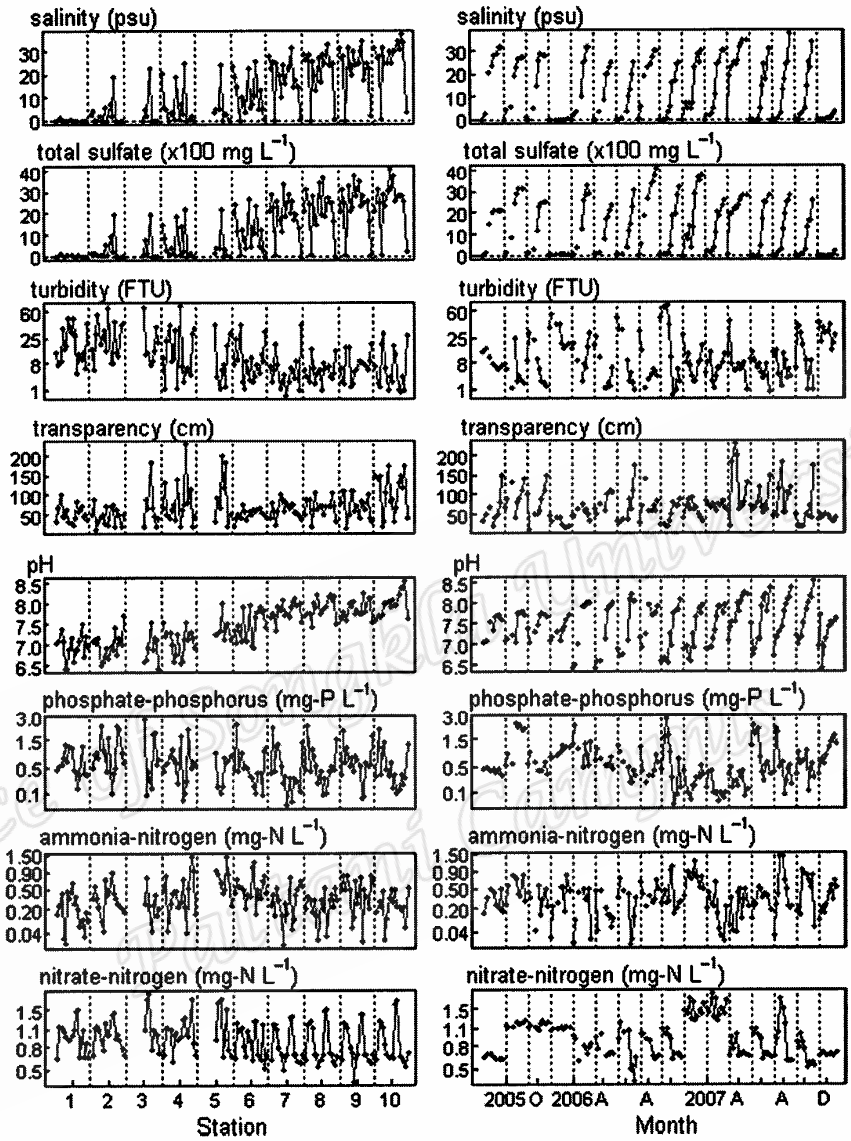

Figure 2. Patterns of physicochemical variables: salinity, total sulfate, turbidity, transparency, water pH, phosphate–phosphorus, ammonia–nitrogen, and nitrate–nitrogen in the Na Thap River from June 2005 to December 2007 by site (left panel) and month (right panel).

nitrogen were classified as unique variables because they did not contribute to the factor analysis.

Relationships between Phytoplankton Density and Physicochemical Variables

To investigate the phytoplankton density in relation to the physicochemical variables, multivariate multiple regression analysis was used to simultaneously fit the outcome variables (31 dominant phytoplankton genera) with the reduced set of physicochemical variables, comprising the five factors and four unique variables obtained from the factor analysis.

The results of the multivariate multiple regression model fitting are shown in Table 3. The coefficients listed are those statistically significant at 5% and 1% (in bold). Since there are 279 regression coefficients in all, denoting the associations

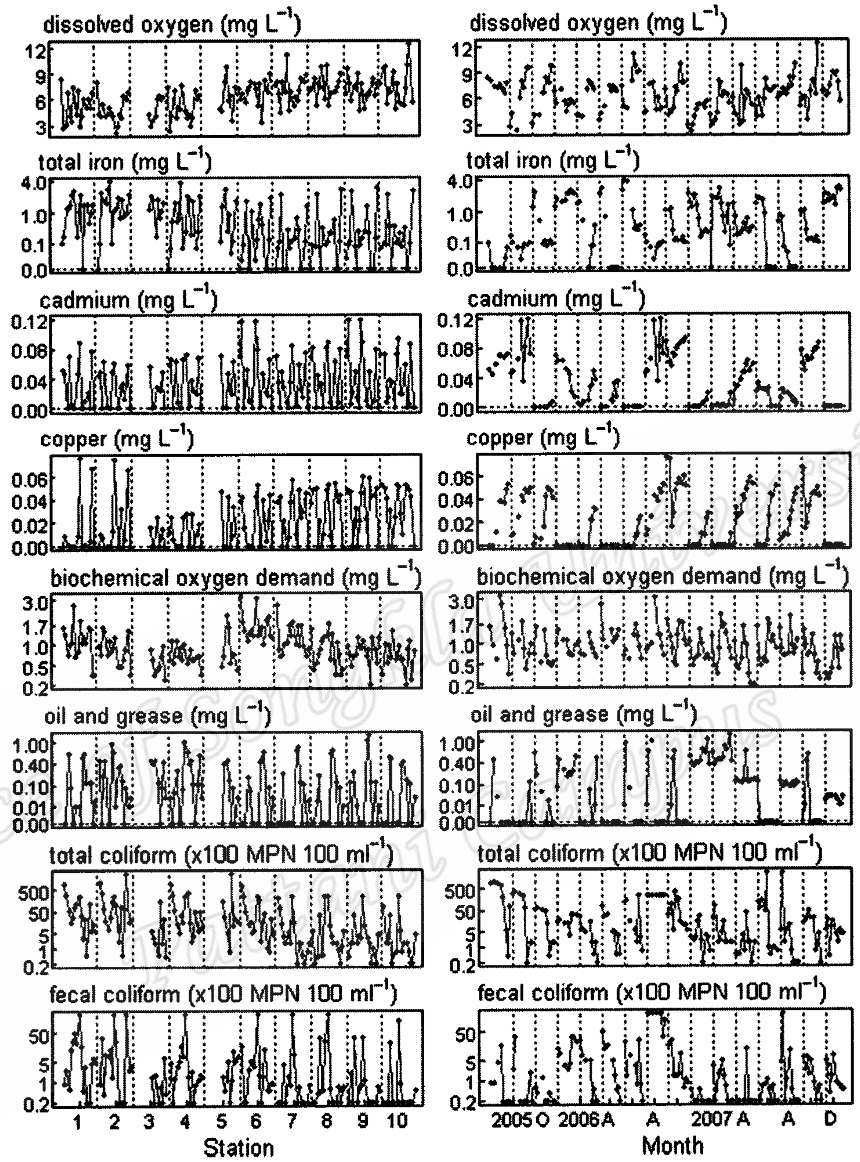

Figure 3. Patterns of physicochemical variables: dissolved oxygen, total iron, cadmium, copper, biochemical oxygen demand, oil and grease, total coliform bacteria, and fecal coliform bacteria in the Na Thap River from June 2005 to December 2007 by site (left panel) and month (right panel).

between the 31 outcome variables and nine environmental predictors, and 5% would be expected to have p values less than 0.05 even if all their corresponding population parameters were zero, the largest 14 p-values less than 0.05 are italicized to indicate failure to achieve "honest" significance (*i.e.,* after allowing for the multiple hypothesis testing). Among the nine physicochemical predictors, factors 1 and 3 had significant effects on changes in the densities of 27 and 18 phytoplankton genera, respectively.

Spatial patterns of phytoplankton density and composition can be distinguished by the concentrations of dissolved salts (factor 1) in the water column, which disclosed clear differences in phytoplankton groups prevailing along the studied area. Positive relationships between factor 1 and a range of

Table 2. *Factor analysis (loadings <0.2 omitted).*

Physicochemical variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Transparency	—	—	**−0.66**	—	—
Salinity	**0.92**	—	—	—	—
Turbidity	—	—	**0.88**	—	—
Water pH	**0.52**	—	—	—	**0.59**
Dissolved oxygen	—	—	—	—	**0.81**
Total sulfate	**0.83**		—	—	—
Nitrate–nitrogen	0.26	−0.20	—	—	**−0.50**
Total iron	—	−0.21	**0.47**	—	−0.20
Cadmium	—	**0.84**	—	—	—
Copper	—	**0.81**	—	—	—
Total coliform bacteria	—	—	—	**1.01**	—
Fecal coliform bacteria	—	—	—	**0.55**	—

phytoplankton genera revealed that a higher level of dissolved salts in the water column promoted the growth of phytoplankton and suggested that these genera were more abundant in the estuarine zone than in the freshwater zone. These genera included diatoms (*Bacillaria* spp., *Bacteriastrum* sp., *Biddulphia* spp., *Chaetoceros* spp., *Coscinodiscus* spp., *Guinardia* sp., *Navicula* sp., *Nitzschia* sp., *Pleurosigma* spp., *Rhizosolenia* spp., *Thalassionema* spp., and *Triceratium* sp.), and dinoflagellates (*Ceratium* spp., *Dinophysis* spp., and *Protoperidinium* spp.). Negative relationships among them indicated that the densities increased as a level of dissolved salts decreased, and *vice versa*, suggesting that the phytoplankton genera preferred to live in the freshwater zone. These genera were cyanobacteria (*Merismopedia* sp.), euglenophytes (*Phacus* sp.), diatoms (*Synedra* sp.), and chlorophytes (*Closterium* spp., *Cosmarium* sp., *Micrasterias* spp., *Mougeotia* sp., *Pandorina* sp., *Pleurotaenium* spp., *Spirogyra* sp., and *Staurastrum* sp.).

Phytoplankton densities were positively related to the clarity of water (factor 3), indicating that the densities increased as the turbidity increased. The genera influenced by this factor included seven chlorophytes (*Closterium* spp., *Cosmarium* sp., *Hyalotheca* spp., *Micrasterias* spp., *Mougeotia* sp., *Pandorina* sp. and *Staurastrum* sp.), sevens diatoms (*Bacillaria* spp., *Biddulphia* spp., *Navicula* sp., *Serirella* sp., *Synedra* sp., *Thalassionema* spp. and *Triceratium* sp.), two euglenophytes (*Euglena* sp. and *Phacus* sp.), one dinoflagellate (*Ceratium* spp.), and one cyanobacteria (*Oscillatoria* spp.).

DISCUSSION

Cyanobacteria, chlorophytes, and euglenophytes were the most common phytoplankton groups in the inland freshwater zone, whereas diatoms and dinoflagellates were more abun-

Table 3. *Coefficients and standard errors (in parenthesis) from fitting the multivariate multiple regression model with all nine predictors. Coefficients with p-values > 0.05 are omitted; those adjudged not honestly statistically significant are shown in italics, and those with p-values < 0.01 are shown in bold. The detailed abbreviation of each genus is shown in Table 1; factor 1 refers to salinity; factor 2 refers to heavy metal; factor 3 refers to water clarity; factor 4 refers to quantity of bacteria; factor 5 refers to dissolved oxygen.*

Genus	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	NH_3-N	PO_4-P	BOD	oilG
Clo	**−1.22** (0.22)	—	**1.21** (0.17)	—	**0.85** (0.19)	—	0.56 (0.24)	**−0.70** (0.22)	—
CMa	**−0.79** (0.18)	**0.52** (0.16)	**0.43** (0.14)	—	—	**−0.75** (0.18)	*0.38* (0.20)	—	**0.56** (0.21)
Hya	—	—	**1.31** (0.21)	0.58 (0.24)	**0.70** (0.24)	—	—	**−0.93** (0.28)	—
Mic	**−0.73** (0.26)	—	**0.81** (0.20)	—	**0.67** (0.23)	—	—	**−0.76** (0.26)	—
Mou	**−1.10** (0.26)	—	**0.75** (0.20)	—	—	**−0.71** (0.26)	—	—	—
Pan	**−1.43** (0.22)	0.45 (0.19)	**0.69** (0.17)	—	0.46 (0.19)	**−0.78** (0.22)	—	−0.48 (0.22)	0.60 (0.25)
PTa	**−0.55** (0.18)	—	—	—	**0.53** (0.16)	—	—	—	—
Spi	**−1.17** (0.35)	—	—	**−1.01** (0.30)	—	−0.79 (0.36)	—	—	—
Sta	**−0.91** (0.21)	0.42 (0.18)	**0.45** (0.17)	—	—	**−0.71** (0.21)	—	—	—
Bac	**1.40** (0.27)	—	**0.55** (0.21)	−0.53 (0.23)	0.56 (0.24)	—	—	−0.61 (0.28)	—
Bid	**1.54** (0.23)	—	*0.38* (0.18)	−0.48 (0.20)	—	—	—	−0.54 (0.24)	—
Bte	**1.07** (0.27)	—	—	—	—	—	—	—	—
CCi	**2.34** (0.32)	**−0.89** (0.27)	—	—	—	*0.68* (0.32)	*0.71* (0.35)	—	—
Cha	**1.87** (0.36)	—	—	—	—	—	—	—	—
Gui	**1.28** (0.29)	—	—	—	*0.50* (0.25)	—	—	−0.74 (0.30)	—
Mel	—	—	—	−0.72 (0.32)	—	—	—	—	—
Nav	0.64 (0.27)	—	**0.72** (0.21)	—	—	—	—	—	—
Nit	**1.72** (0.29)	—	—	—	—	0.72 (0.29)	−0.76 (0.32)	**−0.86** (0.30)	—
Psi	**1.57** (0.32)	—	—	—	*0.53* (0.28)	—	0.87 (0.35)	**−0.66** (0.19)	—
Rhi	**2.34** (0.30)	—	—	—	—	—	—	−0.75 (0.31)	—
Ser	—	*−0.47* (0.25)	**0.73** (0.22)	*0.52* (0.25)	*0.46* (0.25)	—	—	—	—
Syn	−0.74 (0.30)	—	**0.84** (0.24)	—	**0.84** (0.26)	—	**0.92** (0.33)	**−0.98** (0.31)	—
Tha	**2.40** (0.28)	**−0.93** (0.25)	0.49 (0.22)	*−0.54* (0.25)	—	—	—	−0.74 (0.29)	—
Tri	**1.44** (0.23)	—	**0.57** (0.18)	—	0.44 (0.20)	**0.73** (0.23)	—	**−0.68** (0.23)	—
Mer	**−0.79** (0.24)	−0.50 (0.21)	—	—	**0.88** (0.21)	—	—	—	—
Osc	*0.71* (0.36)	—	0.65 (0.29)	—	—	**0.97** (0.37)	**−1.06** (0.40)	—	—
Eug	—	—	**1.23** (0.26)	—	*−0.62* (0.29)	*−0.71* (0.33)	—	—	—
Pha	**−1.13** (0.23)	—	**0.69** (0.18)	—	—	**−1.03** (0.23)	—	—	—
Cer	**1.70** (0.29)	—	**0.67** (0.23)	—	0.63 (0.26)	—	—	*−0.59* (0.30)	—
Pro	**1.86** (0.25)	—	—	**−0.62** (0.22)	—	—	—	—	−0.66 (0.29)
Din	**1.20** (0.28)	**−1.25** (0.24)	—	—	—	**1.07** (0.28)	—	—	—

Abbreviations: NH_3-N = ammonia–nitrogen; PO_4-P = phosphate–phosphorus; BOD = biochemical oxygen demand; and oilG = oil and grease.

dant in the estuarine zone. During the monsoon season, because of heavy rain and lower tidal variation, a large influx of freshwater from the rivers and streams carried excessive nutrients downstream, in turn influencing primary productivity in the lower estuary with the original inhabitants replaced by chlorophytes. This pattern is similar to those occurring in other estuaries in Thailand (Angsupanich and Rakkheaw, 1997) and in other countries (Jackson, Williams, and Joint, 1987; Mallin, Paerl, and Rudek, 1991; Mallin and Pearl, 1994).

Physicochemical variables varied across different spatial and temporal scales of the Na Thap River in response to influences of marine and riverine environments and differences in land use and geographical regions along the basin. The influence of tides extends much further inland than that of salinity, resulting in the upstream region being dominated by freshwater with high turbidity levels and nutrient enrichment (Muylaert, Tackx, and Vyverman, 2005). A similar characteristic was found in the Na Thap River with turbid water upstream and clear water downstream. However, the nutrients were, on average, fairly stable across the basin, with interannual variation for phosphate–phosphorus, spatial variation for ammonium–nitrogen, and temporal variation for nitrate–nitrogen (Figure 2). During the monsoon season in December, the Na Thap River received heavy precipitation and a large freshwater influx reached the estuary, resulting in nutrient and turbidity enhancement and salinity dilution near shore (Mallin *et al.*, 1993).

Tidal variation and nutrient dynamics is more pronounced in tropical estuaries than in temperate estuaries (Nirmal Kumar *et al.*, 2009), resulting in a strong gradient in the environmental variability. In temperate regions, phytoplankton community structure and species assemblage succession have been regulated by different temperature, salinity, and trophic conditions (Gasiunaite *et al.*, 2005). Other environmental determinants have been reported to be low light intensity and high nutrient availability (Popovich *et al.*, 2008), zooplankton grazing (Huang *et al.*, 2004), and a strong tidal-mixing of the water column (Trigueros and Orive, 2001). In tropical regions, phytoplankton density varies from freshwater to estuarine zones and from dry to wet seasons (Varona-Cordero, Gutierrez-Mendieta, and Castillo, 2010). Distributions and compositions of phytoplankton density have been reported to relate to seasonal changes in freshwater flushing (Nirmal Kumar *et al.*, 2009), salinity and turbidity (Pelleyi, Kar, and Panda, 2008), and nutrient availability (Costa, Huszar, and Ovalle, 2009). Our findings in this study were similar to those the results from those previous studies.

Concentrations of dissolved iron decreased with increasing salinity. This tendency is common in other estuaries worldwide (Hunter, 1983; Li *et al.*, 1984; Powell, Landing, and Bauer, 1996), although the unusual trend of increased iron with increasing salinity has been observed in some estuaries (Wang and Liu, 2003), possibly because of sediment resuspension and/or deflocculation of colloidal particles during estuarine mixing. In contrast, the concentration of dissolved cadmium and copper increased with increasing salinity, suggesting desorption of the cations from the suspended solids (Comans and van Dijk, 1988; Duinker and Nolting, 1978). The same behavior of these elements was observed in our study and in other estuaries including the Rhine and Scheldt estuaries (Duinker and Nolting, 1978; Duinker, Nolting, and Michel, 1982). Other metals, including cobalt, lead, manganese, nickel, and zinc, have similar distribution patterns in estuarine systems (Martin and Fitzwater, 1988; Paucot and Wollast, 1997).

Differences in land use and topography along the basin influenced water quality in the Na Thap River. A decreasing trend, from upstream to downstream, of total coliform bacteria and fecal coliform bacteria may be attributed to the much higher-density residential population at the upstream region with consequent sewage and animal waste draining directly into the water. Note that marked concentrations of coliform bacteria at site 6, 9.5 km from the dense population site, may be explained by its reception of wastes and pollutants from intense marine shrimp farms and aquacultural production factories. This result correlates with biochemical oxygen demand, having a decreasing trend between upstream and downstream sites. The results, for both upstream and downstream sites, parallel those of the coliform bacteria. It has been suggested that concentrations of coliform bacteria are directly proportional to the biochemical oxygen demand in aquatic environments (Hiraishi, Saheki, and Horie, 1984). Similarly, a higher concentration of ammonium–nitrogen at the middle sites may be partially attributed to human activities, including changes in land use and water usage.

Relationships between phytoplankton density and the physicochemical variables were investigated using multivariate multiple regression analysis, focusing on the determination of significant factors affecting the changes in density of an individual phytoplankton genus. Salinity and water clarity were the major factors. Other factors were related solely to a particular genus or a small group of genera, suggesting that those species may be present in a certain habitat or can adapt to survive under environmental stress (Reynolds *et al.*, 2002).

The salinity gradient played a major role in determining the distribution of communities of phytoplankton within the estuary. Estuarine species and communities are well adapted to the variations in salinity that are related to tidal cycles and seasonal rainfall patterns. Such variation reduces competition among different phytoplankton groups, possibly causing high rates of estuarine primary productivity. The major estuarine inhabitants in the Na Thap River, as in the other estuaries, were diatoms and dinoflagellates, which were positively correlated with the salinity factor, except for *Serirella* sp. Diatoms dominated over dinoflagellates in cell concentrations and in species diversity throughout the estuary. Small centric diatoms (*Rhizosolenia* spp. and *Thalassionema* spp.) were found in higher concentrations at the downstream sites, whereas large, centric diatoms (*e.g., Coscinodiscus* spp.) were present at the upper estuarine sites and even at the inland sites. Although dinoflagellates were found consistently in relatively low concentrations in the estuary, they have occasionally been reported as the major species responsible for red-tide blooms in freshwater (Berman and Dubinsky, 1985; Hirabayashi *et al.*, 2007; Horne, Javornicky, and Goldman, 1971) or estuarine (O'Shea *et al.*, 1991; Skerratt *et al.*, 2002) systems. Dinoflagellates are known to be dominant in the marine habitats, and under favorable conditions, they can develop into the red-tide blooms causing mass mortality in invertebrates, shellfish, fish, seabirds, and other animals and having adverse effects on human health from contaminated marine mammals (Anderson, 1995).

Our results show that *Cosmarium* sp. dominated the freshwater assemblages and was negatively correlated to salinity and ammonium–nitrogen and positively correlated to heavy metal and turbidity factors. This finding is in accord with the study by Javed and Hayat (1999). Mixed assemblages dominated by cyanobacteria were observed occasionally in the middle sites, which had high levels of ammonium–nitrogen. Although cyanobacteria are known as predominantly freshwater species, our study found that *Oscillatoria* spp. was present throughout the entire study area. Cyanobacteria are commonly thought to be a nuisance, causing the so-called harmful algae bloom, which affects the food chain in aquatic systems, and they are usually linked to changes in nutrient levels defining the trophic status (Marshall, 2009). Cyanobacteria in our study area were low to moderate in density and only occasionally dominated the phytoplankton community, indicating the Na Thap River is a healthy estuarine environment and habitat.

Water clarity was the second significant factor affecting variations in phytoplankton densities in the Na Thap River. Water with high turbidity reduces light penetration into the water column and, therefore, limits phytoplankton photosynthesis. The light limitation controls phytoplankton biomass and prevents phytoplankton from using the available nutrients. Nevertheless, the mechanisms for the development of phytoplankton blooms in freshwater estuaries with high turbidity remain unknown (Cole, Caraco, and Peierls, 1992; Kies, 1997).

In conclusion, the spatial and temporal distributions of phytoplankton density in the Na Thap River were found to be largely controlled by the salinity and turbidity gradients within the different regions of the ecosystem, with chlorophytes and cyanobacteria dominating in the turbid freshwater habitat, and diatoms and dinoflagellates dominating in the clear estuarine environment. The developments of mixed assemblages of riverine and estuarine species varied seasonally throughout the study period and varied predominantly during the monsoon periods, when heavy rainfalls regulated the increasing amount of river flow and nutrient runoff from agricultural, aquacultural, and industrial land into the lower estuary, with subsequent changes in associated water characteristics, destruction of downstream habitats, and loss of estuarine primary productivity. Although unexpected peaks of nutrients and pollutants during the premonsoon season in 2006 did not affect the distributions of phytoplankton, their cause was unclear and deserves further study, probably involving flow rates and the amount of rainfall. With such evidence, changes in phytoplankton densities and compositions in the Na Thap River have been controlled mainly by natural phenomena, rather than by human activity. Our study provides a basic knowledge of the variation in the density of the major phytoplankton groups and community structure and their relationship to the associated water characteristics in the Na Thap River. Understanding such variation provides a basis for further study of the distribution and density of upper aquatic predators, including zooplankton, fish, and other aquatic organisms within the ecosystem studied and other freshwater estuaries.

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3.3 The second article

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Polychaeta Organism Density in Na Thap Estuary

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Abstract

Polychaeta is a class of macrobenthic fauna found in marine and estuarine environments. As part of a larger study of environment health impacts associated with the operation of a thermal power plant in the Na Thap River in Songkhla, Thailand, we examined bi-monthly organism densities of Polychaeta measured at each of five sites along the river downstream from the power plant for before and after its operation began. These densities, after log-transformation to reduce skewness with appropriate handling of zeroes, were found to be related to salinity measured at the same sites on the same occasions. There was also a statistically significant trend in the Polychaeta densities over the whole period.

Keywords: Polychaeta class, Macro-benthic fauna, Salinity, Na Thap River, Data transformation, Regression model

1. Introduction

Scientific studies commonly involve comparisons of means and rates with respect to study factors of interest. For example, an environmental study may investigate the variation of an outcome of interest such as the mean abundance of a species of fish at specific locations along a river. Physical and chemical study factors include water characteristics such as salinity and its related variables (chloride, hardness, total alkalinity and sulphate), water temperature and transparency, turbidity, total suspended solids, dissolved oxygen content, biochemical oxygen demand, heavy metal concentrations, and nitrate and phosphate concentrations. Biological determinants include species at lower levels in the food chain such as phytoplankton and zooplankton abundances.

Polychaeta is a class of macro-benthic fauna that inhabits estuaries of rivers, and its abundance is a biological index of the health of the estuarine environment. Polychaeta densities are related to temperature, transparency, salinity, oxygen demand, pH and minerals (Edgar, 1991). Polychaeta such as *Capitella* sp. and *Theora lubrica* in the family Capitellidae (Kikuchi, 1991; Ferraro *et al.*, 1991) and *Polydora* sp. are biological indicators of organic pollutants. They are usually found in estuaries that contain hydrogen sulfide (H_2SO_4) and high organic matter (Chareonpanich *et al.*, 1994).

For such studies, regression analysis is an appropriate method for measuring differences in these means or rates of interest, and for assessing the statistical significance of their differences. Furthermore, this method can take into account distortions due to the effects of covariates that that can mask or amplify the magnitudes of these differences. Such methods are well established and comprehensively explained in the statistical literature (see, for example, Fox 1997, Venables and Ripley, 2002).

The thermal power plant is located in Chana district, Songkhla province covering 300 acres. It has production capacity of 700 MW by fueling with natural gas from the Thai-Malaysian Joint Development Area in Gulf of Thailand and using approximately 39,000 cubic meters per day of water from Na Thap River for cooling the system. It started to operate the system in January 2008 until present.

In this paper we used these methods to investigate the trend of Polychaeta abundance and its relation with salinity variation in the Na Thap River estuary during a 5-year period from June 2005 to May 2010, as part of an environmental health assessment program of a thermal power plant that began operation at the middle of this period.

2. Materials and Methods

2.1 Data sampling

The data used in this study were collected from the Na Thap River on a bi-monthly basis during a period from June 2005 to May 2010 at 5 sites (stations) in the river, located in brackish and saline water (Figure 1).

Water samples from each site were collected by using 1 liter plastic bottles in size for measuring salinity. Polychaetes were collected by using Ekman dredge 15x15 cm^2 to sieve with 420 micrometre mesh size. The samples were stored in 5% formalin solution in the laboratory. Each species were counted and classified under a stereoscope (Fitter and Manuel, 1986).

2.2 Statistical methods

Linear regression (see, for example, Cook and Weisberg, 1999) is a statistical method widely used to model the association between a continuous outcome and a set of fixed determinants. The model expresses the outcome variable y as an additive function of the determinants. For example, if there are two determinants x_1 and x_2, the model takes the form.

$$y = a + b_1x_1 + b_2x_2 \qquad (1)$$

The model also assumes that the errors are independent and normally distributed with mean 0 and constant standard deviation.

For biological outcomes such as organism counts per unit area or volume, the normality assumption is usually invalid due to skewness in the distribution of the outcomes. In this case the skewness may be reduced substantially by taking logarithms of the outcomes, provided zero outcomes are handled in some way. Clark and Warwick (1994) suggested replacing y by the transformed variable log (1+y). However, for ease of interpretation, results need to be expressed in terms of the organism densities themselves rather than transformed outcomes, so it is necessary to correct the back-transformed means to ensure that the overall mean organism density is preserved. Kongchouy and Sampantarak (2010) gave an appropriate method for modeling proportions using logistic regression, and their method also applies to means modeled using linear regression.

Equation (1) generalizes straightforwardly to any specified number of determinants, including categorical determinants that are treated as factors in the linear regression model. In such cases the parameters of interest are differences between parameters specifying the factor levels and their overall mean, and sum contrasts are needed to obtain appropriate confidence intervals for these differences.

We used the free and open-source basic R program (R Development Core Team, 2007) for all statistical and graphical analysis.

3. Results and Discussion

(Figure 1)

Figure 1 shows map of sampling stations along Na Thap River. The Na Thap River is has a watershed of approximately 232 km^2. It originates at the confluence of Klong Pho Ma and Klong Luek, and after this 26.5 kilometers it flows into the Gulf of Thailand. Therefore, it is water body is a mixture of fresh and sea water subjecting it to receive many influences including tidal regimes, salinity influx, river flows, and surface runoff from the upland regions, resulting in unique characteristics of both the marine and freshwaters. The initial part of the river's body is narrow and rather deep with fresh water and thermal power plant is located in this part. The middle part is wider and shallower than the others and the estuarine part is narrow and deep and adjoins to the Gulf of Thailand.

(Figure 2)

Figure 2 shows histograms of Polychaeta organism densities, before (left panel) and after (right panel) transformation. This transformation reduces the skewness coefficient from 5.13 to 0.13.

(Figure 3)

Figure 3 shows a plot of the transformed Polychaeta organism densities against months elapsed during the study period. Note that the fitted line is corrected by shifting its intercept to make it pass through the point for the mean organism density per square meter (14.4) at the mean number of months elapsed (30.7). The 95% confidence interval for the slope is cantered at the end-point of the fitted line, and scaled to match the increase over the data range. The estimated trend obtained from this model is 1.52 percent per month (95% CI 0.42 – 2.62).

(Figure 4)

The graph on the left of Figure 4 shows that adjusting for salinity reduces the trend very slightly. The adjusted trend estimate is 1.41 percent per month (95% CI 0.34 – 2.46).

The graph on the right of Figure 4 shows a statistically significant relation between Polychaeta abundance and salinity after adjusting for the trend. The increase in the organism density was 2.81 percent (95% CI 1.01 – 4.61) for each unit increase in salinity.

At all stations Polychaete density was statistically significantly related to month and water salinity. The salinity level increased with distance from the thermal power plant to the Gulf of Thailand. The amount of pumping for cooling the system is 39,000 cubic meters of water per day, which is very small compared to the water volume of the river. Thus we have little evidence that the power plant has an impact on the salinity level or temperature of the water.

In November, 2009 – April, 2010, Thailand faced a drought. The average monthly rainfall in the Eastern part of Southern Thailand from November 2009 until April 2010 was lower than the average monthly rainfall from 1950 to 1997. From December 2009 to April 2010 the average monthly rainfall in Chana district of Songkhla province was 15.7 mm, while in the same months from 2005 to 2008, the average monthly rainfall was 76.9 mm (Sithicheewapaak, 2010). Thus, the drought led to intrusion of saltwater in Na Thap River, which can be observed from the increasing salinity along the river.

Sampling stations 2, 4 and 5 are surrounded by communities, shrimp farms, fish cage farms, pig farms, seafood processing factories, and rubber industry (Sapaeing, 2007). Waste water containing organic matter from those areas may cause increasing density of Polychaeta, whereas the location of the power plant is located upstream with less wastewater and organic matter.

4. Conclusion

Polychaeta organism densities in the Na Thap River estuary increased by an average of 1.5 percent per month over the five-year period from June 2005 to May 2010 inclusive, corresponding to a 90% increase (95% CI 25 – 157 percent) over the duration of the study. Given the volatility in the distribution of such organism counts, and the corresponding wide range given by the confidence interval, this result is not particularly conclusive. The impact of environmental factors on Polychaeta organism density in this area should be investigated for further study.

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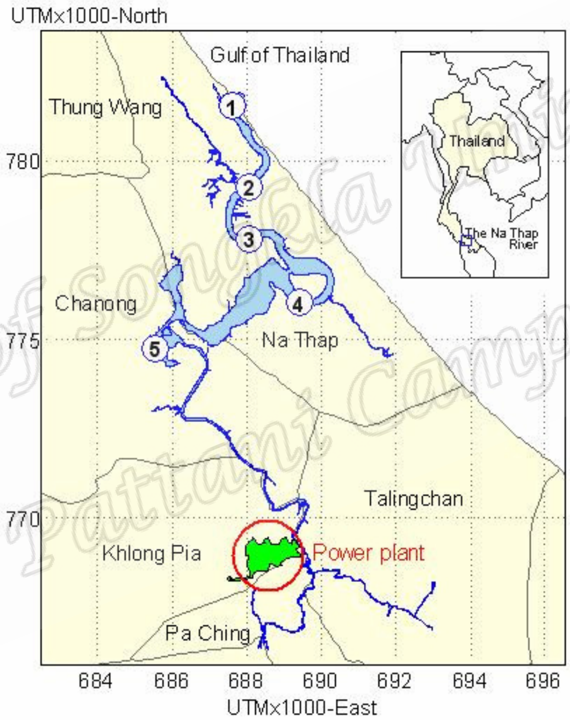

Figure 1. A map with sampling stations

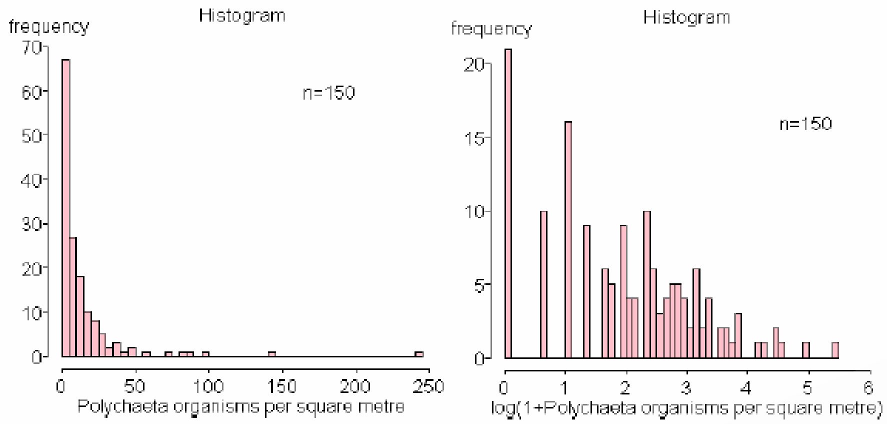

Figure 2. Histograms of Polychaeta organisms per square meter before (left panel) and after transformation using zero-corrected natural logarithms (right panel)

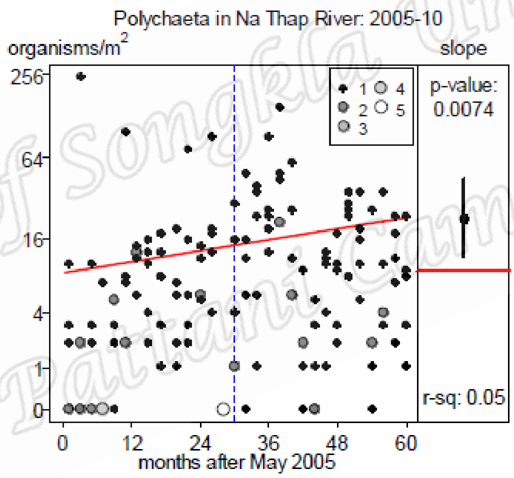

Figure 3. Polychaeta densities observed by months elapsed over duration of study with 95% confidence interval for gradient. The legend gives the number of overlapping points for observations from the 5 sites. The occasions when no Polychaeta were observed are as follows: at one site after 9, 32, 42 and 54 months, at two sites after 1, 3, 5 and 44 months, never at three sites, at four sites after 7 months, and at all five sites after 28 months.

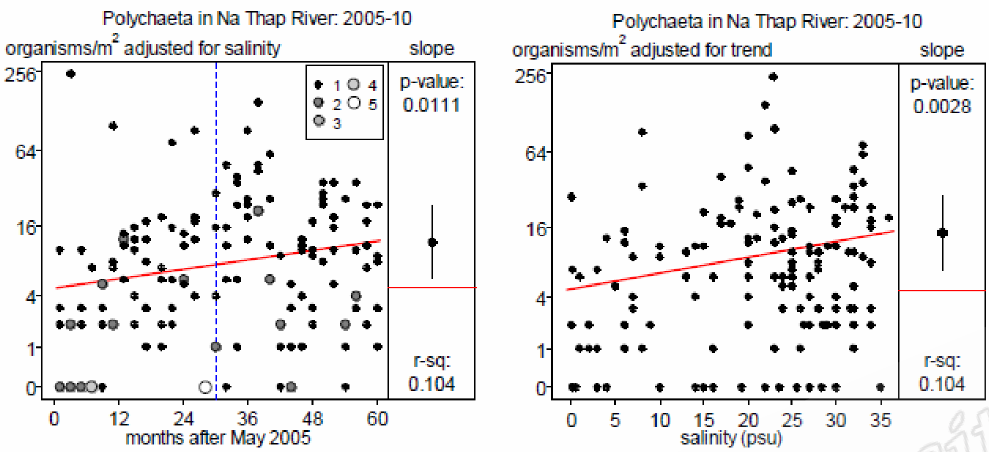

Figure 4. Polychaeta densities by month elapsed adjusted for salinity (left panel), and by salinity adjusted for trend (right panel)