

## Combined Stable Carbon Isotope and C/N Ratios as Indicators of Source and Fate of Organic Matter in the Bangpakong River Estuary, Thailand

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### Abstract

Stable carbon isotopes and C/N ratios of particulate organic matter (POM) in suspended solids and surficial sediment were used to define the spatial and temporal variability in an anthropogenic tropical river estuary, the Bangpakong River Estuary. Samples were taken along salinity gradients during the four different river discharges in the beginning, high river discharge and at the end of the wet season, and low river discharge during the dry season. The values of  $[C/N]_a$  ratio and  $\delta^{13}C$  in the river estuary revealed significant differences from those of the offshore station. Conservative behaviors of  $[C/N]_a$  and  $\delta^{13}C$  in the estuary during the wet season indicated major contribution of terrigenous  $C_3$  plants derived OM. By contrast, during the dry season, marine input mainly dominated OM contribution with an evidence of anthropogenic input to the estuary. These compositions of the bulk sedimentary OM were dominated by paddy rice soils and marine derived OM during the wet and dry seasons, respectively. These results show that the combined stable carbon isotopes and C/N ratios can be used to identify the source and fate of OM even in a river estuary. This tool will be useful to achieve sustainable management in coastal zone.

Keywords: particulate organic matter; stable carbon isotope; the Bangpakong River or Estuary

#### 1. Introduction

An estuary is the region where a terrestrial drainage system meets the sea. With highly variable environments in both space and time, estuaries are the most dynamic and productive ecosystems on earth (Allanson and Baird, 1999). In these systems, heterotrophy generally dominates autotrophy (Gattuso *et al.*, 1998) and the biologically reactive fraction of the riverine organic matter may be partly or entirely mineralized (Abril *et al.*, 2002). One of the key components of the biogeochemical processes in these systems is organic matter (OM), providing substrate for the detritus-based food webs that characterize many estuaries (Wissel and Fry, 2005).

Estuarine OM can be derived from natural and anthropogenic sources; the former including autochthonous and allochthonous inputs and marine-derived material from adjacent coastal water (Graham *et al.*, 2001). Defining the sources and composition of OM in estuaries is crucial for a quantitative understanding of the contribution of terrestrial materials to the energy and nutrient supply of coastal systems (Wu *et al.*, 2004). Transport of terrestrial materials into tropical estuaries is controlled by processing operating in the drainage basins and near the river mouths. Many of the operative processes differ significantly from those temperate rivers (Nittrouer *et al.*, 1995).

In this work, the sources and distribution of OM in a tropical estuary, the Bangpakong River Estuary in the Gulf of Thailand were investigated. This estuary is subject to large seasonal changes in discharge and receives anthropogenic inputs from agricultural, industrial and urban activities. Our approach was to use a combination of elemental and stable carbon isotope analyses to characterize the composition and sources of two reservoirs of OM within the river estuary. Samples were collected along the river estuary channel at four seasonal river discharge regimes. This is the first comprehensive study of OM sources in this regionally important tropical river estuary.

#### Description of the study area

The Bangpakong River Estuary is located in the northeast corner of the Gulf of Thailand (Fig. 1). This river system is the most important watershed in the eastern part of Thailand in terms of water for irrigation, industry, intensive aquaculture and animal farming,

municipal supply and wastewater dilution (Bordalo et al., 2001). The total drainage covers approximately 19,786 km<sup>2</sup> (Boonphakdee et al., 1999) with a human population of about two million. The freshwater inflow to the Bangpakong River comes from Nakorn Nayok and Prachinburi rivers that join 120 km from the inlet and flows naturally to the Gulf without any dam regulations. Mean monthly freshwater discharge varies seasonally reaching a maximum in September (mean 1300 m<sup>3</sup> s<sup>-1</sup>) and a minimum in February (mean 10 m<sup>3</sup> s<sup>-1</sup>) (Boonphakdee *et al.*, 1999). The average depth along the river channel is 8 m. The shallow area of the river estuary, 0.5-2.5 m, is located near the river mouth, where there is a large mud flat. The tide is diurnally mixed with a moderate range of about 1-2.5m (Sojisuporn and Jirasirilert, 1991).

#### 2. Materials and Methods

### 2.1. Sample collection

In order to characterize sources of OM in the Bangpakong River Estuary, samples were taken from 18 stations (St. B12- St. 8) along the transect channel of the river estuary (Fig. 1). Four sampling cruises at contrasting stages of river discharge were carried out on 22 June 2002, 17 October 2002, 7 April 2003 and 30 August 2003.

Surface water was sampled at the middle of the river estuary channel in each cruise. In order to minimize the effect of freshwater from Chao Praya River, suspended matter samples collected from central



Figure 1. Map of Thailand indicating the location of the Bangpakong River and sampling stations in the river estuary (St. B12- St. 8) and the offshore station (St. 9). Marine OM samples were collected at the center of the inner Gulf of Thailand (St. M). Grey area represents intertidal mud flats.

of the inner Gulf of Thailand (St. M) were selected to establish the marine OM end member. Samples of plant leaves of the most abundant trees growing on the bank of the river estuary representing terrigenous  $C_3$  (typical vascular) plants; and soil samples collected from paddy fields and other land sites were collected as potential OM sources.

Water samples were taken 0.5 m below surface using a Kitahara water sampler and filtered immediately through pre-combusted GF/F Whatman glass-fibre filters. All samples were frozen until analysis. Salinity and temperature were vertically measured at 0.5 m depth interval by well-calibrated multi-parameter probes YSI Sonde 6820.

#### 2.2. Analytical methods

POM samples for isotope analysis and for C and N elemental measurements were placed in an evacuated desiccator before acidifying with HCl fumes for 24 hr. (Hedges and Stern, 1984). Suspended particulate matter (SPM) was determined on pre-weighed Whatman GF/F glass-fibre filters. Chlorophyll a (hereafter Chl a) was measured on GF/F filters, which were stored frozen and later Chl a were extracted in 90% acetone (Jeffrey and Humphrey, 1975).

To measure stable isotope ratios of carbon and nitrogen of filters and homogenized terrigenous  $C_3$  plant leaves, they were combusted at 1020 °C in an elemental analyzer (Carlo Erba). The combustion products (CO<sub>2</sub> and N<sub>2</sub>) were introduced to an isotope ratio mass spectrometer (Finnigan MAT) by a He carrier. Isotopic composition of all samples were reported in the standard  $\delta^{13}$ C and  $\delta^{15}$ N notation (‰) relative to PeeDee Belemnite (PDB) standard for carbon and atmospheric N<sub>2</sub> for nitrogen, where:

$$\delta X = [(R_{sample}/R_{std})-1] \ge 1000$$

which *X* is <sup>13</sup>C, and R is <sup>13</sup>C/<sup>12</sup>C for carbon. Instrument drift during analyses was accounted for by running DL-alanine standards every six samples.

One-way analysis of variance (ANOVA) was used to assess the statistical significance of differences among means from various stations and sampling periods. Throughout this paper, statistical differences within 95% confidence interval (*P*-value of 0.05) are discussed.

## 3. Results

#### 3.1. Hydrographic condition

River discharge at the Bangpakong River mouth was calculated as described by Boonphakdee *et al.* 

(1999) primarily on the basis of monthly measurements at Kabinburi gauge station (Royal Irrigation Department – pers. comm.,). River discharges on the four sampling dates [Fig. 2] ranged from 30 to to 712 m<sup>3</sup>sec<sup>-1</sup> on 7 April and 30 August 2003, respectively. Intermediate discharges of 130 and 270 m<sup>3</sup>sec<sup>-1</sup> were recorded on 22 June and 17 October 2002, respectively. Based on these discharge values, we designated the beginning of the wet season, high river discharge, end of the wet season and low river discharge as 22 June 2002, 30 August 2003, 17 October 2002 and 7 April 2003, respectively. The first three cruises were considered as the wet season, whereas the last sampling cruise on 7 April 2003 was representative of the dry season.

Seasonal variations in the distribution of salinity along the estuary are shown in Figs. 3(a-d). During high river discharge, the estuary was stratified [Fig. 3(a)]. On the other hand, surface salinity at the river mouth was up to ~23 and the intrusion of saltwater >0.5 reached 120 km upstream during low river discharge without stratification showing a well-mixed system [Fig. 3(c)].

#### 3.2. OM concentrations and compositions

Concentrations of particulate organic carbon (POC) and particulate organic nitrogen (PON) were significantly different (p<0.001) among the four sampling periods with ranges from 0.03 to 0.32 mg l<sup>-1</sup> and 0.003 to 0.025 mg l<sup>-1</sup> for POC and PON, respectively. Spatial variations in PON and POC concentrations along the river estuary were observed with significant low concentrations of POC and PON at the offshore station (St. 9) (0.05 ± 0.02 and 0.006 ± 0.003 mg l<sup>-1</sup>, respectively). In contrast, high concentrations for each sampling cruise were generally observed where salinity was low (<5) during the wet season, and in the middle estuary (St. B5-B2) during



Figure 2. Daily discharge at the Bangpakong River mouth during the period of study. Sampling dates on 22 June 2002, 30 August 2003 and 17 October 2002 represent the beginning, high river discharge and end of the wet season, and 7 April 2003 is for the dry season, respectively.



Figure 3. Longitudinal distributions of salinity in the Bangpakong estuary during the period of study; (a) the beginning of the wet season, (b) the end of the wet season, (c) low river discharge and (d) high river discharge. River discharge values ( $Q_R$ ) and sampling stations (B12 – St.9) are indicated. The distance is positive seaward from the river mouth (0 km).

POC and PON concentrations in the estuary were strongly correlated ( $r^2$ >0.90, p<0.001) during the wet season [Figs. 4(a, b and d)] but displayed poorer linear correlations ( $r^2$ = 0.53) when river discharge was low in the dry season [Fig.4(c)]. This latter trend indicated significant variability in origin of OM within the estuary at this time. High values of POC/Chl *a* were found in the middle estuary during low river discharge [Fig. 5(c)].

The elemental compositions of POM showed a range of  $[C/N]_a$  ratios varying from 8 to 20.2 with an average of  $13.0 \pm 3.2$  (Table 1). The  $[C/N]_a$  ratios of samples collected during high river discharge (average of  $11.1 \pm 1.8$ ) were lower (p>0.05) than those of samples collected at the beginning and end of the wet season, and during the dry season (averages of  $16.2 \pm 3.0$ ,  $13.1 \pm 1.6$  and  $12.9 \pm 3.5$ , respectively).

Stable carbon isotopic composition of all samples showed depleted  $\delta^{13}$ C values, which varied from -28.8 to -22.2‰. The average  $\delta^{13}$ C values in POM samples were -26.7 ± 1.8, -25.7 ± 2.0, -25.3 ± 0.2 and -26.1 ± 1.0‰ for the beginning and end of the wet season, low river discharge and high river discharge, respectively. Values of  $\delta^{13}$ C at the offshore station with average of -22.6 ± 0.4‰ were significantly higher than those (-26.1 ± 1.2‰) from the rest of the samples (St. B12-St. 8). The  $\delta^{13}$ C values of freshwater samples (salinity<0.5) with an average of -26.9 ± 0.9‰ were slightly lower than those (-25.8 ± 1.1‰) in the estuary, where salinity was > 0.5).

## 3.3. Potential sources of OM in the Bangpakong River Estuary

In order to characterize and predict the origin and fate of OM in the river estuary, we measured several potential natural and anthropogenic sources of OM as shown in Table 2. Values of  $\delta^{13}$ C (-21.5 ± 0.5‰) and [C/N]<sub>a</sub> (6.6 ± 0.3) for marine OM end member samples collected from the central of the inner Gulf of Thailand (St. M) were in the range of marine derived OM (Maksymowska *et al.*, 2000 and references therein).  $\delta^{13}$ C and [C/N]<sub>a</sub> values of freshwater phytoplankton were -27.5 ± 2‰ and 7.3 ± 0.7 and those values for estuarine phytoplankton were -26.5 ± 1.5‰ and 7.8 ± 1.5, respectively. The  $\delta^{13}$ C and [C/N]<sub>a</sub> values for potential anthropogenic sources,

Table 2. Natural and potential anthropogenic OM sourcesin the Bangpakong River Estuary

| Sources                  | n  | [C/N] <sub>a</sub> ratios | $\delta^{13}C$  |
|--------------------------|----|---------------------------|-----------------|
| Natural sources          |    |                           |                 |
| Terrigenous C3 plants    | 15 | $24.6\pm4.2$              | $-29.0 \pm 1.7$ |
| Marine OM                | 6  | $6.6 \pm 0.5$             | $-21.5 \pm 0.4$ |
| Rice paddy soils         | 10 | $16.1 \pm 0.7$            | $-23.3 \pm 1.2$ |
| Freshwater phytoplankton | 6  | $7.3 \pm 0.7$             | $-27.5 \pm 2.0$ |
| Estuarine phytoplankton  | 6  | $7.8 \pm 1.5$             | $-26.5 \pm 1.5$ |
| Mangrove sediment        | 6  | $13.6\pm1.9$              | $-24.4\pm0.6$   |
| Mangrove POM             | 6  | $18.5 \pm 7.9$            | $-26.5 \pm 0.7$ |
| Anthropogenic sources    |    |                           |                 |
| Shrimp feed              | 6  | $7.6 \pm 0.1$             | $-21.0 \pm 0.3$ |
| Shrimp pond POM          | 12 | $7.8 \pm 1.0$             | $-25.3 \pm 2.0$ |
| Shrimp pond sediment     | 12 | $10.4 \pm 1.7$            | $-22.3 \pm 1.6$ |
| Pig farms wastes         | 6  | $6.6\pm0.8$               | $-29.8 \pm 1.2$ |
| Sewage                   | 6  | $12.2 \pm 1.9$            | $-25.3 \pm 1.1$ |

including intensive shrimp farming (i.e., shrimp feed, POM and sediment from shrimp ponds), organic waste from pig farms, sewage of the Chacheongsoa and Chonburi wastewater treatment plants, soils from rice paddies ranged from -21 to -30‰ and from 7 to 12, respectively (Table 2). In contrast, the  $\delta^{13}$ C and [C/ N]<sub>a</sub> values of natural sources of continentally-derived OM, such as terrigenous C<sub>3</sub> plants, natural soils, freshwater & estuarine phytoplankton, and marine OM ranged from are likely to cover the range of those values in POM (Table 2; Fig. 6).

### 4. Discussion

4.1. Distribution of OM in surface water of the Bangpakong River Estuary



Figure 4. Plots of PON (triangles) and POC (circles) against salinity for POM in the Bangpakong estuary during (a) the beginning of the wet season, (b) the end of the wet season, (c) low river discharge and (d) high river discharge. Conservative mixing lines for PON and POC are indicated by broken and dotted lines, respectively.

Distributions of POC and PON in the Bangpakong Estuary during the wet season were mostly conservative due to influences of large river inputs [Figs 5(a, b and c)] consistent with conservative mixing of SPM ( $r^2>0.8$ ; plot not shown). These conservative behaviors were to be expected from conservative mixing over the salinity ranges in the estuary.

Relationships between POC or PON and salinity during low river discharge in the dry season [Fig. 4 (c)] were low ( $r^2 < 0.05$ ) in contrast to those in the wet season. POC and PON were present at higher concentrations in the mid salinity region (10<salinity <16), where turbidity was high, than in the low salinity region (salinity <10) where turbidity was low. These showed removal and additional behaviors of PON and POC along salinity gradients in the estuary. These variations differed from those of unpolluted estuaries where POC in the turbidity zone was relatively constant (Abril *et al.*, 2002). These spatial variations in the Bangpakong Estuary indicated that it was a site



Figure 5. Relationships of salinity and (a)?[C/N]<sub>a</sub> ratios, (b)  $\delta^{13}$ C (c) POC/Chl *a* ratios for POM in the Bangpakong estuary. Samples were collected from stations where salinity > 0.5. Solid circles(•), solid squares( $\blacksquare$ ), open circles(O), and open triangles( $\triangle$ ) represent the beginning of the wet season, end of the wet season, low and high river discharge, respectively.

of significant OM cycling, where the internal (e.g. flocculation, autochthonous phytoplankton production) and/or external inputs (e.g. anthropogenic loads); and removals of particulate material clearly exceeded the effect of mixing. However it is difficult to estimate the behavior of the riverine particles during this period because we were unable to sample the pure freshwater end members.



Figure 6. Plots of the stable carbon isotopic compositions ( $\delta^{13}$ C) and [C/N]<sub>a</sub> ratios of POM from the Bangpakong River Estuary during the four sampling periods. The symbols of plotted panels correspond to the station numbers in Fig. 1 and Table 1. Data ranges of four OM origins; Terr C3: terrigenous C3 vascular plants, FW phytopl.: freshwater phytoplankton, Est. phytopl.: estuarine phytoplankton and Mar. OM.: marine OM are indicated. The broken lines in each panel are expected area where OM origin end members would plot.

# 4.2. Source of OM in surface water of the Bangpakong River Estuary

Sources of OM in the estuary are diverse and can include phototrophs, sewage-derived POC, zooplankton, fecal matter and aggregates of inorganic and organic materials absorbed onto particle including suspended sediment (Wienke and Cloern, 1987). Even though the composition of these OM sources can undergo significant changes during degradation, they are useful in assessing the contributions of different end-members (Goni *et al.*, 2003; Sugimoto *et al.*, 2006).

Although the distributions of OM in the Bangpakong Estuary were largely influenced by riverborne materials, both [C/N] and  $\delta^{13}$ C did not exhibit strong conservative mixing behaviors [Figs.5(a and b]. Enriched  $\delta^{13}$ C values at the offshore station, ranging from -23.2 to -22.2‰, during the four sampling cruises supported the conclusion that suspended matter was a mixture of marine derived OM. Low [C/N], ratio (8.1) of the offshore samples in the dry season was indicative of contributions by marine phytoplankton. Large variations in  $[C/N]_{a}$  and  $\delta^{13}C$  along salinity gradients indicate the replacement of terrestrial POM in the plume by *in situ* production of marine phytoplankton. Offshore transports during the wet season were expected to be controlled by freshwater discharge. Terrestrial matter was distributed to offshore water before being respired or deposited in the estuary.

Isotopic carbon and  $[C/N]_a$  of POM during low discharge were more scattered than those of the wet season indicating various sources of OM along the estuary. Low  $[C/N]_a$  (8.1) and enriched  $\delta^{13}C$  (-22.7‰) in the offshore samples in the dry season was indicative of contributions by marine phytoplankton(Fry and Sherr, 1983). Estuarine phytoplankton were likely major contributors to POM in low salinity regions (<7). POC/ Chl *a* values in the POM (61-237) were well within the values for healthy algae (20-200)(Cifuentes *et al.*, 1996).

OM in the mid salinity regions (8<salinity<20) show a broad range of  $[C/N]_a$  (11.3-19.5) with depleted d<sup>13</sup>C (-25.8 to -27.0‰) suggesting the presence of allochthonous OM which was supported by high POC/ Chl *a* (>500) ratios [Fig 5(c)]. Cifuentes *et al.* (1988) and Cifuentes *et al.* (1996) distinguished allochthonous OM in their POM samples by extremely high POC/ Chl *a* values. To estimate elemental and isotopic characters of these inputs, samples from stations of which POC/Chl *a* ratio higher than 800 were used, and their elemental and isotopic ratios were averaged. The calculations resulted in a  $\delta^{13}$ C of -26 ± 1.1‰, and [C/N]<sub>a</sub> of 16.1 ± 3.6 which are higher than those reported for similar material from a tropical estuary in Ecuador (Cifuentes *et al.*,1996). This comparison suggests that during low discharge in the dry season, the Bangpakong estuary had a comparatively high allochthonous contribution. This is consistent with an estimate of a large nitrogen input to the estuary (Boonphakdee and Fujiwara, submitted for publication) from anthropogenic activities including agriculture, low salinity shrimp farms, animal husbandry and domestic wastes (Szuster and Flaherty, 2002).

## 4.3. Quantitative assessment of OM sources in the Bangpakong River Estuary

The OM source in the Bangpakong River Estuary was assessed with a mixing model based on the bulk compositions of POM and sedimentary OM. Based on data plotted on Fig. 6, we selected terrigenous  $C_3$  plants, riverine/estuarine phytoplankton, rice paddy soils and marine OM as the four end members for the calculations. In addition to combinations of  $[C/N]_a$  ratios and isotopic carbon, salinity was added to the mixing equations to eliminate the effect of marine OM on that in freshwater. Due to none of freshwater samples corrected during the low river discharge, estuarine phytoplankton was used as one of the four end members instead of freshwater phytoplankton.

Once all sources were established, we solved the following system of linear equations for contributions of the four sources to each sample:

 $\begin{array}{l} F_{1}\delta^{13}C_{1}+F_{2}\delta^{13}C_{2}+F_{3}\delta^{13}C_{3}+F_{4}\delta^{13}C_{4}=\delta^{13}C_{sample}\\ F_{1}[C/N]_{1}+F_{2}[C/N]_{2}+F_{3}[C/N]_{3}+F_{4}[C/N]_{4}=[C/N]_{sample}\\ F_{1}Sal_{1}+F_{2}Sal_{2}+F_{3}Sal_{3}+F_{4}Sal_{4}=Sal_{sample}\\ F_{1}+F_{2}+F_{3}+F_{4}=1 \end{array}$ 

Where  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$  are fractional contributions of the four sources, terrigenous C<sub>3</sub> plants, freshwater / estuarine phytoplankton, land soil and marine OM, respectively. As with other mixing models based on geochemical parameters, a major assumption is that the end member compositions were representative of the OM sources in the study area ( $\delta^{13}C_1 = -29.5\%$ ;  $\delta^{13}C_2 = -27.5$  and -26.5% (for freshwater and estuarine phytoplankton, respectively);  $\delta^{13}C_3 = -23.3\%$ ;  $\delta^{13}C_4 = -21.5\%$ ; [C/N]<sub>1</sub> = 24.6; [C/N]<sub>2</sub> = 7.3 and 7.8 (for freshwater and estuarine phytoplankton, respectively);  $[C/N]_3 = 16.1$ ;  $[C/N]_4 = 6.7$ . These values are still in the ranges reported by Fry et al.(1984); Maksymowska et al. (2000); Gordon and Goni (2003) and Goni et al.(2003). Salinity was nil for rice paddy soil, freshwater phytoplankton and terrigenous C<sub>2</sub> plants sources, while salinities for estuary phytoplankton and marine OM, were 5 and 30, respectively.

The fractional OM contributions of POM samples in the Bangpakong River Estuary were indicative of



Figure 7. Fractional contributions for the four-end member mixing model of OM compositions for POM from the Bangpakong River Estuary during (a) the beginning of the wet season, (b) the end of the wet season, (c) low river discharge and (d) high river discharge.

temporal and spatial variations in OM sources (Fig. 7). In the beginning of the wet season, terrigenous  $C_{2}$ plants accounted for the majority of the OM in most freshwater samples, whereas freshwater phytoplankton and marine derived OM sources seemed to contribute more at the end of the wet season when river discharge was high. As well at this time rice paddy soils became more important OM sources. These inter-seasonal variations displayed the impact of river discharge changes on the OM contributors in this river system. High river discharge, the result of heavy precipitation in the basin, almost certainly enhanced soil erosion and transportation of soil particles to the river. During low river discharge, evidence of anthropogenic contributions were observed in the middle estuary [Fig. 5(c)] with the high POC/Chl a (>500) and [C/N], ratios (>12). This suggested a large input of OM derived from anthropogenic activities to the Bangpakong estuary as described by Bordalo et al. (2001) and Boonphakdee and Fujiwara, (submitted for publication). In the future, however, DO<sup>13</sup>C and biomarker analyses could be applied to approve this explanation.

The end member mixing estimates also indicated that soil-derived OM contributing to the majority of OM present in freshwater sediments. Elevated estimates of marine OM contribution were quite high in estuarine samples. However, the contribution of terrigenous C<sub>3</sub> plant in the turbidity maximum area (St. 6-8) and the offshore station during the wet season can be observed [Figs 7(a-d)]. These contributions may be attributed to the deposition of fine plant fragments, consistent with their elemental and isotopic compositions. Goni et al.(2003) suggested that an increase in salinity and fluid mud in a high turbidity area of an estuary may trap fine sediment and associated organic matter. As indicated previously, nitrogen incorporation during OM decay was likely to be the cause of the high N contents in the sediment. Although no concurrent measurements of phytoplankton and marsh were made during this study, the results also demonstrated the efficient mineralization of autochthonous OM in the Bangpakong River prior to its sequestration in the sediment.

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