

## Water chemistry variability in the lower intertidal zone of an estuary in the Seto Inland Sea, Japan: seasonal patterns of nutrients and particulate compounds

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### Abstract

From April 1994 to April 1996, we carried out monthly surveys on the physical and chemical variability of ebbing water at two intertidal stations near the emerged tidal flat and surface water at a nearby subtidal station, in a tidal estuary of the Seto Inland Sea, Japan. The fresh water runoff was a major source of *new* nitrogen (nitrate + nitrite =  $-2.1 \times \text{salinity} + 74.3$ ,  $r^2 = 0.76$ ,  $p < 0.001$ , at the inner intertidal station). In contrast, the absence or weak correlation of ammonium, phosphate and silicate with salinity in ebbing water highlighted the importance of *in situ* biologically-mediated processes, including the excretory activity of intertidal dominant bivalves (*Ruditapes philippinarum* and *Musculista senhousia*) and primary producer nutrient uptake. Among suspended particulate matter (SPM), the negative correlation of Chl *a* with salinity ( $p < 0.001$ ) suggested that an upper intertidal zone, where high microphytobenthic assemblages occur, may represent a considerable source of microalgal biomass. Whereas, a stronger correlation of POC with pheo-pigments ( $r^2 \geq 0.92$ ) than with Chl *a* ( $r^2 \leq 0.44$ ), a low fraction of living phyto-Carbon (ca. 5%) and a high pheo-pigment/Chl *a* ratio (ca. 3) indicated a high portion of refractory algal material and a close water–sediment coupling on the flat, in which abundant macrozoobenthos is likely to play an important role. In spite of the strong variability of this ecosystems, this study also demonstrated that highly significant seasonal patterns can be recognized in our study area. However, patterns varied depending on the different nutrient species, SPM and sites. In ebbing water, ammonium concentrations were significantly higher in the cold period (i.e. November–April: water temperature  $10.0 \pm 4.4$  °C), when primary producer biomass decreases, while Chl *a* and pheo-pigment content were 4.3 and 4.8 higher in the warm period (May–October: water temperature  $23.5 \pm 4.2$  °C), respectively. At the subtidal site, nutrients were 1.9 (silicate)–2.9 (phosphate) and SPM was 1.8 (POC)–2.1 (pheo-pigments) times higher in the warm period. On an annual basis, mean pheo-pigment and POC content was 12–25 times and 4–8 times higher on the intertidal zone than on the subtidal zone, respectively, while nutrient concentrations were within the same order of magnitude. Such a qualitative difference (i.e. nutrients vs. SPM) between sites suggests that particle deposition and/or removal is likely to occur along the estuary, resulting in a limited SPM content at the surface layer of the subtidal site, while nutrients are more directly transported into the surface layer of the subtidal zone by lower salinity water mass intrusion from the intertidal zone, most importantly during the warm period.

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## Introduction

An evaluation of the variability of the water chemistry in the intertidal zone represents an important background of information to study the dynamics of biophylic elements (i.e. carbon, nitrogen, phosphorus and silicon). Within a tidal estuary, the short-term (hours/days) physical and chemical variability of the water is strongly related to the meteorological conditions (Magni & Montani, 1997) and the changes of fresh water runoff (Schubel & Pritchard, 1986; Page et al., 1995), and to the close link between water chemistry and tidal hydrology (Vörösmarty & Loder, 1994; Yin et al., 1995; Montani et al., 1998).

Irrespective of short-term changes, seasonal patterns of dissolved and particulate compounds can also be recognizable. An evaluation and quantification of seasonal patterns of the water chemistry in the intertidal zone may represent an additional and integrative approach to unveil the extent of *in situ* biological processes, such as nitrogen uptake by primary producers and excretion by macrozoobenthos, which relate to the ecosystem metabolism of production and respiration of an estuary (D'Avanzo et al., 1996). Studies on the seasonal distribution of nutrients in surface water have been undertaken by controlled sampling regimes, such as around high tide (Hernández-Ayón et al., 1993; Balls, 1994). Other works have used either intact cores (Hansen & Blackburn, 1992; Overnellet al., 1995; Cowan et al., 1996; MacIntyre & Cullen, 1996) or *in situ* chambers (Yamada & Kayama, 1987; Yamamuro & Koike, 1993) to quantify the temporal variability of nutrient fluxes at the sediment–water interface. In natural conditions, the characteristics of near-bottom water have also been investigated to assess the change of dissolved and/or particulate compounds (Ward & Twilley, 1986; Cowan et al., 1996), and to determine either the biochemical composition (Navarro et al., 1993; Bonn & Duinevelt, 1996) or the sedimentation (Clavier et al., 1995) of suspended particulate matter (SPM). Little information is, however, available on the seasonal variability of both nutrients and SPM within the intertidal zone during low tide.

This study is part of a multidisciplinary project which aims at quantifying the cycling of biophylic elements in a tidal estuary of the Seto Inland Sea (Magni, 1998; Montani et al., 1998). On the tidal flat, at low tide, we previously followed the development of benthic microalgae assemblages (Magni & Montani, 1997), and investigated the faunal composition and temporal distribution of macrozoobenthos (Magni &

Montani, 1998) and quantified its contribution on the *in situ* processes of benthic nutrient regeneration over a 2-year period (Magni et al., 2000). In the present work, our first objective was to evaluate whether seasonal pattern of nutrient concentrations at low tide could be recognized, in spite of a high short-term variability (Montani et al., 1998), and to compare the variability of major nutrient species over the short-(hours) and the long-(seasonal) period. As a more comprehensive evaluation of the chemistry of ebbing water, we extended the investigations on the seasonal variability of SPM. Finally, we aimed to assess on a seasonal and annual basis possible qualitative differences (i.e. nutrients vs. SPM) between ebbing water and surface water at an innermost subtidal site. We will concurrently discuss the relative influence of external variables (i.e. atmospheric conditions and river runoff) and internal processes (i.e. primary producers and consumers metabolism) on the water chemistry variability in our study area.

## Materials and methods

### *Study area and sampling activities*

Sampling was carried out at two intertidal stations near the emerged flat and at an adjacent subtidal station on a tidal estuary in the Seto Inland Sea, Japan (Figure 1). On the intertidal zone, the inner station (Stn. H1) was set at a creek on the river Shin where we previously investigated for a year the short-term (days) physical and chemical variability of ebbing water and surface sediments (Magni & Montani, 1997). The second one (Stn. H2) was located at the low water level (LWL) and was 150 m distant from Stn. H1 (Figure 1). Due to the temporal variation of the LWL, sampling site varied within a distance of ca. 50 m along the shore line. At both Stns. H1 and H2, the water depth ranged between 20 and 50 cm. Care was taken in our sampling strategy to minimize possible contingent effects of currents and waves on sediment resuspension and exportation, in order to have realistically comparable samples through the year. This was also pursued by sampling constantly in days of relatively calm weather to overcome a possible concurrent effect of wind. During our field observations, the wind speed varied most remarkably from the lowest values of October (monthly mean of  $2 \text{ m s}^{-1}$ , both in 1994 and 1995) to the highest ones of January (monthly mean of 3.0 and  $3.3 \text{ m s}^{-1}$  in 1994 and 1995, respectively), for two consecutive

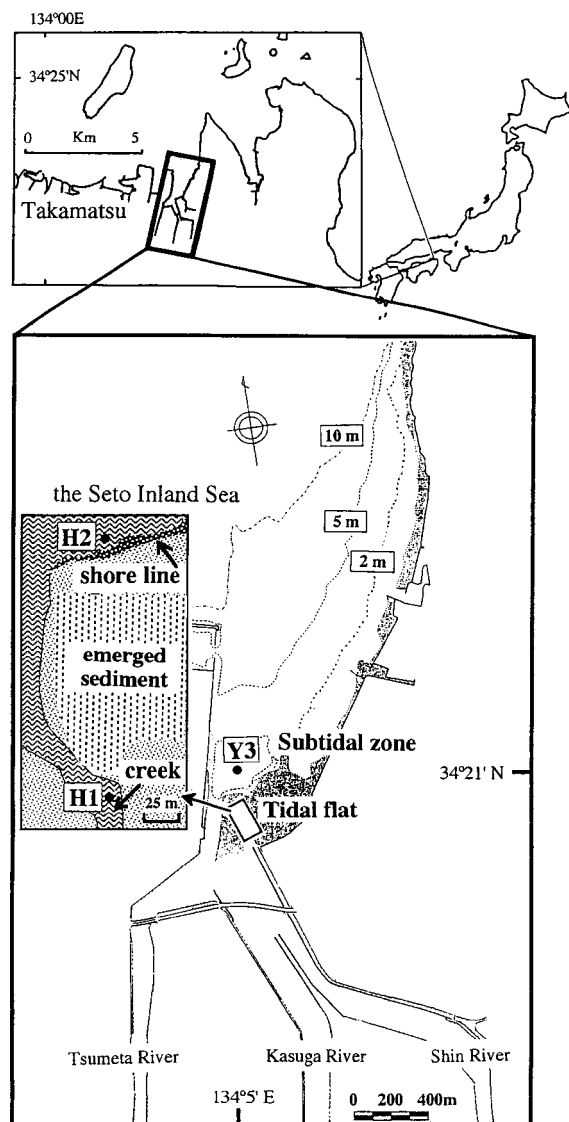


Figure 1. Study area and location of the sampling stations (Stns. H1, H2 and Y3).

years (Takamatsu Observatory of the Meteorological Agency). As a replicates, we simultaneously collected, in 5 l polyethylene bags, surface ebbing water samples at both stations twice. This occurred before and after (1 hour interval) the collection of sediment samples for chemical analysis (i.e. Chlorophyll *a*, pheo-pigments, TOC and acid-volatile sulfides) and the quantitative determination of abundance and faunal composition of macrozoobenthos on a transect line set between Stns. H1 and H2 on the emerged tidal flat (Magni & Montani, 1998; Magni et al., 2000). Concurrently, at both stations, measurements were made of temperat-

ure and dissolved oxygen concentration (D.O. meter, UK 2000) and salinity (portable YSI salinometer).

On the subtidal zone, sampling was carried out at an adjacent station (Stn. Y3, Figure 1) set 200 m distant from Stn. H2. For practical reasons, the field work at Stn. Y3 was not simultaneous to the intertidal survey. This prevented us from a direct comparison between the intertidal and the subtidal sites. Accordingly, we did not attempt to assess the spatial variability of the water chemistry along the estuary on the basis of one-to-one sampling occasion, as we previously demonstrated occurring within 1–2 h (Montani et al., 1998). Whereas, we aimed to have an insight on the extent of variability of the water chemistry at Stn. Y3 as the subtidal site closest to the intertidal zone and to further investigate, on a seasonal and annual basis, differences in distributional patterns between dissolved (nutrients) and particulate (SPM) compounds and between sites. On the subtidal zone, following a similar procedure to that adopted on the tidal flat, hydrological measurements (temperature, salinity and dissolved oxygen) were made using a STDDO (Alec, Model ADO 1050-D) and surface water samples were collected, using a clean bucket, for chemical analysis. Our study area is located in a mixed-semidiurnal type estuary with a mean tidal range of ca. 2 m (Montani et al., 1998). A complementary survey on the short-term (1 h interval) variability of nutrients during a complete tidal cycle, which linked the river to the rear to the innermost part of the subtidal zone, highlighted the close hydrodynamic connection between the intertidal and the subtidal sampling sites of the present study (Montani et al., 1998). We found that main sources of nutrients came either from the fresh water runoff or the intertidal zone, but not the subtidal zone (Montani et al., 1998).

We carried out the field surveys approximately on a monthly basis, from April 1994 to April 1996. Air temperature, rainfall and solar radiation daily data were obtained from the Takamatsu Observatory of the Meteorological Agency, near the estuary under investigation.

#### *Sample treatment and analysis*

In the laboratory, water samples were filtered for the analysis of nutrients [ $\text{NH}_4^+$ -N, ( $\text{NO}_3^- + \text{NO}_2^-$ )-N,  $\text{PO}_4^{3-}$ -P and Si ( $\text{OH}$ )<sub>4</sub>-Si], pigments (Chl *a* and pheo-pigments), particulate organic carbon (POC) and for the determination of suspended solids (SS). For nutri-

ent analysis, subsamples were filtered through Nucleopore filters (0.4  $\mu\text{m}$  pore-size) and concentrations were determined with a Technicon autoanalyzer II, according to Strickland & Parsons (1972). For Chl *a* and phaeo-pigments analysis, Whatman GF/F (0.7  $\mu\text{m}$  pore-size) glass fiber filters were used. Pigments were extracted using a 90% acetone solution. After 24 h extraction in the dark at 4 °C, Chl *a* and phaeo-pigments were spectrophotometrically analyzed according to Lorenzen's (1967) method, as described by Parsons et al. (1984). For POC analysis and the determination of suspended solids (SS), the same volume of water used for pigment analysis (200 ml to 1 l) was filtered on pre-combusted (450 °C for 2 h) and pre-weighed Whatman GF/F filters. The filters, stored at -20 °C, were later freeze-dried and weighed for the determination of SS. Pre-treatment with 12 N HCl vapour to remove carbonates and neutralization with NaOH was carried out before the samples were analyzed for POC, using a CHN analyzer (Yanako, Model MT-3).

To analyse the significance of the correlation among the examined parameters and seasonal differences of the data set, ANOVA (Sokal & Rohlf, 1995) was performed.

## Results

### *Temperature, salinity and dissolved oxygen*

The temperature of ebbing water varied from 3.4 °C (December 1995) to 32.2 °C (July 1994) (i.e. Stn. H1, Figure 2) and closely paralleled the temporal variation of air temperature (Figure 2). At Stn. Y3, the temperature range was comprised between 9.3 °C (February 1995) and 28.1 °C (September 1994) (Figure 2). Annual mean of water temperature was  $16.9 \pm 8.0$  °C,  $16.2 \pm 7.6$  °C and  $18.5 \pm 6.5$  °C at Stns. H1, H2 and Y3, respectively (Table 1).

Salinity averaged  $24.7 \pm 7.9$  psu and  $23.9 \pm 7.9$  psu, at Stns. H1 and H2, respectively (Table 1). During high rainfall, from May 1995 to July 1995, salinity at Stn. H1 ranged between 3.4 psu and 8.3 psu (Figure 2). In contrast, salinity was maximum (32.2 psu) in November 1995, when rainfall was least. At Stn. Y3, the range of salinity variation appeared to be narrower, as from 22.3 psu (July 1995) to 33.8 psu (February 1995) (Figure 2), with an annual mean of  $30.3 \pm 3.2$  psu (Table 1).

Dissolved oxygen (D.O.) concentration ranged from 3.2 mg l<sup>-1</sup> (October 1994) to 15.1 mg l<sup>-1</sup> (April

1996) and from 5.1 mg l<sup>-1</sup> (July 1995) to 15.8 mg l<sup>-1</sup> (March 1996) at Stns. H1 and H2, respectively (Figure 2). At these stations, D.O. concentration was usually oversaturated in early spring and tended to decrease in late summer–early autumn. At Stn. Y3, D.O. variation was more restricted and comprised between 4.0 mg l<sup>-1</sup> (November 1994) and 9.4 mg l<sup>-1</sup> (January 1996). Annual mean of D.O. concentration was  $8.2 \pm 2.8$  mg l<sup>-1</sup>,  $9.7 \pm 4.1$  mg l<sup>-1</sup> and  $7.6 \pm 1.3$  mg l<sup>-1</sup> at Stns. H1, H2 and Y3, respectively (Table 1).

### *Nutrients*

The seasonal variability of nutrient concentrations at Stns. H1 and H2 was rather distinct and varying depending on the nutrient species. At Stn. Y3, all nutrient concentrations tended to increase during the spring–summer period (Figure 3). At this station, we also found an exceptional peak of dissolved inorganic nitrogen (DIN) concentrations in February 1996 (Figure 3).

Ammonium (NH<sub>4</sub><sup>+</sup>-N) concentration varied from 12.6  $\mu\text{mol l}^{-1}$  (August 1994) to 69.2  $\mu\text{mol l}^{-1}$  (October 1994) and from 3.7  $\mu\text{mol l}^{-1}$  (March 1996) to 123  $\mu\text{mol l}^{-1}$  (January 1996) at Stns. H1 and H2, respectively (Figure 3). Ammonium concentration in ebbing water tended to be higher at Stn. H2 than at Stn. H1 (Table 1), and was highest in winter for 2 consecutive years (Figure 3). At Stn. Y3, the annual mean ammonium ( $15.7 \pm 12.9$   $\mu\text{mol l}^{-1}$ ) was ca. 1/2 time that of Stns. H1 and H2 (Table 1).

Nitrate + nitrite [(NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>)-N] concentration (Figure 3) varied from 5.4  $\mu\text{mol l}^{-1}$  (May 1994) to 80.7  $\mu\text{mol l}^{-1}$  (May 1995) at Stn. H1 (mean of  $21.9 \pm 17.9$   $\mu\text{mol l}^{-1}$ , Table 1) and from 0.9  $\mu\text{mol l}^{-1}$  (March 1996) to 50.1  $\mu\text{mol l}^{-1}$  (June 1995) at Stn. H2 (mean of  $19.7 \pm 14.3$   $\mu\text{mol l}^{-1}$ , Table 1). Major peaks coincided with a decrease of salinity and an increase of the rainfall, as from May 1995 to July 1995 (Figure 2). During this period, we observed a drop of the ratio NH<sub>4</sub><sup>+</sup>-(NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>)-N (< 1, not shown), as likely caused by fresh water runoff and a major import of nitrate + nitrite (Figure 3). Whereas at both Stns. H1 and H2, nitrate + nitrite concentration was usually 2–3 times lower than ammonium concentration, up to 4–5 times in winter. At Stn. Y3, nitrate + nitrite concentration varied from 1.0  $\mu\text{mol l}^{-1}$  (February 1995) to 55.7  $\mu\text{mol l}^{-1}$  (February 1996), with an annual mean of  $13.4 \pm 13.0$   $\mu\text{mol l}^{-1}$  (Table 1). Nitrate + nitrite concentration was usually lower at Stn. Y3 than at Stns. H1 and H2. However, the difference in



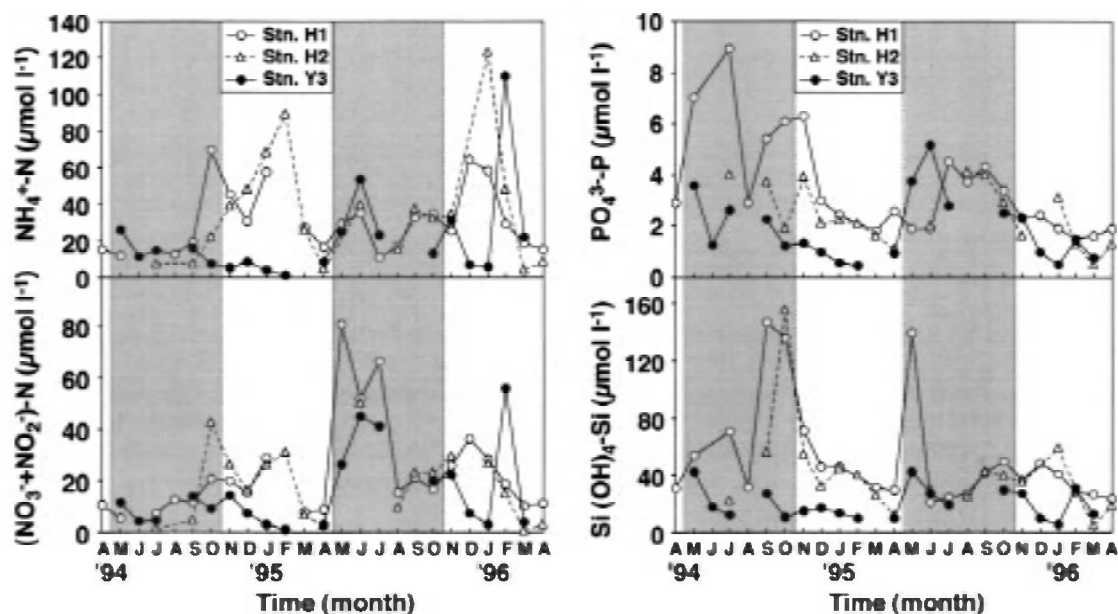


Figure 3. Temporal variation of ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate + nitrite nitrogen [ $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ ], phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) and silicate [ $\text{Si}(\text{OH})_4\text{-Si}$ ] concentrations at Stns. H1, H2 and Y3. Data of Stns. H1 and H2 represent the mean of two samples collected within an hour from each other. Shaded areas: As described in Figure 2.

nitrate + nitrite concentrations between intertidal and the subtidal stations was less than that of ammonium (Table 1).

Phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) concentration varied from  $1.5 \mu\text{mol l}^{-1}$  (February 1996) to  $8.9 \mu\text{mol l}^{-1}$  (July 1994) at Stn. H1, from  $0.5 \mu\text{mol l}^{-1}$  (March 1996) to  $4.1 \mu\text{mol l}^{-1}$  (August 1995) at Stn. H2, and from  $0.5 \mu\text{mol l}^{-1}$  (February 1995) to  $5.2 \mu\text{mol l}^{-1}$  (June 1996) at Stn. Y3 (Figure 3). Phosphate concentration was highest at Stn. H1 between May 1994 and November 1994 (Figure 2). Both in 1994 and in 1995, in contrast to the seasonal trend of ammonium, phosphate concentration tended to decrease during the cold months at all three stations. The annual mean concentration of phosphate varied from  $1.8 \pm 1.3 \mu\text{mol l}^{-1}$  (Stn. Y3) to  $3.5 \pm 1.9 \mu\text{mol l}^{-1}$  (Stn. H1) (Table 1).

Silicate [ $\text{Si}(\text{OH})_4\text{-Si}$ ] concentration varied from  $21.1 \mu\text{mol l}^{-1}$  (June 1995) to  $146 \mu\text{mol l}^{-1}$  (September 1994) and from  $5.2 \mu\text{mol l}^{-1}$  (March 1996) to  $155 \mu\text{mol l}^{-1}$  (October 1994) at Stns. H1 and H2, respectively (Figure 3). In ebbing water, sharp peaks occurred in September 1994, October 1994 and May 1995, which coincided with periods of high rainfall (Figure 2). Similarly to phosphate, silicate concentration decreased in winter. At Stn. Y3, silicate concentration varied from  $6.1 \mu\text{mol l}^{-1}$  (February 1995) to  $42.5 \mu\text{mol l}^{-1}$  (May 1995). The annual mean concentration

of silicate was relatively higher at Stn. H1 ( $50.1 \pm 34.9 \mu\text{mol l}^{-1}$ ), intermediate at Stn. H2 ( $37.5 \pm 26.8 \mu\text{mol l}^{-1}$ ) and low at Stn. Y3 ( $19.5 \pm 10.9 \mu\text{mol l}^{-1}$ ) (Table 1).

#### Nutrient stoichiometric balance

The N/P ratio widely fluctuated between 2.5 (July 1994) and 61.3 (May 1995) at Stn. H1 and between 2.2 (July 1994) and 57.9 (February 1995) at Stn. H2 (Figure 4). At these stations, major peaks occurred in winter of both 1994–95 and 1995–96, and between May and June 1995. At Stn. Y3, the N/P ratio ranged between 5.3 (February 1995) and 34.7 (March 1996). An unusual peak at Stn. Y3 occurred in February 1996 (N/P = 114) (Figure 4), in coincidence with an exceptional nitrogen enriched sample (Figure 3). The Si/N ratio ranged mostly between 0.4 and 1.6, with peaks in September 1994 at both Stns. H1 and H2 and in February 1995 at Stn. Y3 (Figure 4). The Si/P ratio at Stns. H1 and H2 ranged between 5 and 25, with two highest peaks in October 1994 (Stn. H2) and May 1995 (Stn. H1) (Figure 4).

#### Suspended particulate matter (SPM)

At Stns. H1 and H2, the SPM (Chlorophyll *a*, pheopigments, particulate organic carbon and suspended

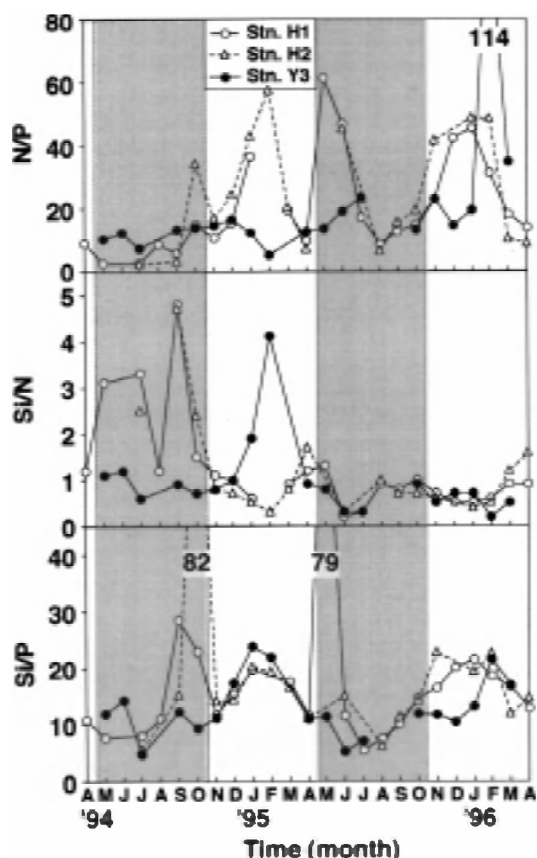


Figure 4. Temporal variation of elemental N/P, Si/N and Si/P ratios at Stns. H1, H2 and Y3. Data of Stns. H1 and H2 represent the mean of two samples collected within an hour from each other. Shaded areas: As described in Figure 2.

solids) content sharply increased for 2 consecutive years during the spring–summer period (Figure 5).

Chlorophyll *a* (Chl *a*) varied from  $0.3 \mu\text{g l}^{-1}$  (March 1996) to  $46.2 \mu\text{g l}^{-1}$  (August 1994) at Stn. H1, and from  $1.1 \mu\text{g l}^{-1}$  (November 1995) to  $25.9 \mu\text{g l}^{-1}$  (August 1995), at Stn. H2 (Figure 5). At Stn. Y3, Chl *a* varied from  $0.8 \mu\text{g l}^{-1}$  (February 1995) to  $6.1 \mu\text{g l}^{-1}$  (July 1994). At this station, we found an occasional peak in February 1996 ( $47.3 \mu\text{g l}^{-1}$ , Figure 5), which corresponded to an exceptional dissolved nitrogen-enriched sample (Figure 3).

Pheo-pigments varied from  $0.5 \mu\text{g l}^{-1}$  (February 1996) to  $89.4 \mu\text{g l}^{-1}$  (October 1994) at Stn. H1, and from  $2.6 \mu\text{g l}^{-1}$  (January 1995) to  $115 \mu\text{g l}^{-1}$  (October 1994) at Stn. H2 (Figure 5). Pheo-pigment distribution did not parallel that of Chl *a* (Figure 5). At Stns. H1 and H2, both in 1994 and in 1995, Chl *a* content was maximum in summer (July–August), while pheo-pigment peaks were highest in October

1994 and June 1995 (Figure 5). At Stn. Y3, pheo-pigments varied from  $0.1 \mu\text{g l}^{-1}$  (December 1994) to  $2.0 \mu\text{g l}^{-1}$  (June 1995), with a relative peak in February 1996 ( $14.4 \mu\text{g l}^{-1}$ ) (Figure 5). On an annual basis, the pheo-pigment content in ebbing water was higher than that of Chl *a* (Table 1). In contrast, at Stn. Y3 the pheo-pigment content was 3 times lower than the Chl *a* content (Table 1).

The POC varied from  $0.58 \text{ mg l}^{-1}$  (March 1996) to  $16.5 \text{ mg l}^{-1}$  (October 1994) at Stn. H1, and from  $0.97 \text{ mg l}^{-1}$  (September 1994) to  $19.9 \text{ mg l}^{-1}$  (October 1994) at Stn. H2 (Figure 5). At Stn. Y3, POC values ranged between  $0.20 \text{ mg l}^{-1}$  (February 1995) and  $1.18 \text{ mg l}^{-1}$  (June 1995), with a peak of  $4.36 \text{ mg l}^{-1}$  in February 1996 (Figure 5).

Suspended solid (SS) content varied from  $15.6 \text{ mg l}^{-1}$  (February 1996) to  $401 \text{ mg l}^{-1}$  (October 1994) and from  $20.5 \text{ mg l}^{-1}$  (January 1995) to  $478 \text{ mg l}^{-1}$  (June 1995) at Stns. H1 and H2, respectively. At Stn. Y3, SS varied from  $5.9 \text{ mg l}^{-1}$  (March 1996) to  $38.8 \text{ mg l}^{-1}$  (May 1995).

#### Ratios among SPM components

The pheo-pigment/Chl *a* ratio in ebbing water showed a wide temporal variability (Figure 6). At Stns. H1 and H2, this ratio was, with few exceptions, higher than 1, up to  $> 10$  in October 1994 and March 1996 (Figure 6). In contrast, at Stn. Y3 the pheo-pigments/Chl *a* ratio varied between  $< 0.1$  (December 1994) and 1.1 (June 1995) (Figure 6). The ratio between the POC and the Chl *a* content (C/Chl *a*) at Stns. H1 and H2 indicated major peaks in October 1994 and March 1996, as found for the pheo-pigment/Chl *a* ratio (Figure 6). At Stn. Y3, the C/Chl *a* ratio fluctuated to a lesser extent. At this station, a noticeable increase occurred during the period of much rainfall, as in May and June 1995, in coincidence with a parallel decrease of salinity (Figure 2) and a peak of all nutrient concentrations (Figure 3). The range of temporal fluctuation of the organic content in the suspended solids (C/SS ratio) was relatively larger at Stn. Y3, varying from  $17.1 \text{ mg g}^{-1}$  (May 1995) to  $162 \text{ mg g}^{-1}$  (February 1996) (Figure 6).

## Discussion

#### Physical variability

The large seasonal variation of water temperature at

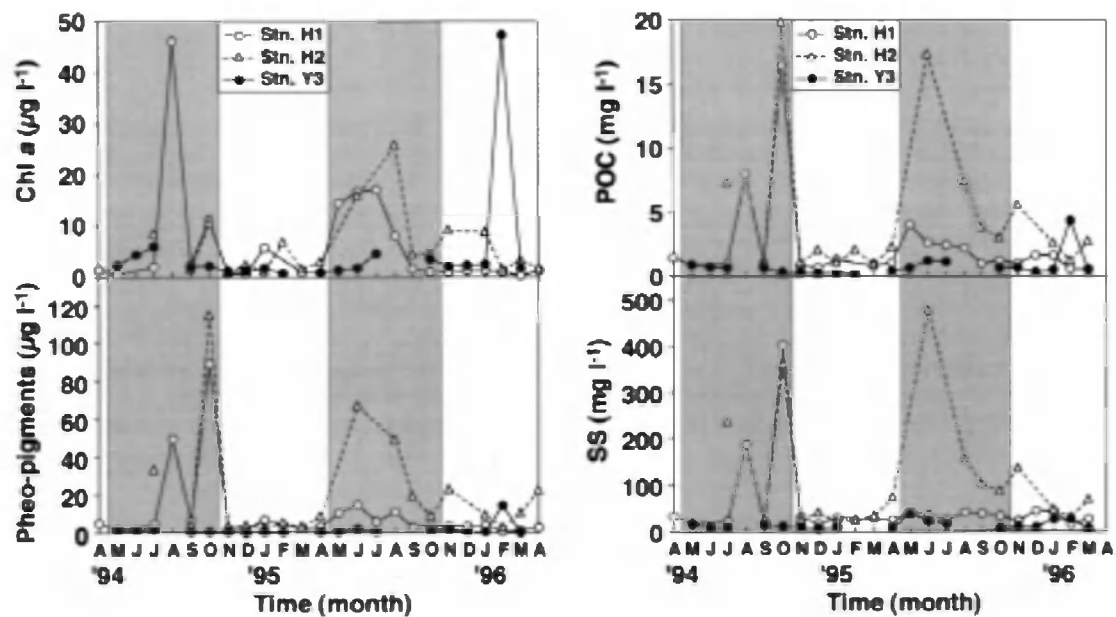


Figure 5. Temporal variation of suspended particulate matter (SPM) at Stns. H1, H2 and Y3 (Chl *a*: Chlorophyll *a*, POC: particulate organic carbon, SS: suspended solids). Data of Stns. H1 and H2 represent the mean of two samples collected within an hour from each other. Shaded areas: As described in Figure 2.

Stns. H1 and H2 was the result of the direct and strong influence of atmospheric conditions (i.e. air temperature) during low tide. Heat transfer in summer and water cooling in winter through the surface water were most effective at Stn. H1, while more limited at Stn. Y3 (Figure 2). The range of ca. 30 °C highlighted the great environmental variability of our study area as compared to that reported in flats/beaches located at higher latitudes (Beukema et al., 1988; Bamber 1993).

A linear regression analysis between the monthly mean salinity in ebbing water (Stns. H1 and H2) and the sum of rainfall occurred in the last three days preceding each sampling and that on the sampling date indicated a highly significant negative correlation between these two variables (rainfall =  $-2.7 \times \text{salinity} + 80.7$ ,  $r^2 = 0.60$ ,  $p < 0.001$ ,  $n = 24$ , plots not shown). Assuming a direct effect of rainfall to the extent of fresh water runoff and considering the rapid intrusion of low salinity water mass into the intertidal zone during low tide (Montani et al., 1998), we infer that the temporal variation of rainfall may strongly influence salinity in ebbing water. Most noticeably, the high rainfall between May 1995 and July 1995 corresponded to the strongest decrease of salinity in ebbing water (Figure 2). At Stn. Y3, the overall restricted temporal change of salinity may be ascribed to the decreasing impact of fresh water runoff and the

progressive mixing between intertidal and sea water masses (Montani et al., 1998).

Dissolved oxygen (D.O.) concentration in ebbing water tended to be oversaturated in early spring (Figure 2). This occurred in relation to the sharp increase of temperatures and solar radiation, most noticeably in 1996 (Figure 2), and the rapid development of a high macroalgal biomass (*Ulva* sp., field observations). In contrast, the seasonal deterioration of atmospheric conditions in late spring–early summer (i.e. increased rainfall and decrease of solar radiation, such as in 1995, Figure 2) and/or early autumn (i.e. decrease of solar radiation and temperatures) corresponded to a drop of D.O. concentration. This may be attributable to a decrease of primary producer activity and an increase of decomposition processes and benthic respiration (Magni & Montani, 1998).

#### *Nutrients: river runoff and "in situ" processes*

As an attempt to evaluate the impact of fresh water runoff on the water chemistry at individual stations, we plotted nutrient concentrations vs. salinity (Figure 7). On the intertidal zone, we found a significant negative correlation for nitrate + nitrite, particularly strong at Stn. H1 ( $r^2 = 0.76$ ,  $p < 0.001$ ; Figure 7). In contrast, at both Stns. H1 and H2, the correlation of ammonium



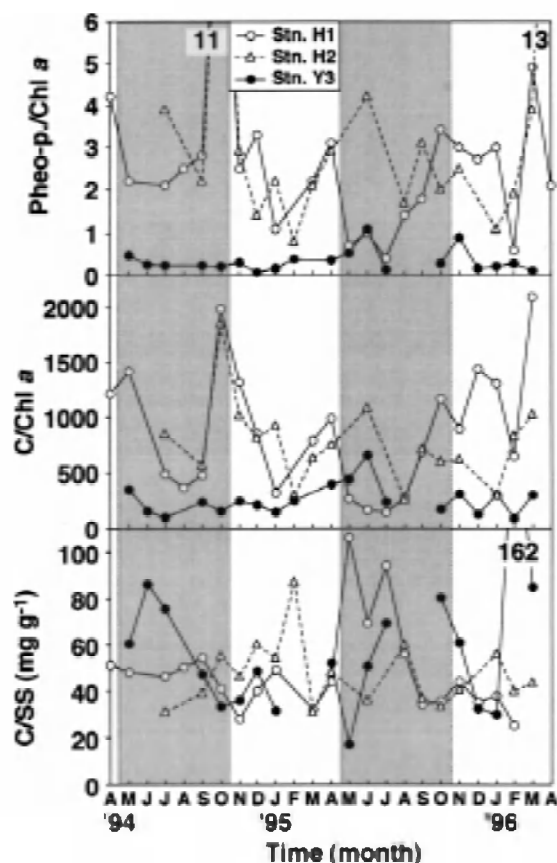


Figure 6. Temporal variation of the ratios among components of suspended particulate matter (SPM) (Pheo-p/Chl *a*: pheo-pigment/Chlorophyll *a*, C/Chl *a*: particulate organic carbon (POC)/Chlorophyll *a*, C/SS: particulate organic carbon (POC)/suspended solids (SS) ratios). Data of Stns. H1 and H2 represent the mean of two samples collected within an hour from each other. Shaded areas: As described in Figure 2.

and silicate with salinity was not significant and that of phosphate was significant, but rather weak, only at Stn. H2 ( $r^2 = 0.28$ ,  $p < 0.01$ ; Figure 7). At Stn. Y3, all nutrient species, but ammonium, correlated significantly with salinity, being strongest that of phosphate ( $r^2 = 0.51$ ,  $p < 0.001$ ; Figure 7).

The distributional pattern of nitrogen compounds in ebbing water was similar to that observed during a 24-h time series survey over a tidal cycle on a transect line set along the estuary (Montani et al., 1998). During both the short-term (24-h) and the present seasonal (2 years) investigations, the river runoff was a major source of *new* nitrogen (nitrate + nitrite) for this estuary. In contrast, we found that in both surveys ammonium was added at an intermediate salinity range (i.e. 20–28 psu, Figure 7). The low correlation

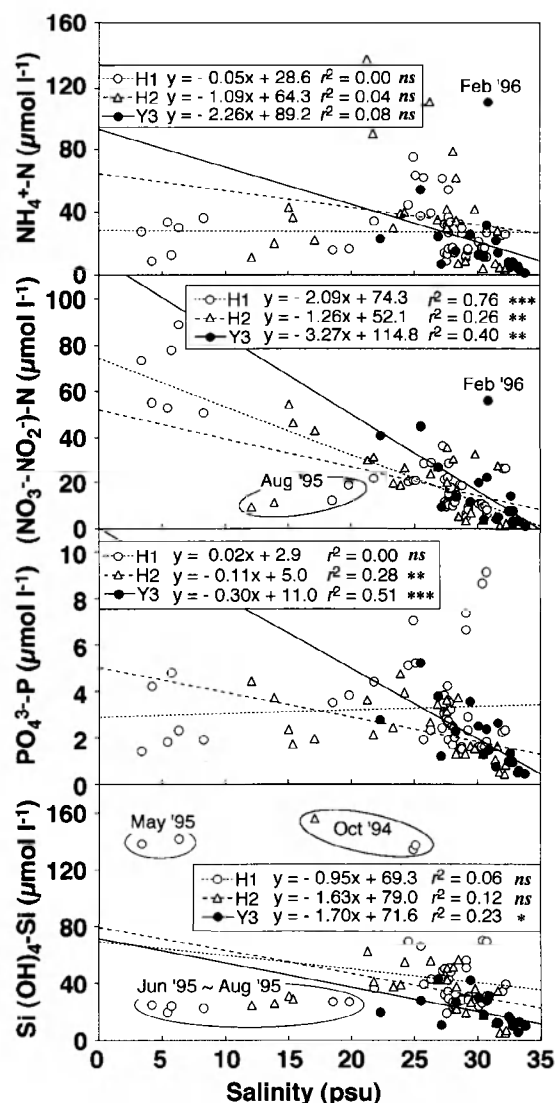


Figure 7. Plots of ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate + nitrite nitrogen [ $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ ], phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) and silicate [ $\text{Si}(\text{OH})_4\text{-Si}$ ] against salinity at individual stations. At Stns. H1 and H2, duplicate values from samples collected within an hour each other are plotted. Circled nitrate + nitrite nitrogen values indicate the sampling occasion in which relatively low nitrate + nitrite concentrations occurred at intermediate salinity. Circled silicate values highlights the periods of major addition (October 1994 and May 1995) or removal (from June 1995 to August 1995) of silicate during the 2-year period of investigation. Lines are best fitting linear regressions (ANOVA, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns: not significant).

between ammonium concentration and salinity is, therefore, attributable to processes of nutrient regeneration within the estuary rather than to the riverine import. In an associated piece of work carried out

on the nearby tidal flat, we demonstrated that dominant bivalves (*Ruditapes philippinarum* and *Musculista senhousia*) have a major impact on the year-round processes of benthic nutrient regeneration within the intertidal zone, with an excretion rate of ammonium up to  $> 30 \text{ mmol l}^{-1} \text{ NH}_4^+ \text{-N m}^{-2} \text{ d}^{-1}$ , as in August and September 1994 (Magni et al., 2000). These observations support our hypothesis that *in situ* biologically-mediated processes importantly contribute to explain the low correlation between ammonium concentration and salinity in ebbing water.

In addition, we found that at both Stns. H1 and H2, ammonium concentration was higher in winter (Figure 3). This is in contrast with a seasonal pattern which shows summer maximum of ammonium due to the enhancement of benthic processes of decomposition (Balls, 1992; Cowan et al., 1996). We argue that higher ammonium concentrations in winter may also relate to the seasonal change of macroalgal (*Ulva* sp.) biomass and its ammonium requirements. As recently reported for several lagoon and embayments (Piriou & Menesguen, 1992; Viaroli et al., 1992; Rivers & Peckol, 1995), also in our study area the macroalga *Ulva* sp. displays a marked seasonal pattern. In particular, it tends to grow heavily in spring and summer. It has been shown that the development of *Ulva* spp. is strongly influenced by several environmental and chemical factors, such as light and water temperature (Steffensen, 1976; Lapointe & Tenore, 1981) and nitrogen loading (Piriou & Menesguen, 1992). In addition, Rivers & Peckol (1995) showed that the ammonium uptake rate of *Ulva* sp. is extremely high at both subsaturating ( $15 \mu\text{mol l}^{-1}$ ) and saturating ( $75 \mu\text{mol l}^{-1}$ ) concentrations. Accordingly, Viaroli et al. (1996) found a marked seasonal pattern of nutrient benthic fluxes in a macroalgal-dominated system. The authors indicated that ammonium was kept at low levels during the *Ulva* sp. growth period due to macroalgal uptake. In light of these considerations, we infer that during the spring-summer period much of the ammonium released into the water column as an excretion product by the macrozoobenthos can be efficiently utilized by the macroalgae itself. Accordingly, macroalgal development (up to  $10 \text{ kg WW m}^{-2}$ , *in situ* estimation) helps to explain the often oversaturated D.O. concentrations, particularly in early spring (Figure 2), which relate to biological events of primary production by the macroalgal itself (Viaroli et al., 1992). In contrast, in winter the macroalgal biomass is much reduced or absent. As a result, the ammonium available within the intertidal zone, including

that released by macrozoobenthos, would remain not utilized, rather than biologically taken up by primary producers.

In ebbing water, similarly to ammonium, we did not observe an important addition of phosphate at salinity  $< 20$  psu (Figure 7). This suggests that also phosphate is strongly affected by *in situ* processes of biogenic regeneration (Magni et al., 2000) and algal removal. As for ammonium, the increasingly significant slope and correlation of phosphate with salinity from Stn. H1 to Stn. Y3 (Figure 7) further suggest that this nutrient species may be exported from the intertidal zone into the subtidal zone at intermediate salinity values.

In the present study, we did not observe a significant correlation between silicate and salinity in ebbing water (Figure 7). This is in contrast to the patterns of nitrate + nitrite (Figure 7) and to our observations during the short-period (hours), in which silicate showed conservative mixing (Montani et al., 1998). Balls (1994) indicated that conservative mixing of dissolved nutrients from Scottish east coast rivers was a function of estuarine flushing time which controls the extent to which internal processes can modify nutrients inputs. In our study area, the relatively longer residence time of ebbing water within the intertidal zone during summer, due to a relatively higher low water level (Magni & Montani, 1998), may have affected the conservative mixing of silicate and facilitated its removal along the estuary. In particular, the relatively low silicate concentrations at salinity  $< 20$  psu, as from June to August 1995 (Figure 7), and the increase of Chl *a* in summer (Figure 5) suggest the effect of biological processes, such as silicate uptake by microalgae, within the intertidal zone.

#### *Suspended particulate matter (SPM)*

In ebbing water, Chl *a* peaked in summer of both 1994 (Stn. H1) and 1995 (Stn. H2) (Figure 5). Each year, the coincident limited increase of pheo-pigments (Figure 5) and sharp decrease of the C/Chl *a* ratio (Figure 6) were further evidences of recently formed microalgal biomass. In a previous study, we found that at an upper intertidal station, 1.1 km distant from Stn. H1, microphytobenthic assemblages strongly develop during periods of favourable conditions (i.e. calm and fine weather), up to  $700 \text{ mg Chl } a \text{ m}^{-2}$  in the uppermost 2 cm of the sediments (Magni & Montani, 1997). Whereas, such a high microphytobenthic biomass may considerably decrease within a few days/weeks as it is

Table 2. Annual mean of the ratios among nutrient species and SPM (suspended particulate matter) components at the intertidal (Stns. H1 and H2) and the subtidal (Stn. Y3) stations. At Stn. Y3, data of February 1996 are excluded from the mean. S.D.: standard deviation; n.: number of samples; C/SS: carbon content (POC) in suspended solids (SS) expressed as  $\text{mg g}^{-1}$

Param.	N/P	Si/N	Si/P	Pheo/Chl <i>a</i>	C/Chl <i>a</i>	C/SS
Stn. H1	19.1	1.2	16.4	2.8	855	54.1
S.D.	15	1.0	13.9	2.5	691	20.9
n.	54	54	54	48	50	50
Stn. H2	25.6	1.0	16.9	3.1	727	48.9
S.D.	17.9	0.9	13.2	2.7	375	19.4
n.	31	31	31	31	30	30
Stn. Y3	15.5	1.0	12.4	0.3	269	52.6
S.D.	6.7	0.9	5.3	0.3	135	21.1
n.	18	18	18	18	18	17

easily washed out, e.g. during rainfall events (Magni & Montani, 1997). The negative correlation of Chl *a* with salinity at both Stns. H1 and H2 ( $r^2 = 0.74$  and  $r^2 = 0.64$ , respectively,  $p < 0.001$ , plots not shown) suggests that the upper intertidal zone may represent an important source of microalgal biomass to the lower part of the flat, where it is likely to be periodically transported during the ebb flow.

In order to assess the characteristics of SPM, we used the C/Chl *a* ratio as an index to evaluate the amount of living phyto-fraction in the POC content of our samples. Including the microphytobenthos (either transported from the upper intertidal zone or *in situ* produced) as an important component of the microalgal standing stock in the water, we used a mean value of 45 as an approximate C/Chl *a* ratio for both phytoplankton (Parsons et al., 1984) and estuarine benthic diatom populations (De Jonge, 1980) to assess the qualitative composition of POC. By comparing this value with the annual mean of the C/Chl *a* ratio at individual stations (C/Chl *a* = 855, 727 and 269, at Stns. H1, H2 and Y3, respectively; Table 2), it results that 5–6% and 17% of the organic carbon standing stock, on the intertidal and the subtidal zones, respectively, may be accounted as living phyto-Carbon.

At both Stns. H1 and H2, the low percentage of living microalgal fraction in POC went together with a stronger correlation of POC with pheo-pigments ( $r^2 = 0.97$  and  $r^2 = 0.92$ , respectively) than with Chl *a* ( $r^2 = 0.22$  and  $r^2 = 0.44$ , respectively) (Figure 8). POC

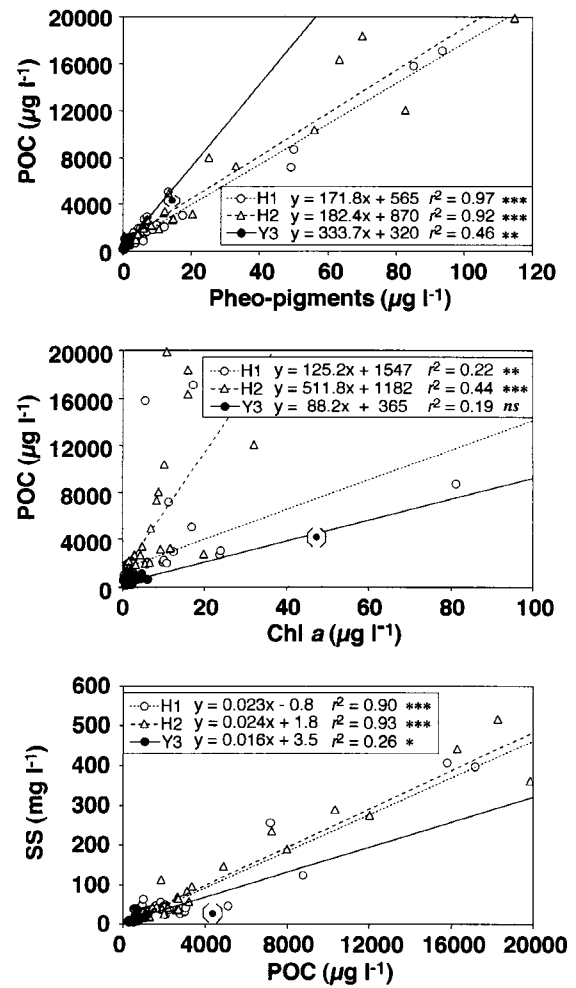


Figure 8. Plots of particulate organic carbon (POC) against suspended solids (SS), pheo-pigments and Chlorophyll *a*. At Stn. Y3, the value in bracket (February 1996) is excluded from the regression line. For Stns. H1 and H2, duplicate values from samples collected within an hour from each other are plotted. Lines are best fitting linear regressions (ANOVA, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns: not significant).

also correlated strongly with suspended solids (SS), at both Stns. H1 and H2 ( $r^2 = 0.90$  and  $0.93$ , respectively,  $p < 0.001$ ) (Figure 8). Interestingly, the high pheo-pigment/Chl *a* ratio at Stns. H1 and H2 was closer to that found in the adjacent emerged surface sediments (Magni & Montani, 1997, 1998) than to that at Stn. Y3 (Figure 6, Table 2). These observations indicate that a relatively high fraction of detrital and/or surface sediment algal materials characterizes the SPM in ebbing water, as an evidence of a close water-sediment coupling. Physical events related to the effect of solar radiation on shallow water and emerged sed-

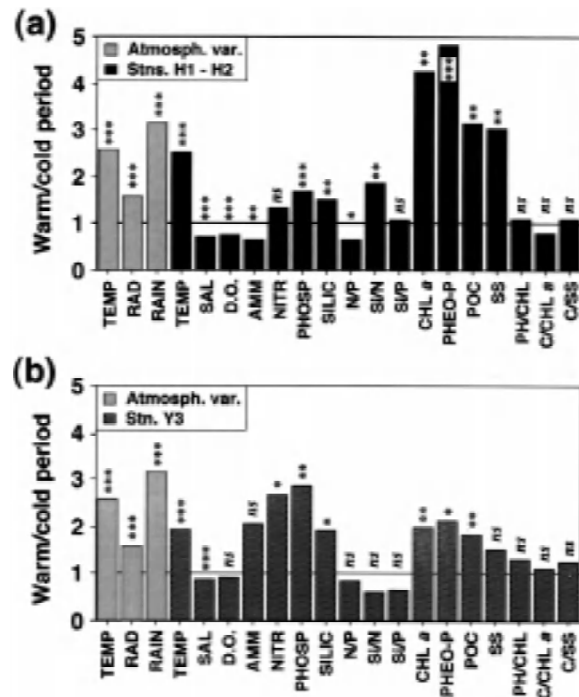


Figure 9. (a) and (b) Dimensionless ratio, irrespective of the year, between the warm period (May–October) and the cold period (November–April) of the physical and chemical parameters investigated in surface ebbing water (i.e. Stns. H1 and H2, (a) and surface water at an adjacent subtidal site (i.e. Stn. Y3, (b)). At Stn. Y3, samples of February 1996, which had exceptionally high dissolved inorganic nitrogen concentrations (Figure 3) and SPM content (Figure 5), are not included in the mean values of the cold period. Legend: TEMP: air temperature; RAD: solar radiation; RAIN: rainfall; TEMP: water temperature; SAL: salinity; D.O.: dissolved oxygen; AMM:  $\text{NH}_4^+$ -N; NITR:  $(\text{NO}_3^- + \text{NO}_2^-)$ -N; PHOSP:  $\text{PO}_4^{3-}$ -P; SILIC: Si  $(\text{OH})_4$ -Si; N/P, Si/N and Si/P: nutrient stoichiometric ratios; CHL *a*: Chlorophyll *a*; PHEO-P: phaeo-pigments; POC: particulate organic carbon; SS: suspended solids; PH/CHL: PHEO-P/CHL *a* ratio; C/CHL: POC/CHL *a* ratio; C/SS: POC/SS ratio. Symbol on top of each bar indicate the significance of difference between the warm and the cold period (ANOVA, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns: not significant).

iments may be responsible for a faster degradation of algal materials in ebbing water. The presence of a high biomass of filter feeders in our study area (Magni & Montani, 1998; Magni et al., 2000) also suggests that the grazing pressure by macrozoobenthos may account as a biological factor reducing the living phyto-Carbon fraction in ebbing water, as they feed on bottom sea water and resuspended microphytobenthos (Numaguchi, 1990; De Jonge & Van Beusekom, 1992; Brotas & Plante-Cuny, 1998).

#### Evaluation of seasonal and spatial variability of dissolved and particulate compounds

In order to quantify the extent of temporal variability and to assess the significance of seasonal patterns of dissolved and particulate compounds, we distinguished, irrespective of the year, the warm period from May to October and the cold period from November to April, based on the water temperature recorded at Stns. H1, H2 and Y3 (Figure 2). We used the averaged data of both the warm and the cold periods to obtain a dimensionless ratio for the physical and chemical parameter investigated at both the intertidal (Stns. H1 and H2) and the subtidal (Stn. Y3) sites (Figure 9a,b, respectively).

The difference in water temperature between the two periods was very large at both sites, with seasonal means of  $23.5 \pm 4.2$  °C (warm period) and  $10.0 \pm 4.4$  °C (cold period) on intertidal zone, and  $24.2 \pm 2.4$  °C and  $12.7 \pm 3.0$  °C, respectively, on the subtidal zone. The marked seasonal pattern of environmental conditions was also indicated by strong differences of air temperature and solar radiation (Figure 9a,b). In addition, rainfall was significantly higher in the warm period and accordingly salinity was significantly lower, during the same period, at both sites (Figures 9a and b). Concurrently, on the intertidal zone nitrate + nitrite concentrations did not differ significantly between the warm and the cold period, although they correlated negatively with salinity, particularly at Stn. H1 (Figure 7). Differently, concentrations of the other nutrient species, which showed no (ammonium and silicate) or limited (phosphate) correlation with salinity (Figure 7), were significantly higher either in the cold period (ammonium) or in the warm period (phosphate and silicate). These observations indicated that on the intertidal zone a marked seasonality of nutrient concentrations is significantly affected by *in situ* processes, in spite of the strong effect of external and physical variables (i.e. river runoff, rainfall, salinity). The seasonal pattern of individual nutrient species accordingly determined that of their stoichiometric ratios, with N/P and Si/N significantly higher in winter and summer, respectively (Figure 9a). On the subtidal site (Stn. Y3), ammonium did not correlate significantly with salinity, while, differently from the intertidal site, nitrate + nitrite, phosphate and silicate did (Figure 7). The concomitant significantly higher concentrations of the latter species nutrient species in the warm period, but not those of ammonium (Figure 9b), and the close hydrodynamic link between the two

sites (Montani et al., 1998) further suggest that the intertidal zone itself plays an important seasonal role as a nutrient supplier to the subtidal zone.

Among SPM, Chl *a*, phaeo-pigments and POC were significantly higher in the warm period, both on the intertidal and the subtidal zone (Figure 9a,b). On the intertidal zone, SS, which strongly correlated with POC (Figure 8), also showed a significant seasonal pattern (Figure 9a). In addition, seasonal differences in SPM content were more marked on the intertidal zone than on the subtidal zone, up to a Chl *a* and phaeo-pigment content 4.3 and 4.8 higher in the warm period, respectively, at the former site (Figure 9a). Whereas the phaeo-pigment/Chl *a*, POC/Chl *a* and POC/SS ratios, approximating value 1 both on the intertidal (Figure 9a) and the subtidal zone (Figure 9b), indicated the absence of seasonality in the qualitative composition of SPM as such evaluated.

Overall, the extent of seasonal variability between dissolved and particulate compounds was larger on the intertidal zone (Figure 9a) than on the subtidal zone, where nutrients were 1.9 (silicate)–2.9 (phosphate) and SPM was 1.8 (POC)–2.1 (phaeo-pigments) times higher in the warm period than in the cold period (Figure 9b). This proved the larger variability of the former site also on qualitative (i.e. nutrients vs. SPM) basis. On an annual basis too, spatial differences between the two sites were larger for the SPM content than for the nutrient concentrations (Table 1). Annual mean of phaeo-pigment and POC content was 12 (Stn. H1) to 25 (Stn. H2) times and 4 (Stn. H1) to 8 (Stn. H2) times higher on the intertidal zone than on the subtidal zone, respectively (Table 1). Such a qualitative difference between sites suggests that particle deposition and/or removal is likely to occur within the intertidal zone, resulting in a limited SPM content at the surface layer of Stn. Y3, while nutrients are more directly transported toward the subtidal zone, through the surface layer, by lower salinity water mass intrusion from the intertidal zone (Montani et al., 1998).

## Conclusion

As an integration of associated pieces of work (Magni & Montani, 1997, 1998; Montani et al., 1998; Magni et al., 2000), this study provides substantial information on the physical and chemical variability of the water in the lower part of the intertidal zone under investigation. The controlled condition of low tide adopted for sampling in the intertidal zone minimized the

strong variability related to the ebb advection and the tidal flood (Montani et al., 1998). As for the short-term (Montani et al., 1998), the fresh water was a major contributor of nitrate + nitrite to the estuary. In contrast, ammonium, phosphate and silicate concentrations were more affected by *in situ* biologically-mediated processes. Among particulate compounds, the negative correlation between Chl *a* and salinity ( $r^2 \geq 0.64$ ,  $p < 0.001$ ) indicated that an upper intertidal zone (Magni & Montani, 1997) is also an important source of microalgal biomass to the estuary. Whereas, a stronger correlation of POC with phaeo-pigments ( $r^2 \geq 0.92$ ) than with Chl *a* ( $r^2 \leq 0.44$ ), a low fraction of living phyto-Carbon (ca. 5%) and a high phaeo-pigment/Chl *a* ratio (ca. 3) in ebbing water indicated a high portion of refractory algal material on the flat. These observations and the results from associated pieces of work (Magni & Montani, 1998; Magni et al., 2000) suggest that abundant intertidal macrozoobenthos play an important role in the water-sediment coupling on the flat.

Furthermore, in spite of the strong short-term variability of this ecosystem (Magni & Montani, 1997; Montani et al., 1998), this study also demonstrated that highly significant seasonal patterns can be recognized in our study area. However, patterns varied depending on the different nutrient species and SPM, and the possibly contrasting effect of physical variables (i.e. river runoff, rainfall, salinity, temperature) and *in situ* biological processes (i.e. primary producer and consumer activity and metabolism). Finally, the intertidal zone appeared to play an important role on the variability of the water chemistry of the adjacent subtidal site, not only on the short-term (Montani et al., 1998) but also on a seasonal basis, as a supplier of nutrients and SPM, particularly during the May–October warm period.

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