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Richard Sempere, Gustave Cauwet. Occurrence of organic colloids in the stratified estuary of the Krka River (Croatia).. *Estuarine, Coastal and Shelf Science*, 1995, 40 (1), pp.105-114. 10.1016/0272-7714(95)90016-0 . hal-01171591

HAL Id: hal-01171591

<https://hal.science/hal-01171591>

Submitted on 18 Feb 2019

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1. Sempéré, R., Cauwet, G., (1995) Occurrence of organic colloids in the stratified estuary of the Krka River (Croatia). *Estuarine Coastal and Shelf Science*. 40: 105-114. DOI: [10.1016/0272-7714\(95\)90016-0](https://doi.org/10.1016/0272-7714(95)90016-0).

Occurrence of Organic Colloids in the Stratified Estuary of the Krka River (Croatia)

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Received 18 October 1993 and in revised form 18 January 1994

Keywords: colloidal organic carbon; dissolved organic carbon; halocline; Krka Estuary

Along a vertical profile of a stratified estuary, we determined organic carbon in the following fractions: low molecular weight (LMW-OC < 0.02 μm), colloidal (0.02 μm < COC < 0.7 μm), dissolved (DOC < 0.7 μm) and particulate (POC > 0.7 μm). The results showed abundant concentrations of organic colloids accounting for: 40% of total organic carbon (TOC) in the overlying brackish water (salinity 2), 22% in the underlying marine water (salinity 38) and 19% ($\pm 5\%$) for intermediate salinity samples (salinity 3–31). Upon contact with seawater (in the salinity range 2–5) both DOC and COC concentrations decreased drastically (44 and 47 μM respectively), whereas POC slightly increased (11 μM), being consistent with previous laboratory and field studies, and indicating that at low salinities, the colloidal fraction is actively involved in the DOC physicochemical aggregation. In the halocline, we observed that organic colloids and relatively degraded particles accumulated together in the lowest part of the interface (salinity 31). Because previous studies in the same area have shown accumulation of degraded cells and fragments in the bottom of the halocline, we suggest that large colloids (COC in this study) at the interface probably originated from fragmentation of non-living organisms or aggregates. Furthermore, it is likely that these processes which are enhanced in a highly stratified estuary, are partly responsible for the DOC non-conservative dilution observed along this profile.

Introduction

In aquatic environments colloids are defined as material in the range 0.001–1 μm (Vold & Vold, 1983; Goldberg, 1988). Thereby, they comprise a large spectrum of organic and inorganic species including clays, macromolecules, aggregates as well as viruses and some bacteria and flagellates (Sharp, 1973; Cauwet, 1978; Stumm & Morgan, 1981; Rassoulzadegan & Sheldon, 1986; Bergh *et al.*, 1989). Although several studies have reported a significant concentration of colloids particularly in particle-rich media such as the oceanic euphotic layer, the coastal or estuarine waters (Sigleo *et al.*, 1982;

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Koike *et al.*, 1990; Wells & Goldberg, 1991; Benner *et al.*, 1992; Ogawa & Ogura, 1992; Sempéré *et al.*, 1994), very little is known about their distribution and how they might affect biogeochemical processes in natural waters.

However, in the estuary numerous studies reported physicochemical aggregation phenomena of high molecular weight terrigenous-derived dissolved organic matter (DOM) (Sholkovitz, 1976; Mayer, 1985; Whitehouse *et al.*, 1989; Sempéré & Cauwet, 1993) highlighting a colloid→particle transfer which may precede sedimentation (Pau, 1980; McCave, 1984). On the other hand, it has been proposed that some organic colloids may have originated from fragmentation of particles (Lampert, 1978; Cho & Azam, 1988; Karl *et al.*, 1988; Koike *et al.*, 1990; Smith *et al.*, 1992; Sempéré *et al.*, 1994) indicating in this case a particle→colloid pathway. Moreover, recent discoveries indicated possible assimilation of colloidal DOM by choanoflagellates as well as tunicates (Marchant & Scott, 1993; Tranvik *et al.*, 1993). Therefore, qualifying and quantifying processes involving the colloidal fraction, may help to a better understanding of the microbial food-web as well as the organic matter transfer in estuary.

The highly stratified estuary of the Krka River (Croatia), is well suited for investigations dealing with the transformations of organic matter because different processes such as primary production, degradation of particles by bacteria as well as physicochemical aggregation/disaggregation have been observed along a vertical salinity gradient established in less than 1 m (Zutic & Legovic, 1987; Vilicic *et al.*, 1989; Biscan *et al.*, 1991; Cauwet, 1991; Fuks *et al.*, 1991; Legovic *et al.*, 1991a; Svetlicic *et al.*, 1991). In spring 1990, we simultaneously collected water samples along a vertical profile from the Krka Estuary and analysed them for organic carbon content in the so-called 'low molecular weight', 'colloidal', 'dissolved' and 'particulate' fractions. By comparison between these parameters and previous studies, we discuss the physicochemical and biological processes which may involve large organic colloids in the size range 0.02–0.7 μm .

Materials and methods

In May 1990, seven water samples were collected from the Krka Estuary (Figure 1; station E) above, below and at 4–10-cm intervals vertically in the halocline. For the sampling a scuba diver used a multichannel peristaltic pump which avoids disturbance by air bubbles (Kniewald *et al.*, 1987; Cauwet, 1991). In this study, the halocline which was defined as the interval in which $\Delta S/\Delta Z > 5 \text{ m}^{-1}$ (Vukojevic, 1983, cited in Legovic *et al.*, 1991b), separated the upper brackish water layer from the lower marine layer at *c.* 2 m below the surface. After collection, the water was filtered under reduced vacuum with an all-glass filter holder through a 47-mm-diameter pre-heated (45 °C) glass fibre filter (GF/F). The filtration was stopped after 1 l to avoid colloid adsorption on the filter. The rinsed filters were dried at 40 °C for 24 h and were used later for total suspended matter (TSM) and POC determinations, whereas the filtrates were collected in pre-heated (450 °C) graduated glass bottles (1 l) tightly closed with a Teflon-lined cap.

The subsamples were subsequently ultrafiltered under a laminar flow air bench through a 0.02 μm laboratory synthesized tubular membrane of zirconium/aluminium oxide (Larbot *et al.*, 1989) by the method described previously (Sempéré, 1991; Sempéré *et al.*, 1994). The laboratory experiments (with Dextran solutions) indicated that the membrane cutoff was around 500 000 dalton. Briefly, 1 l of each pre-filtered sample was 1.7 times concentrated by cross-flow ultrafiltration on the membrane

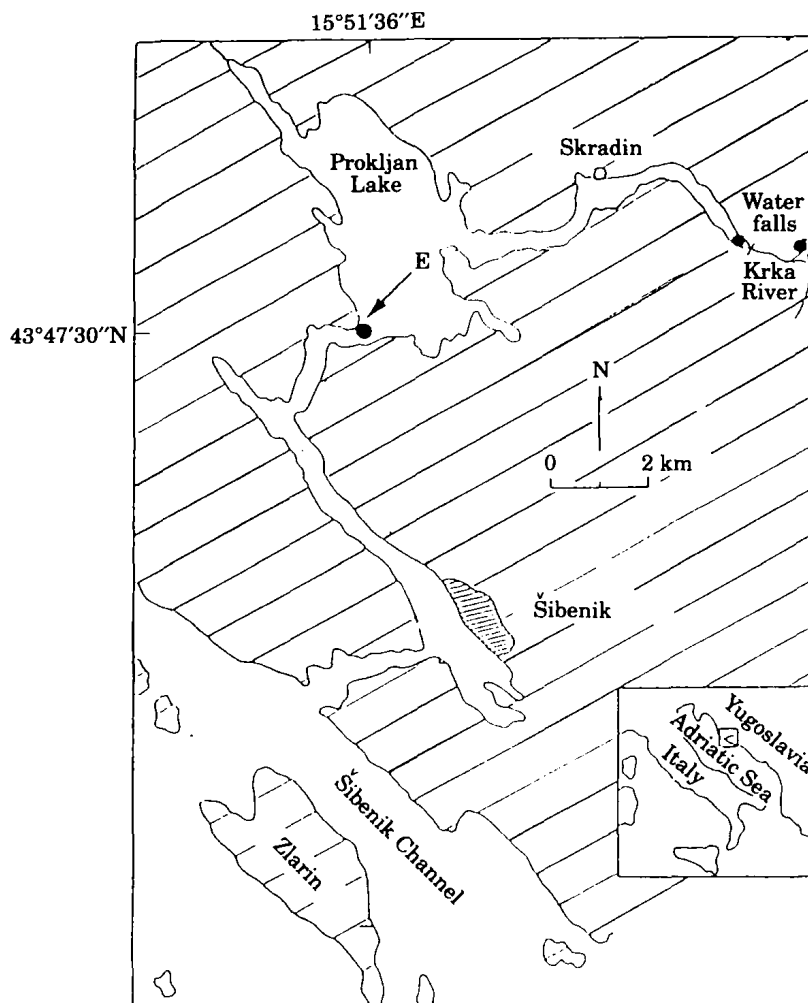


Figure 1. Location of the Krka Estuary (Croatia) and sampling station (E).

inserted in a Teflon/Kel F unit. These conditions facilitated a good conditioning of the membrane and avoided membrane clogging. Ultrafiltrate and concentrate were collected in pre-heated (450 °C) graduated glass bottles (1 l) and closed with a Teflon-lined cap. Total procedure time for each sample was *c.* 5 h. Aliquots (8 ml) of filtered and ultrafiltered samples (ultrafiltrate+concentrate) were taken in pre-heated (450 °C) 10-ml glass vials poisoned with HgCl_2 (10 mg l^{-1}), covered with a Teflon-lined cap, and stored at 4 °C in the dark. Therefore, by the separative methods described above, organic carbon was operationally fractionated in the following fractions: LMW-OC < 0.02 μm ; 0.02 < COC < 0.7 μm ; DOC < 0.7 μm ; POC > 0.7 μm .

For DOC (ultrafiltrate, concentrate and initial GF/F filtrate) measurements, after acidification (pH 2) with a 2 M HCl solution, the samples were bubbled for 5 min, with CO_2 -free pure air to purge inorganic carbon. Dissolved organic carbon was measured by means of the uv-persulphate oxidation method (Collins & Williams, 1977) modified by Cauwet (1984). The standard deviation was *c.* 3 μM for < 83 μM and less than 2% above. The carbon blank of the water used for the standard solutions (83, 167, 250, 333 μM)

TABLE 1. Depth collection, salinity, particulate organic carbon (POC) and dissolved inorganic carbon (DIC) in water samples collected from the Krka Estuary, May 1990

| Depth (m) | Salinity | POC (μM) | POC% | DIC(μM) |
|-----------|----------|-----------------------|------|----------------------|
| 1.50 | 2 | 48 \pm 1 | 18 | 3815 \pm 8 |
| 1.90 | 3 | 36 \pm 1 | 16 | 3931 \pm 8 |
| 1.96 | 5 | 59 \pm 1 | 17 | 3591 \pm 8 |
| 2.06 | 19 | 82 \pm 2 | 26 | 3239 \pm 8 |
| 2.08 | 24 | 108 \pm 2 | 22 | 2951 \pm 8 |
| 2.12 | 31 | 106 \pm 2 | 20 | 2934 \pm 8 |
| 5.00 | 38 | 32 \pm 1 | 15 | 2488 \pm 8 |

$$\text{POC\%} = (\text{POC} \times 100) / \text{TSM}.$$

was estimated to be 15 μM by pure air circulation inside the system and subtracted from the standards. In this study, COC represented (after volume corrections) the difference between $\text{DOC}_{\text{concentrate}}$ and $\text{DOC}_{\text{ultrafiltrate}}$ and thus its uncertainty was estimated to *c.* 4 μM from the square root of the sum of the standard deviation calculation. Our uv-persulphate unit might have underestimated DOC values by a factor of 1.0–1.2 (Cauwet *et al.*, 1990a; Sempéré & Cauwet, 1993; Cauwet, 1994). Dissolved inorganic carbon (DIC) was measured by a uv-persulphate analyser with a standard deviation of 8 μM . After removal of carbonates, POC was determined by GF/F filter combustion (LECO CS 125 analyser) with a standard deviation of 2%.

Results and discussion

Description of the halocline, particle characteristics

Table 1 gives the physical parameters (depth collection, salinity) as well as DIC and POC concentrations and POC% [(POC \times 100)/TSM] of the water samples along the profile. The DIC distribution showed a normal (Meybeck, 1982; Cauwet, 1991) conservative behaviour [Figure 2(a); $r^2=0.96$, $n=7$] establishing confidence in our sampling precision. The POC concentration range was 32–108 μM (average 67 μM) comprising 15–26% (average 19%) of TSM and 21–44% (average 32%) of total organic carbon (TOC). These high POC% for corresponding TSM data reflect a situation in which aquatic production dominated the charge of the river (Meybeck, 1982; Ittekkot, 1988; Cauwet *et al.*, 1990b).

Particulate organic carbon concentrations increased with the salinity from the brackish water to the bottom of the halocline, thus, the strong salinity gradient (from 3 to 31 in 22 cm) and particulate content (POC and POC%) of the profile [Figure 2(b,c)] are in good agreement with those described previously (Zutic & Legovic, 1987; Cauwet, 1991, and references therein; Legovic *et al.*, 1991a,b). (1) A well-defined halocline (at *c.* 2 m below the surface) separating the overlaying brackish water (first sample salinity 2) and the underlying marine water (salinity 38). (2) A particle accumulation in the bottom of the halocline (depth=2.08–2.12 m; salinity 24–31) as a consequence of the density and shear stress barrier (Cauwet, 1991). However, it is important to notice that POC% maximum (depth=2.06 m; salinity 19) was situated above the POC maximum (depth=2.08 m; salinity 24) indicating that the particles are preferentially degraded in the lowest part of the interface. This later observation is consistent with the results of Zutic and Legovic (1987) and Vilicic *et al.* (1989) who reported in the bottom of the

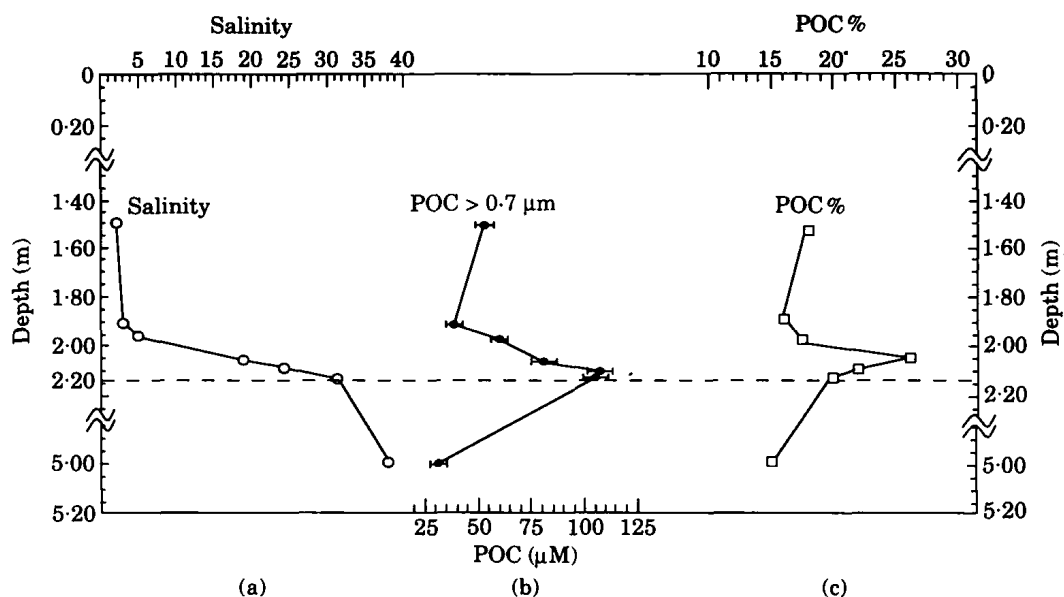


Figure 2. Vertical profiles for (a) salinity, (b) particulate organic carbon ($\text{POC} > 0.7 \mu\text{m}$) and (c) organic content of total suspended matter [$\text{POC}\% = (100 \times \text{POC})/\text{TSM}$]. POC standard deviation is within 2%, horizontal error bars are smaller than the symbol size.

TABLE 2. Depth collection, salinity, dissolved organic carbon (DOC), colloidal organic carbon (COC) and low molecular weight organic carbon (LMW-OC) in the water samples collected from the Krka Estuary, May 1990

| Depth (m) | Salinity | DOC (μM) | COC (μM) | LMW-OC (μM) | COC/DOC (%) | % Initial DOC |
|-----------|----------|-----------------------|-----------------------|--------------------------|-------------|---------------|
| 1.50 | 2 | 147 \pm 3 | 79 \pm 3 | 75 \pm 2 | 54 | 108 |
| 1.90 | 3 | 134 \pm 3 | 46 \pm 4 | 99 \pm 2 | 34 | 108 |
| 1.96 | 5 | 103 \pm 2 | 32 \pm 3 | 80 \pm 2 | 31 | 108 |
| 2.06 | 19 | 147 \pm 3 | 32 \pm 4 | 121 \pm 2 | 22 | 104 |
| 2.08 | 24 | 144 \pm 3 | 40 \pm 4 | 109 \pm 2 | 28 | 103 |
| 2.12 | 31 | 133 \pm 3 | 41 \pm 4 | 98 \pm 2 | 31 | 104 |
| 5.00 | 38 | 121 \pm 2 | 33 \pm 4 | 92 \pm 2 | 27 | 103 |

DOC $< 0.7 \mu\text{m}$; $0.02 < \text{COC} < 0.7 \mu\text{m}$; LMW-OC $< 0.02 \mu\text{m}$.

% Initial DOC = $(\text{DOC}_{\text{ultrafiltrate}} + \text{COC}) \times 100 / \text{DOC}_{\text{initial water}}$

halocline, accumulation of chlorophyll degradation product (phaeophytin) as well as heterotrophic activity, the chlorophyll being produced in the overlying water.

Dissolved and colloidal organic carbon distribution

Table 2 gives DOC and COC data for the samples. Mass balances measured on the samples indicated that an average of $105 \pm 2\%$ was found in the concentrate and ultrafiltrate fractions indicating that contamination and loss were minimized during the procedure. Dissolved organic carbon concentrations ranged from 103 to $147 \mu\text{M}$ (average $133 \mu\text{M}$) and are similar with those already published (Cauwet, 1991). The COC concentration range was 32–79 μM (average $43 \mu\text{M}$) being maximum at low

TABLE 3. Depth collection, salinity, and relative abundance of particulate organic carbon (POC), colloidal organic (COC) and low molecular weight organic carbon (LMW-OC) in the water samples collected from the Krka Estuary, May 1990

| Depth (m) | Salinity | POC/TOC (%) | COC/TOC (%) | LMW-OC/TOC (%) |
|-----------|----------|-------------|-------------|----------------|
| 1.50 | 2 | 25 | 40 | 38 |
| 1.90 | 3 | 21 | 27 | 58 |
| 1.96 | 5 | 36 | 20 | 49 |
| 2.06 | 19 | 36 | 14 | 53 |
| 2.08 | 24 | 43 | 16 | 43 |
| 2.12 | 31 | 44 | 17 | 41 |
| 5.00 | 38 | 21 | 22 | 60 |

POC > 0.7 μm ; DOC < 0.7 μm ; 0.02 μm < COC < 0.7 μm ; LMW-OC < 0.02 μm ;
POC + DOC = TOC.

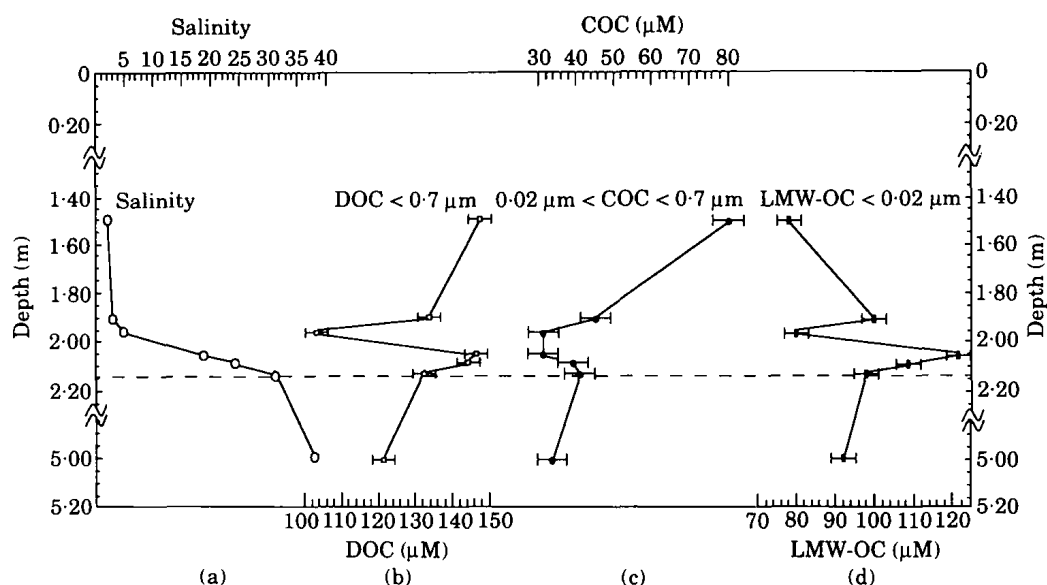


Figure 3. Vertical profiles for (a) salinity, (b) dissolved organic carbon (DOC < 0.7 μm), (c) colloidal organic carbon (0.02 < COC < 0.7 μm) and (d) low molecular weight organic carbon (LMW-OC < 0.02 μm). Horizontal error bars are instrument standard deviations (indicated in Tables 1 and 2) and if not shown are smaller than the symbol size.

salinity (2); COC comprised 22–54% (average 32%) of DOC (Table 2) and 14–40% (average 22%) of TOC (Table 3), indicating that organic colloids represented a significant fraction of organic carbon during the spring season in the Krka Estuary. Interestingly, the results show a clear non-conservative behaviour of DOC (Figures 3 and 4) as well as COC and LMW-OC (Figure 3), a point which will be discussed later.

In the overlying brackish water the colloidal fraction was relatively the most abundant (COC/DOC = 54%; COC/TOC = 40%). We found that upon contact with seawater (salinity 2–5) the brackish-water DOC decreased sharply by 44 μM , moreover, Tables 2, 3 and Figure 3 indicate that this can principally be attributed to its colloidal fraction. This fact has been observed in other estuaries by different authors (Sholkovitz, 1976;

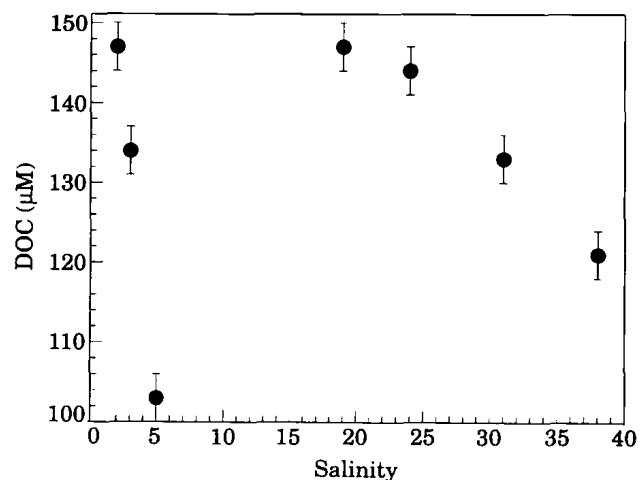


Figure 4. Dissolved organic carbon (DOC) concentration versus salinity for the water samples collected from the Krka Estuary, May 1990. Horizontal error bars are instrument standard deviations (indicated in Table 2).

Whitehouse *et al.*, 1989; Sempéré & Cauwet, 1993), and certainly reflects the physico-chemical flocculation of some terrigenous-derived dissolved organic matter (mainly humic acids) through colloidal state into particles due to ionic strength changes (Sholkovitz, 1976; Mayer, 1985). This phenomenon might explain why we observed [Figure 2(b)] in the salinity 5 sample, a small increase of POC concentration (11 µM), the rest of the newly generated particles having probably sunk towards the sediment through the interface. This assumption seems consistent with the previous work of Hadzija *et al.* (1985) indicating a preferentially terrigenous source of sedimented organic material (rich in carbohydrate-humic substances complexes) in the estuarine sediments. Slightly above the halocline (depth=2.06 m; salinity 19) we observed a DOC sub-maximum due to the LMW-OC. It is important to notice that at the same level, we observed the highest organic content of particles [see POC%, Figure 2(c)]. Although in this study, we do not have simultaneous biological data available, several authors in previous work located the chlorophyll maximum above the halocline as well (Zutic & Legovic, 1987; Vilicic *et al.*, 1989). If these informations were confirmed together, they may suggest a production of LMW-OC by healthy phytoplankton above the interface.

From the higher part of the halocline (depth=2.06 m; salinity 19) to its lowest part (depth=2.12 m; salinity 31), the LMW-OC concentrations decreased slightly from 121 to 98 µM, whereas COC increased slightly from 32 to 41 µM. These results showed that at the interface level (depth=2.12 m; salinity 31), organic carbon is in colloidal and particulate states for, respectively, 17 and 44% (Table 3). Actually, the residence time of particles as well as the number of heterotrophic bacteria in an interface is larger than anywhere else in the water column (Mayer, 1982; Zutic & Legovic, 1987; Legovic *et al.*, 1991a), then particulate organic matter including dead organisms and also various aggregates, represents a potential source for transformations such as flocculation, disaggregation or DOM releases by organisms. However, it has been reported that in seawater, autolysis of dead cells releases mainly low molecular weight compounds (Carlucci *et al.*, 1984) whereas the ultrafiltration membrane we used in this study, retains mostly large colloids with a molecular weight higher than 500 000 dalton (Sempéré *et al.*, 1994).

Although in estuaries, physicochemical aggregation of dissolved organic matter into colloids induced by seawater usually appears mainly at low salinities (salinity less than 10; Sholkovitz, 1976; Mayer, 1985), we can not totally disregard the flocculation process in saline waters (Baskaran *et al.*, 1992; Moran & Buesseler, 1992). However, we feel that the apparent increase of organic colloids near the interface may be due to the fragmentation of larger particles by physical disaggregation (Karl *et al.*, 1988) or biological processes (such as micro-organism degradation as well as interspecies grazing; Lampert, 1978; Koike *et al.*, 1990; Smith *et al.*, 1992). Furthermore, Koike *et al.* (1990) reported that the grazing of bacteria by flagellates in the open ocean is responsible for some submicrometre particle (essentially organic) production. Interestingly, Sigleo *et al.* (1982) gave a similar composition (30% carbohydrates, 10% lipids and 10% nucleotides) for colloidal material and phytoplankton in the Patuxent River. It is likely that these processes occur together at the interface in proportions difficult to determine.

Non-conservative distribution of DOC

These phenomena may certainly explain partly the clear non-conservative DOC behaviour (Figure 4; $r^2 < 0.1$, $n = 7$) along the vertical profile at this season. Several authors have already reported no clear relationship between salinity and DOC (Cauwet, 1991; Suzuki & Tanoue, 1991; Miller *et al.*, 1993; Sempéré & Cauwet, 1993). On the other hand, although the flocculation of the colloidal fraction of DOC was generally admitted, DOC conservative dilution has been observed many times (Moore *et al.*, 1979; Mantoura & Woodward, 1983; Whitehouse *et al.*, 1989). Dissolved organic carbon conservative dilution concept along an estuary is usually related to internal physicochemical and biological processes (Mantoura, 1987). Flowing from a karstic environment, the Krka River generally has clear water and a very low DOC concentration constant throughout the year, therefore, the biological production in the estuary will play an important role in the carbon budget (Cauwet, 1991). However, upon the first contact with seawater, and probably because of the sensitive sampling technique we observed the fate of DOC by physicochemical processes (29% as regard to the theoretical dilution by seawater salinity 5). Moreover, the special character of the interface (which acts as a kind of density barrier) probably contributes to biologically regulate the concentration of colloidal and low molecular weight fractions of DOC slightly above and in the halocline. All these organic matter transformations which in other kinds of estuaries may occur near the sediment or in the bottom nepheloid layer (Sempéré *et al.*, 1994) might firstly not be detected by surface water sampling, and secondly affect the DOC mass balance in the estuary.

Acknowledgements

This study is part of a French-Yugoslav research programme on stratified estuaries, supported by EEC contract No. CI1-0544-F. We thank D. Petricoli for sample collection, Professors I. Koike, H. Ogawa and N. Ohkuchi from the O. R. I. Tokyo Japan for useful comments on the manuscript, Drs B. Cosovic, N. Vdovic, V. Vojvodic and V. Zutic from the Centre For Marine Research of the Rudjer Boskovic Institute, Zagreb Croatia for helpful discussions. The authors are also very grateful to Manami Motoya and Dr J. Sanderson for the English correction of the manuscript and to the anonymous referees for improving the quality of the manuscript.

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