

## NOTE

**Intertidal biofilms on rocky substratum can play a major role in estuarine carbon and nutrient dynamics**Catarina M. Magalhães<sup>1,\*</sup>, Adriano A. Bordalo<sup>1</sup>, William J. Wiebe<sup>2</sup><sup>1</sup>Laboratory of Hydrobiology, Institute of Biomedical Sciences, University of Porto, 4099-003 Porto, Portugal<sup>2</sup>Department of Marine Sciences, University of Georgia, Athens, Georgia 30602, USA

**ABSTRACT:** Problems associated with increasing eutrophication of estuaries have stimulated many investigators to examine primary productivity and nutrient dynamics and transformations within and between the water column and sediments. Surprisingly, rocky surfaces, both natural and man-made, while comprising significant areas of many estuaries, have received virtually no investigation. In the Douro River estuary (Portugal), a flooded granitic valley, human intervention has progressively replaced intertidal soft sediments with hard surface areas. In this study we examine the role of the inorganic nutrient and metabolic dynamics of intertidal rocky biofilms in the lower estuary. Hourly net primary production ( $389 \pm 168 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) greatly exceeded hourly net respiration rates ( $50 \pm 18 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ), results comparable to those of adjacent muddy and sandy sediments. These rocky biofilms consistently removed  $\text{NO}_3^-$  and  $\text{SiO}_4^{4-}$  from the water column ( $-595.4 \pm 236.7$  and  $-305.7 \pm 209.6 \text{ } \mu\text{moles m}^{-2} \text{ h}^{-1}$ , respectively), whereas the net flux of  $\text{NH}_4^+$  depended on light conditions ( $-123.15 \pm 22.45$ ,  $144.54 \pm 151.90 \text{ } \mu\text{moles m}^{-2} \text{ h}^{-1}$ , for light and dark treatments respectively).  $\text{NO}_2^-$  and  $\text{PO}_4^{3-}$  net fluxes were low, with no clear trends in flux direction ( $-0.57 \pm 9.1 \text{ } \mu\text{moles NO}_2^- \text{ m}^{-2} \text{ h}^{-1}$  and  $1.4 \pm 10.5 \text{ } \mu\text{moles PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ). Although rocky substrata constitute only 21% of the total planar intertidal zone of Douro estuary, they were responsible for 43% of the nitrate and 45% of the silicate uptake when compared to sandy and muddy sediment hourly rates within the total intertidal area. Thus intertidal rocky biofilms can represent important sites for biogeochemical activity.

**KEY WORDS:** Douro River estuary · Intertidal rocky biofilms · Nutrient flux · Oxygen flux · Biogeochemistry

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Worldwide, eutrophication of estuaries and coastal waters constitutes a serious, rapidly increasing problem (e.g. Jickells 1998). While intertidal and subtidal estuarine sediments are recognized as important sites for nutrient transformation and sequestration via biogeochemical cycling (Ogilvie et al. 1997, Cabrita & Brotas 2000, Schulz & Zabel 2000), many estuaries also possess extensive areas of hard surfaces (e.g. rocky

coastlines, fjords, drowned sandstone and granitic valleys, carbonate platforms). Further, man-made structures (e.g. breakwaters and jetties, pilings and walls for marinas and port facilities) progressively replace sediments with hard surfaces. These surfaces provide substrate for the proliferation of biofilms with a wide variety of metabolically diverse microorganisms.

Biogeochemical benthic-water coupling in rocky rivers and coral reef flats (carbonate platforms) are documented (e.g. Wetzel 1983, D'Elia & Wiebe 1990), and coastal rocky shores have received much attention by community ecologists studying animal, macrophyte and microalgal distribution, controls of trophic structure, and food webs of these communities (Hillebrand & Sommer 2000, Knox 2000 and references therein, Worm et al. 2000). However, the extent of biogeochemical processes within the thin layer of organic matter that characterizes hard surfaces of many marine coastal habitats has scarcely been considered. For example Decho (2000) reviewed microbial biofilms in intertidal systems, and Knox (2000) published a book on the ecology of seashores, yet neither mention this topic.

In this study, rates of primary production and respiration, and net fluxes of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SiO}_4^{4-}$  between hard surface biofilms and the water column of the intertidal zone of the Douro River estuary were examined and compared with comparable data from adjacent intertidal sandy and muddy sediments.

**Materials and methods. Site description:** The Douro River and its tributaries drain 17% of the Iberian Peninsula (Fig. 1). Confined 21 km upstream by a dam, the estuary averages 8 m depth, has a semidiurnal tidal range of 2 to 3 m, temperatures between 8 and 25°C, salinities from 0 to 35‰ and water residence times between 0.3 and 16.5 d (Vieira & Bordalo 2000). The estuary receives an average of  $455 \text{ m}^3 \text{ s}^{-1}$  of freshwater, con-

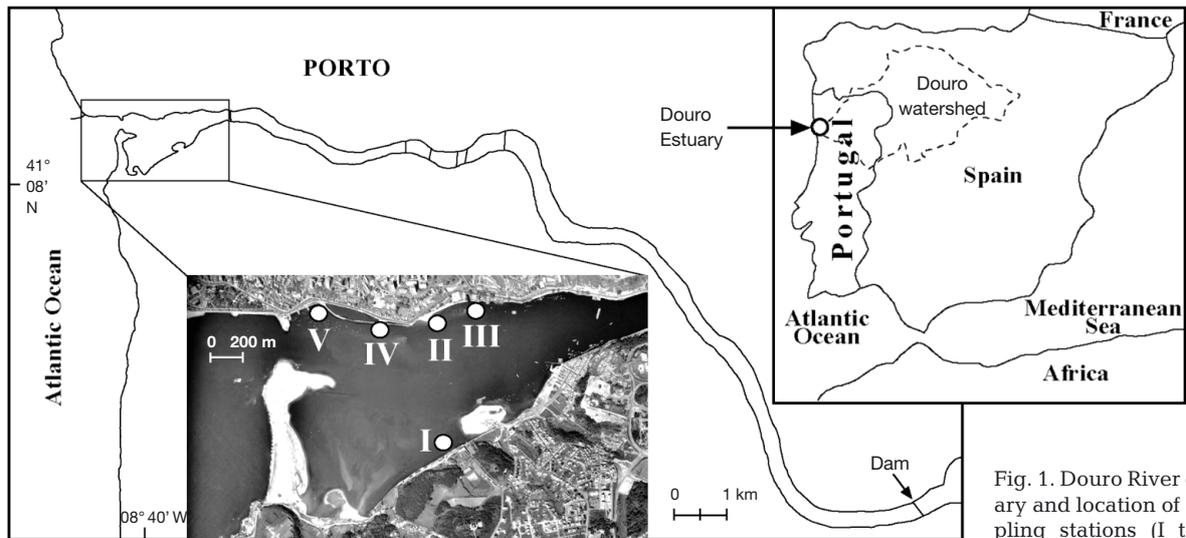


Fig. 1. Douro River estuary and location of sampling stations (I to V)

taining, on average,  $8 \mu\text{M NH}_4^+$ ,  $80 \mu\text{M NO}_3^-$ ,  $2 \mu\text{M NO}_2^-$ ,  $2 \mu\text{M PO}_4^{3-}$  and  $43 \mu\text{M SiO}_4^{4-}$  (Bordalo et al. unpubl. data). In addition, largely untreated sewage from ca. one million inhabitants is discharged within its lower reach. Throughout the estuary, much of the historic intertidal marsh and sediment flats have been dredged or filled to make way for housing, parks, roads and port facilities. Today the total planar (2D) area of the estuary is  $7.5 \text{ km}^2$ , of which approximately  $1.5 \text{ km}^2$  is intertidal sand and muddy sediment, and  $0.39 \text{ km}^2$  intertidal, natural and man-made hard surfaces. Because of its rugosity, the actual (3D) surface area of the intertidal hard surface rises to  $0.63 \text{ km}^2$ . The hard surfaces support extensive microbial biofilms intertwined with the macroalga *Enteromorpha* and benthic microalgae (mostly diatoms, unpubl. data); a patchy distribution of the macroalga *Fucus* co-occurs with these biofilms.

**Flux measurements:** The exchange of oxygen and inorganic nutrients (ammonium, nitrate, nitrite, phosphate and silicate) between biofilm-covered rocks and water was measured on samples taken in the spring of 2000 from 5 mid-intertidal sites (between March and June) (Fig. 1, Table 1). Rocks were collected at low tide, placed in plastic bags and returned immediately to the laboratory; 30 l of estuarine water was also collected adjacent to the sampling sites. In the laboratory, the estuarine water was filtered through a  $0.2 \mu\text{m}$  mem-

brane filter; triplicate light and dark (wrapped with several layers of aluminum foil) Plexiglas chambers (11 cm diameter and 12 cm depth) were prepared for incubation. Individual rocks were placed at the bottom of each chamber, the chambers filled carefully with the filtered estuarine water, and all air bubbles removed. Light and dark chambers were incubated under natural light ( $>800 \mu\text{Einstein m}^{-2} \text{ s}^{-1}$ ) at *in situ* temperature (Table 1). Triplicate light and dark control chambers consisted of only the filtered ( $0.2 \mu\text{m}$ ) estuarine water. After incubation, each rock was molded with aluminum foil and the foil weighed after drying. The weight was converted to area ( $\text{cm}^2$ ), using a linear regression between foil weight and the corresponding area. The coefficient of variation of this method was 1.8%.

Primary production and respiration were estimated from the difference between the initial ( $T_0$ ) and time 1 ( $T_1$ ) dissolved oxygen concentrations in the triplicate light and dark incubation chambers. During this period of incubation (1 to 1.5 h), the chamber water volume did not change as the  $T_0$  overlying water sample (ca. 6% of the total volume) was replaced immediately with filtered estuarine water. Net oxygen fluxes were calculated according to Hargrave et al. (1983), following the equation:

$$F_{\text{O}} = \left( \frac{T_1 - T_0}{A \times t} \right) \times V \times 10^4 \quad (1)$$

Table 1. Characteristics of the estuarine water used for incubations (mean  $\pm$  SD)

Site	Date (dd/mm/yy)	Temperature ( $^{\circ}\text{C}$ )	$\text{O}_2$ ( $\text{mg l}^{-1}$ )	Salinity (‰)	$\text{NH}_4^+$ ( $\mu\text{M}$ )	$\text{NO}_3^-$ ( $\mu\text{M}$ )	$\text{NO}_2^-$ ( $\mu\text{M}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	$\text{SiO}_4^{4-}$ ( $\mu\text{M}$ )
I	14/03/00	$15.3 \pm 2.2$	$8.8 \pm 0.2$	14.0	$11.2 \pm 0.2$	$83.2 \pm 0.1$	$0.8 \pm 0.1$	$1.7 \pm 0.3$	$53.6 \pm 2.6$
II/III	01/06/00	$17.1 \pm 0.2$	$6.6 \pm 0.0$	4.5	$13.5 \pm 0.1$	$125.4 \pm 0.8$	$2.4 \pm 0.2$	$1.1 \pm 0.0$	$73.6 \pm 4.0$
IV/V	08/06/00	$19.5 \pm 0.6$	$6.5 \pm 0.4$	9.5	$8.6 \pm 0.6$	$91.6 \pm 2.1$	$1.1 \pm 0.1$	$1.4 \pm 0.2$	$70.3 \pm 4.9$

where  $F_O$  is the flux of oxygen ( $\text{mg m}^{-2} \text{h}^{-1}$ ),  $(T_1 - T_0)$  the difference in oxygen concentration between time zero and time one of the incubation ( $\text{mg O}_2 \text{ l}^{-1}$ );  $A$  is the rock surface area in  $\text{cm}^2$ ;  $t$  is the incubation time (h);  $V$  is the estuarine water volume in each chamber (l) and  $10^4$  is the conversion factor from  $\text{cm}^2$  to  $\text{m}^2$ .

Immediately after sampling, the oxygen samples were fixed and dissolved oxygen determinations performed in triplicate, using a modification of the Winkler method (Carpenter 1965). Oxygen values were corrected for the oxygen variations in the control chambers. Hourly respiration and primary production rates were calculated per square meter of biofilm as follows: Hourly net primary production rate (NPP) = mean oxygen production ( $\text{mg O}_2 \text{ m}^{-2}$ ) in transparent chambers/incubation time (h); Hourly respiration rate ( $R$ ) = mean oxygen consumption ( $\text{mg O}_2 \text{ m}^{-2}$ ) in dark chambers/incubation time (h). Oxygen data were converted to carbon assuming that 1 mg of oxygen produced or respired is equivalent to 0.375 mg of organic carbon (Uthicke & Klumpp 1998).

For inorganic nutrients a  $T_0$  sample was taken from each chamber, and the volume of water removed was replaced with estuarine water, as described below. Subsequently, 4 ( $T_1$  to  $T_4$ ), 50 ml subsamples of overlying water were taken approximately hourly from each

chamber during the 4.5 to 5.5 h incubation period to assess linearity of the rates (Fig. 2). Water was gently mixed manually every 20 min. In controls (0.2  $\mu\text{m}$  filtered estuarine water), subsamples were taken only at times  $T_0$  and  $T_4$ . All subsamples were immediately syringe-filtered through 0.45  $\mu\text{m}$  acid-washed membrane filters and stored at  $-21^\circ\text{C}$  in acid-cleaned polyethylene flasks until inorganic nutrient analysis. Nutrient analyses of each sample were performed in triplicate. Orthophosphate, nitrite, ammonium and reactive silicate were quantified using methods described in Grasshoff et al. (1983). Samples for dissolved silicate analysis did not have contact with glass and after thawing were kept for 1 wk at  $5^\circ\text{C}$ ; this procedure completely depolymerizes the silicate (Grasshoff et al. 1983). Nitrate was assayed using an adaptation of the spongy cadmium reduction technique (Jones 1984), with the nitrite value subtracted from the total. The precision of all determinations was in the range of 0.1 to 8%, depending on the particular nutrient concentration. For each incubation period, inorganic nutrient concentrations ( $\mu\text{M}$ ) were corrected for the corresponding volume of chamber water ( $\mu\text{mol}$ ) by multiplying the concentration value of each inorganic nutrient by the actual volume (l) of each chamber at each time.

Flux rates of inorganic nutrients were calculated using the slope of the linear relationship between the changes in the nutrient content ( $\mu\text{mol}$ ) in the water chamber with the time of incubation (h) (e.g. Barbanti et al. 1992), following the equation:

$$F_N = \left(\frac{\alpha}{A}\right) \times 10^4 \quad (2)$$

where  $F_N$  is the flux of each inorganic nutrient ( $\mu\text{mol m}^{-2} \text{h}^{-1}$ ) and  $\alpha$  is the slope of the linear regression ( $\mu\text{mol h}^{-1}$ ). When net nutrient flux rates were either positive or negative, the fluxes were linear over the entire incubation period (Fig. 2). Null net fluxes were observed 7 times (out of 50); in these cases each of the replicate chambers showed no change in nutrient concentration with incubation time. Significant differences in control chamber nutrient concentrations between  $T_0$  and  $T_4$  were never observed (ANOVA,  $p > 0.05$ ).

**Total organic matter and chl a:** Surface triplicate subsamples (1 cm diameter) were collected by scraping each rock at the end of the experiment and placing them into glass vials for chl *a* and total organic matter (OM) analyses. Samples for chl *a* measurements were kept frozen ( $-21^\circ\text{C}$ ) until analysis. Extraction was performed in a mixed solution of acetone, methanol and water (45:45:10 ratio, respectively), according to Joye et al. (1996). Also, Hill & Hawkins (1990) found that methanol extraction is a more accurate and precise method than acetone extraction to estimate chlorophyll

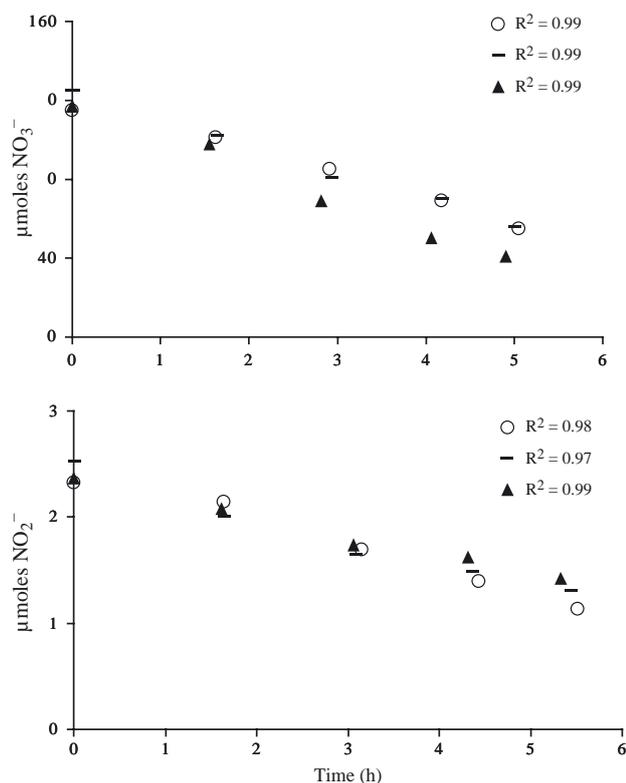


Fig. 2. Examples of typical linearity between water nutrient concentrations and incubation period. Different symbols represent individual values for triplicate chambers

Table 2. Spatial variation of chl *a* and total organic matter (OM) content (mean  $\pm$  SD). na: not available

Site	g chl <i>a</i> m <sup>-2</sup>	g OM m <sup>-2</sup>	% OM
I	na	na	na
II	0.44 $\pm$ 0.26	41.07 $\pm$ 23.99	11.96 $\pm$ 2.56
III	0.80 $\pm$ 0.26	71.54 $\pm$ 34.73	9.34 $\pm$ 4.16
IV	1.24 $\pm$ 0.31	124.53 $\pm$ 19.76	16.23 $\pm$ 9.67
V	0.84 $\pm$ 0.22	101.45 $\pm$ 33.47	16.86 $\pm$ 13.90

content in intact rocks. Chl *a* concentration was determined spectrophotometrically, using standard equations (Parsons et al. 1984). Samples for organic matter content were processed immediately by drying to constant weight at 60°C, followed by ignition in a muffle furnace at 500°C for 4 h and reweighing (data are presented either as percentage of total organic matter or g of total organic matter per m<sup>2</sup> of biofilm).

**Statistical analysis:** Data analysis was performed at the 95% confidence level ( $p < 0.05$ ). Data were tested for normality using the Kolmogorov-Smirnov test, and homoscedasticity using Leven's test (Zar 1996). In order to compare the means of each variable between the different stations, a 1-way ANOVA (1 degree of freedom) was performed (Zar 1996).

**Results and discussion.** Water column temperature, oxygen concentration, salinity and inorganic nutrient concentration for each sampling date are given in Table 1.

The average concentration of chl *a* on hard surfaces was  $0.8 \pm 0.4$  g m<sup>-2</sup>. The average total organic matter per square meter of biofilm was  $84.7 \pm 41.8$  g, and the mean value for percent total organic matter was  $13.4 \pm 8\%$ . This shows that, in spite of the high total organic matter per square meter of biofilm, there was also a high percentage of non-organic material, as just  $13.4 \pm 8\%$  of these biofilms are organic matter. Spatial data for the variables are shown in Table 2. The positive linear relationship found between chl *a* and total organic matter ( $r = 0.84$ ,  $p < 0.001$ ,  $n = 24$ ) suggests that the majority of the organic matter in these biofilms was macroalgae.

Hourly NPP (light incubation) exceeded hourly *R* (dark incubation) by 5 to 11 times (Fig. 3, Table 3). NPP and *R* were similar between sites (Fig. 3); only Site III

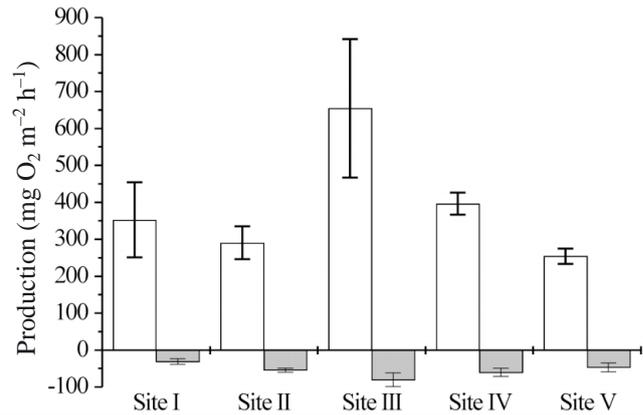


Fig. 3. Hourly net production (white bars = light incubation) and respiration (grey bars = dark incubation) rates at each sampling site (mean  $\pm$  SD)

showed significantly higher NPP and *R* rates (ANOVA,  $p < 0.05$ ). The mean hourly biofilm NPP averaged  $146.0 \pm 58.1$  mg C m<sup>-2</sup> h<sup>-1</sup>, similar to values found in adjacent sediments (Magalhães et al. 2002) and in sediments elsewhere (e.g. Colijn & de Jonge 1984, Barranguet et al. 1996). NPP showed a positive correlation with respiration rates ( $r = 0.64$ ,  $p < 0.05$ ,  $n = 30$ ), comparable to results from other intertidal sediment-water flux studies (e.g. Cammen 1991, Magalhães et al. 2002).

The direction and magnitude of light and dark incubated inorganic nutrient fluxes between hard substrates and water varied, depending on the nutrient examined (Fig. 4). Ammonium concentrations in incubation water decreased linearly over time in light incubations (negative fluxes) but showed variable responses in dark incubations (Fig. 4a). In contrast, nitrate, which in addition to providing fixed nitrogen for algae and bacteria is considered a serious contributor to coastal eutrophication, was consistently removed at similar rates in both light and dark incubations (Fig. 4b). These findings suggested an ammonium assimilation preference by the primary producers that inhabit the rocky biofilms. The preferential use of NH<sub>4</sub><sup>+</sup> instead of NO<sub>3</sub><sup>-</sup> by macrophytes and microalgae has been documented (e.g. Korb & Gerard 2000, Tungaraza et al. 2003). Nitrite concentrations in incubation water ranged from 0.8 to 2.4  $\mu$ M (Table 1); light vs dark incubation did not affect the flux direction or magnitude

Table 3. Comparison of net inorganic nutrient fluxes ( $\mu$ mol m<sup>-2</sup> h<sup>-1</sup>), net primary production (NPP) and hourly respiration rates (*R*) (mg O<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) in hard surface-water and sediment-water interfaces, for the Douro River estuary. Sandy and muddy sediment data from Magalhães et al. (2002). Mean  $\pm$  SD

	NH <sub>4</sub> <sup>+</sup> (light)	NH <sub>4</sub> <sup>+</sup> (dark)	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SiO <sub>4</sub> <sup>4-</sup>	NPP	<i>R</i>
Rocky biofilms	-123.2 $\pm$ 22.5	144.5 $\pm$ 151.9	-595.4 $\pm$ 236.7	-0.57 $\pm$ 9.1	1.4 $\pm$ 10.5	-305.7 $\pm$ 209.6	389.4 $\pm$ 168.3	50.4 $\pm$ 17.5
Sandy sediment	-64.1 $\pm$ 25.1	-18.3 $\pm$ 22.9	-338.6 $\pm$ 170.1	-3.8 $\pm$ 3.6	-4.8 $\pm$ 3.1	-169.3 $\pm$ 57.0	427.5 $\pm$ 173.0	37.9 $\pm$ 23.5
Muddy sediment	-55.8 $\pm$ 33.0	33.5 $\pm$ 67.1	-316.7 $\pm$ 142.9	2.6 $\pm$ 9.4	-3.9 $\pm$ 3.5	-127.2 $\pm$ 71.6	261.5 $\pm$ 51.0	44.6 $\pm$ 13.1

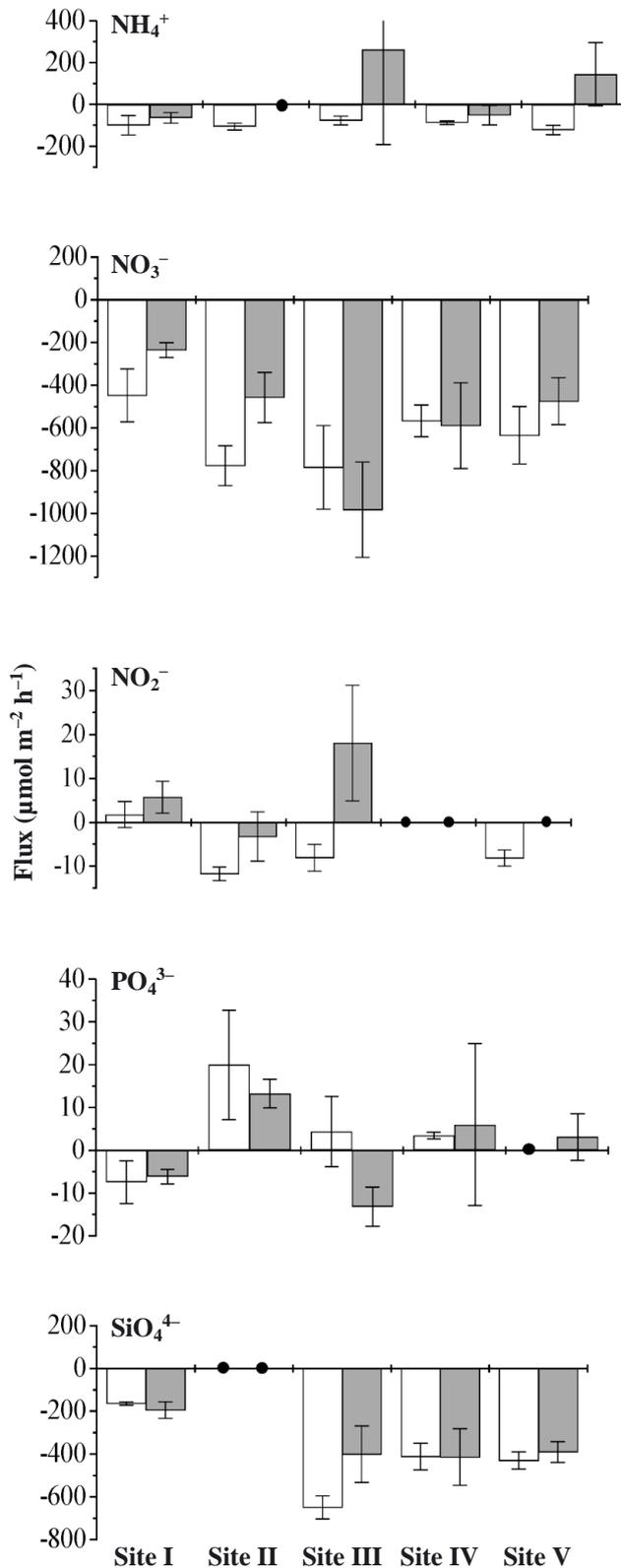


Fig. 4. Mean  $\pm$  SD of Douro River estuary net fluxes of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  between the water column and rock biofilms (grey bars = dark incubation; white bars = light incubation; ● = null flux)

(Fig. 4c). Nitrite, produced via ammonium oxidation and during denitrification, is generally found in low concentrations, and benthic fluxes are reported to be minimal (e.g. Ogilvie et al. 1997, Cabrita & Brotas 2000).

No spatial variability was found in light net nitrate fluxes, since the ANOVA analysis yielded no spatially significant differences, while Site III showed significantly higher dark uptake rates than Sites I, II and V (ANOVA,  $p < 0.05$ ). In all cases molar ratios of C:N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) net fluxes in light chambers ( $20.4 \pm 4.1$ ;  $10.4 \pm 1.8$ ;  $25.0 \pm 11.3$ ;  $18.9 \pm 0.6$ ;  $10.7 \pm 1.7$ , for Sites I, II, III, IV and V, respectively) were not far from the optimal stoichiometric ratio of C:N = 7 given by Hillebrand & Sommer (1999) for periphyton. Further, Worm et al. (2000) found C:N ratios of *Enteromorpha* in spring and summer from 10 to about 40, depending in part on the ambient water nitrogen concentration. On the other hand, the lack of statistical relationships between NPP and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  net uptake rates, and the absence of significant differences between light and dark incubations in nitrate net fluxes, supports the idea that primary production alone is unlikely to be the major process controlling nitrogen uptake rates. Denitrification, as previously found for intertidal and subtidal sediments (cf. Seitzinger 1990, Ogilvie et al. 1997), could also be largely responsible for the nitrate uptake rates reported here, and experiments are underway to assess this possibility.

Phosphate net fluxes showed no consistent trend in flux direction or magnitude (Fig. 4d). Phosphate, in addition to its biological importance, also rapidly adsorbs to clays and other particulate inorganic compounds (de Jonge & Villerius 1989, Sundby et al. 1992). As a result, clear biologically mediated benthic-water fluxes are not commonly seen. In this study, the absence of significant, positive relationships between net  $\text{PO}_4^{3-}$  uptake rates and NPP, as well as the dominance of positive fluxes, suggest that net  $\text{PO}_4^{3-}$  uptake rates measured did not primarily reflect phosphate assimilation by epilithic primary producers.

Both light and dark dissolved-silicate fluxes were negative (removal from water), with one exception when no net flux occurred in either light or dark incubations (Fig. 4e). Dissolved silicate, while required by diatoms for production of frustules (Tréguer et al. 1995), can also be adsorbed by hydrated oxides of aluminium, iron, manganese and magnesium (Edwards & Liss 1973), and thus the uptake can result from both biological and abiotic processes. However, since 2 samples gave no net uptake (Fig. 4e), biological processes were probably responsible for the kinetics seen. Sigmon & Cahoon (1997) demonstrated the potential importance of benthic diatoms as regulators of water column  $\text{SiO}_4^{4-}$  concentrations, but in this case dark and light flux rates differed. Correlation analysis between

NPP rates and net  $\text{SiO}_4^{4-}$  fluxes in light chambers revealed a significantly positive relationship between NPP and net influx of dissolved silicate ( $r = 0.69$ ,  $p < 0.05$ ,  $n = 15$ ), i.e. the higher the NPP, the higher the uptake of  $\text{SiO}_4^{4-}$ . These results suggest that diatoms, which appear visually abundant in the biofilms, could account for a significant amount of the  $\text{SiO}_4^{4-}$  uptake. Also, net  $\text{SiO}_4^{4-}$  uptake rates found in this study were generally within the values reported by Tréguer et al. (1991) for rates of pelagic biogenic silicate production ( $90$  to  $950 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) (Fig. 4e).

The comparisons between hard surface and sediment water fluxes of oxygen and nutrients within the Douro River estuary reported in Table 3 show that NPP and respiration rates were virtually identical in the 2 systems (ANOVA,  $p > 0.05$ ). This is in some ways remarkable given that the chl *a* in spring averaged  $188 \pm 32 \text{ mg m}^{-2}$  for muddy sediments,  $631 \pm 91 \text{ mg m}^{-2}$  for sandy sediments and  $830 \pm 38 \text{ mg m}^{-2}$  for rocky substrates. This emphasizes the importance of the microalgal contribution to the NPP, even when macroalgae dominate the plant biomass. The fact that only 34% of the variability found in NPP is explained by the chl *a* content strengthens this idea ( $R^2 = 0.34$ ,  $p = 0.05$ ,  $n = 12$ ). This is reminiscent of the finding by Pomeroy (1959), who found that the thin film of diatoms on the intertidal sediments of a *Spartina alterniflora* marsh contributed 20% of the marsh productivity.

In the case of inorganic nutrient net fluxes, while no statistical differences were found for  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{PO}_4^{3-}$  between these intertidal environments, hard surfaces removed significantly higher amounts of  $\text{NO}_3^-$  (ANOVA,  $p < 0.001$ ) and  $\text{SiO}_4^{4-}$  (ANOVA,  $p < 0.001$ ) from the water column than did adjacent muddy and sandy sediments (Table 3). In order to compare the biogeochemical significance of  $\text{NO}_3^-$  and  $\text{SiO}_4^{4-}$  fluxes between intertidal hard substrates and sediments, mean hourly values were calculated and integrated for the total exposed area of each component. While rocky biofilms occupy just 21% of the total linear (2D) area of the intertidal zone of Douro River estuary, because of rugosity and higher flux rates, they were responsible for approximate 43% of the  $\text{NO}_3^-$  uptake and 45% of the  $\text{SiO}_4^{4-}$  uptake, in terms of hourly removal capacity for the total intertidal area. These data provide a substantial case that hard surface zones of estuaries can represent an important, albeit overlooked, component of estuarine and coastal carbon and nutrient cycling.

From a general biogeochemical perspective, it was unexpected that the metabolic rates and nutrient flux directions and magnitudes would produce such similar results for intertidal muddy sediment, sand and rock substrata, given the vastly different 3D physical structure of the biotic communities. The results presented here emphasize the importance of the boundary sur-

face layer of the rock substrata for processing carbon and nutrients.

In summary, inorganic nutrient and oxygen fluxes of rocky surfaces within the intertidal zone of the Douro River estuary function at comparable, and in some cases higher, rates than are found in adjacent sandy and muddy sediments. The results strongly suggest that these overlooked components of the intertidal zones deserve inclusion in assessing biogeochemical fluxes of other estuarine systems and along coastlines with significant areas of hard surface.

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#### LITERATURE CITED

- Barbanti A, Ceccherelli VU, Frascari F, Reggiani G, Rosso G (1992) Nutrient regeneration processes in bottom sediments in a Po delta lagoon (Italy) and the role of bioturbation in determining the fluxes at the sediment-water interface. *Hydrobiologia* 228:1–21
- Barranguet C, Plant-Cuny MR, Alivon E (1996) Microphytobenthos production in the Gulf of Fos, French Mediterranean coast. *Hydrobiologia* 333:181–193
- Cabrita MT, Brotas V (2000) Seasonal variation in denitrification and dissolved nitrogen fluxes in intertidal sediments of the Tagus estuary, Portugal. *Mar Ecol Prog Ser* 202: 51–65
- Cammen LM (1991) Annual bacterial production in relation to benthic microalgal production and sediment oxygen uptake in an intertidal sandflat and an intertidal mudflat. *Mar Ecol Prog Ser* 71:13–25
- Carpenter JH (1965) The accuracy of the Winkler method for dissolved oxygen. *Limnol Oceanogr* 10:135–140
- Colijn F, de Jonge VN (1984) Primary production of microphytobenthos in the Ems-Dollard estuary. *Mar Ecol Prog Ser* 14:185–196
- Decho AW (2000) Microbial biofilms in intertidal systems: an overview. *Cont Shelf Res* 20:1257–1273
- de Jonge VN, Villerius LA (1989) Possible role of carbonate dissolution in estuarine phosphate dynamics. *Limnol Oceanogr* 34:332–340
- D'Elia CF, Wiebe WJ (1990) Biogeochemical nutrient cycles in coral-reef ecosystems. In: Dubinsky Z (ed) *Coral reefs*. Elsevier, Amsterdam, p 49–74
- Edwards AMC, Liss PS (1973) Evidence for buffering of dissolved silicon in fresh waters. *Nature* 243:341
- Grasshoff K, Ehrhardt M, Kremling K (1983) *Methods of seawater analysis*. Verlag Chemie, Weinheim
- Hargrave BT, Prouse NJ, Philips GA, Neame PA (1983) Primary production and respiration in pelagic and benthic communities at two intertidal sites in the upper Bay of Fundy. *Can J Fish Aquat Sci* 40:229–243

- Hill AS, Hawkins SJ (1990) An investigation of methods for sampling microbial films on rocky shores. *J Mar Biol Assoc UK* 70:77–88
- Hillebrand H, Sommer U (1999) The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnol Oceanogr* 44:440–446
- Hillebrand H, Sommer U (2000) Effect of continuous nutrient enrichment on microalgae colonizing hard substrates. *Hydrobiologia* 426:185–192
- Jickells TD (1998) Nutrient biogeochemistry of the coastal zone. *Science* 281:217–222
- Jones MN (1984) Nitrate reduction by shaking with cadmium: alternative to cadmium columns. *Water Res* 18:643–646
- Joye SB, Mazzotta ML, Hollibaugh JT (1996) Community metabolism in microbial mats: the occurrence of biologically-mediated iron and manganese reduction. *Estuar Coast Shelf Sci* 43:747–766
- Knox GA (2000) The ecology of seashores. CRC Press, Boca Raton, FL
- Korb RE, Gerard VA (2000) Nitrogen assimilation characteristics of polar seaweeds from different nutrient environments. *Mar Ecol Prog Ser* 198:83–92
- Magalhães C, Bordalo AA, Wiebe WJ (2002) Temporal and spatial patterns of intertidal sediment-water nutrient and oxygen fluxes in the Douro River estuary, Portugal. *Mar Ecol Prog Ser* 233:55–71
- Ogilvie B, Nedwell DB, Harrison RM, Robinson A, Sage A (1997) High nitrate, muddy estuaries as nitrogen sinks: the nitrogen budget of the River Colne estuary (United Kingdom). *Mar Ecol Prog Ser* 150:217–228
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Elmsford, NY
- Pomeroy LR (1959) Algal productivity in salt marshes of Georgia. *Limnol Oceanogr* 4:386–397
- Schulz HD, Zabel M (2000) Marine geochemistry. Springer-Verlag, Berlin
- Seitzinger SP (1990) Denitrification in aquatic sediments. In: Revsbech NP, Sorensen J (eds) Denitrification in soil and sediment. Plenum Press, New York, p 301–322
- Sigmon DE, Cahoon LB (1997) Comparative effects of microalgae and phytoplankton on dissolved silica fluxes. *Aquat Microb Ecol* 13:275–284
- Sundby B, Gobeil C, Silverberg N (1992) The phosphorus cycle in coastal marine sediments. *Limnol Oceanogr* 37:1129–1145
- Tréguer P, Lindner L, Van Bennekom AJ, Leynaert A, Panouse M, Jacques G (1991) Production of biogenic silica in the Weddell-Scotia Seas measured with <sup>32</sup>Si. *Limnol Oceanogr* 36:1217–1227
- Tréguer P, Nelson DM, Van Bennekom AJ, DeMaster DJ, Leynaert A, Quéguiner B (1995) The silica balance in the world ocean: a reestimate. *Science* 268:375–379
- Tungaraza C, Rousseau V, Brion N, Lancelot C, Gichuki J, Baeyens W, Goeyens L (2003) Contrasting nitrogen uptake by diatom and *Phaeocystis*-dominated phytoplankton assemblages in the North Sea. *J Exp Mar Biol Ecol* (in press)
- Uthicke S, Klumpp DW (1998) Microphytobenthos community production at a near-shore coral reef: seasonal variation and response to ammonium recycled by holothurians. *Mar Ecol Prog Ser* 169:1–11
- Vieira MEC, Bordalo AA (2000) The Douro estuary (Portugal): a mesotidal salt wedge. *Oceanol Acta* 23:585–594
- Wetzel RG (1983) Limnology, 2nd edn. Saunders College Publ, Philadelphia, PA
- Worm B, Lotze HK, Sommer U (2000) Coastal food web structure, carbon storage, and nitrogen regulated by consumer pressure and nutrient loading. *Limnol Oceanogr* 45:339–349
- Zar JH (1996) Biostatistical analysis. Prentice-Hall, Upper Saddle River, NJ

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