Nutrient dynamics in the intertidal pools of the Mondego estuary: II - seasonal efflux of PO₄-P and NH₄-N in bare bottom and vegetated pools

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NUTRIENT DYNAMICS IN THE INTERTIDAL POOLS
OF THE MONDEGO ESTUARY.
II – SEASONAL EFFLUX OF PO₄-P AND NH₄-N IN BARE BOTTOM
AND VEGETATED POOLS

Abstract

In intertidal estuaries, such as the Mondego estuary, not much is known about the importance of the low water intertidal pools, with regard to sediment/water exchanges of ammonia and phosphate, as a result of anabolic/catabolic balances. Therefore, the main topic of this work was a comparative study of the role and the importance of the nutrient efflux in intertidal bare bottom (sandy and muddy) and in *Spartina maritima* covered pools, during day and night periods. Seasonal variation and daily variations were studied in July and November 1997.

Although, the availability of nutrients is reflected by the organic content in sediments, *S. maritima* dynamics clearly influence ammonia and phosphate efflux rates, especially in summer and during the day. Considering the fact that sediments constitute a major source of nutrients for rooted macrophytes, during the day there might be a certain competition between two processes: *Spartina maritima* facilitated adsorption capacity and the efflux from the sediment.

Ammonia and phosphate fluxes were higher in bare bottom muddy pools, followed by *Spartina maritima* covered pool and sandy bare bottom pools. It was also possible to observe the importance of plant dynamics. Ammonia efflux rates were higher during fall, due to *Spartina* was less productive, while the *Spartina* kept the efflux low during summer (flowering season). On the system level, the results express the importance of intertidal low water pools to the internal nitrogen loading, particularly in the bare bottom muddy pools.

This study also shows that in July, high temperatures and anoxic conditions, especially during the night, promoted the phosphate efflux to the overlaying water column. Moreover, *S. maritima* also controlled phosphate efflux from the sediment during the day, but in absence of enough light for primary production to take place, during the night, phosphate efflux increases, especially in July. Although in a seasonal scale, the daily mean

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(day/night) efflux rates were similar. On the system level, results also evidences the importance of these intertidal pools to the internal dissolved inorganic phosphate loading, especially the muddy bare bottom pools, which showed a clear seasonal variation.

Introduction

Many studies have focused on the importance of freshwater runoff from land and the exchange of water with adjacent open sea regarding biogeochemical processes in estuaries (e.g. Valiela and Teal 1979, Hinga 1990, Staver et al. 1996, Pardal 1998, Flindt et al. 1999).

It is know, that in estuaries vertical distribution and relative abundance of inorganic nitrogen compounds (NH_4^+ , NO_2^- , and NO_3^-) in the sediment are controlled by the redox state (Valiela 1995). In anaerobic sediments ammonium is far more abundant and it has been shown that ammonium diffuses upward to the overlying water (Valiela 1995). Rocha (1998) described a mechanism where, in warm climates intertidal estuaries and during the day, temperature gradient between the exposed sediment and water at the time of flooding causes a free convection of ammonia to the water column. On the other hand, if the water is warmer than the sediment surface, which is the intertidal pool situation, ammonia efflux only occurs by diffusion (Rocha 1998).

The movement of particulate organic matter transports most of the phosphorous to the sediment. In an intertidal system, sediments get exposed to air and subsequent drying conditions during ebb tide, and it has been show (Baldwin 1996 in Mitchell and Baldwin 1998) that under this desiccated/oxidation conditions, sediments have a substantially higher phosphate sorption capacity than submerged sediments. Nevertheless, in the intertidal pools sediments are always submersed, and may represent 30-40% of the system area (Flindt et al. 2002, Lillebø et al. 2002). Phosphorus dynamics is also influenced by sediment resuspension and macrophyte coverage (Flindt and Kamp-Nielsen 1998) and bacteria might directly or indirectly mediate phosphorous release from sediments (Mitchell and Baldwin 1998). On the other hand, temperature seems to be of crucial importance to the efflux of phosphorus from the sediment (e.g. Asmus et al. 2000), with a resulting increased of phosphorus internal loading (e.g. Perttälä et al 1995, Valiela 1995, Pardal 1998, Sfriso 1988).

The importance of benthic mineralisation to the trophic chain increase as the water column gets shallower (e.g. Nixon and Pilson 1983 in Rocha 1998), and particularly in these ephemeral pools system, physic and chemical conditions change in few hours, reaching high water temperature and low oxygen concentrations, especially during summer night ebbs tides. Moreover, submersed rooted macrophytes link the nutrients in sediments with the overlying water, which may have important implications for nutrient cycling within such systems (e.g. Valiela 1995, Kamp-Nielsen and Flindt 1993, Flindt et al. 1999). But the relative contribution from the sediment pore-water and the overlying water column to N /P supply is not very well documented (Flindt et al. 1999). Therefore, the main topic of this work was a comparative study of the seasonal

nutrient efflux in intertidal bare bottom (sandy and muddy) and macrophyte (*Spartina maritima*) covered pools during 24-hours tidal cycles.

Material and methods

Field procedure

In the present study *Spartina maritima* salt marsh area was chosen for being the most representative regarding vegetated tidal pools in the Mondego estuary (Marques et al. 2002, Pardal 1998, Lillebø et al. 1999) (Fig 1). A tidal pool in this area was selected to represent an average pool size in respect to surface area and depth. Simultaneously, the same study was performed in two bare bottom areas characterised respectively by muddy and sandy sediments. Pools were studied during 24-hours tidal cycles in July 97 and November 97, representing respectively summer and fall situations. From the pool formation until the pool was submerged again, water temperature, salinity and dissolved oxygen were measured, and water samples (10 ml each) were taken for dissolved reactive phosphate and ammonia analysis. The sampling frequency was one hour. All the water samples were filtered in the field through a Whatman GF/F glass-fiber filter and stored in ice. In the laboratory they were kept frozen at -18°C until analysis. Each pool was measured for surface area and depth at each sampling date. Flux chambers were placed in the main channel (under day and night situations, July 2001) for comparison of the efflux rates in the low water pools.

Fig 1. *Spartina maritima* salt marsh area in the Mondego estuary with the location of the three types of tidal pools.

Analytical procedure

Analysis of dissolved reactive phosphate and ammonia were carried out using a rapid flow autoanalyser (RFA 300 Alpkem) and performed according to Alpkem methodologies (Alpkem, 1990).

Calculations

Phosphate, ammonia and oxygen fluxes were calculated taking into account the initial concentrations in the pool, which corresponded to the very first measurement just after the formation of the tidal pool (t_0). So, for each consecutive sample ($t_1, t_2, \dots, t_n$) the calculated rates were given by the difference between concentration in time (t_n) and the initial concentration, and then divided by the number of hours between the two samples.

$$\text{Rate} = \left[\frac{C_f - C_i}{t_n - t_0} \right]$$

All data were previously standardised ($\mu\text{mol l}^{-1} \text{m}^{-2}$) to avoid bias caused by differences in the pools size. For the same reason, all data were also standardised (mg m^{-2}) to calculate the total amount of nutrients per hour.

Calculations of the mean efflux rates ($\text{mg m}^{-2} \text{d}^{-1}$) were performed considering: a) the mean daily efflux rates (day and night) from each type of pool (Lillebø et al. 2002); b) a visual estimation of the contribution of each type of pool to the estuarine area (1.9 km^2 : 17% *Spartina*, 50% mud and 33% sand); c) a visual estimation of the area covered by pools during low tide (40%); d) the mean efflux rates in July could represent a mean value for spring and summer, and that the mean efflux rates in November could represent a mean value for fall and winter. In the main channel it was assumed that there is no significant seasonal variation.

It is known that nutrient fluxes are strongly dependent on temperature dynamics, and several studies have demonstrated this promoting effect of rising temperatures (e.g. Nixon et al. 1976, Fisher et al. 1982 *all in* Asmus et al. 2000). As a preliminary approach, we therefore used a simple dynamic temperature model to simulate the yearly variation in the ammonia fluxes. The model equations are:

$$\text{NH}_4\text{-flux}_{\text{actual}} = \text{NH}_4\text{-flux}_n \cdot K(n\text{-temp}_{\text{actual}})$$

$$\text{Accumulated NH}_4\text{-flux} = \text{NH}_4\text{-flux}_{i-1} + \text{NH}_4\text{-flux}_i$$

Where the $\text{NH}_4\text{-flux}_n$ is the measured phosphate flux at a certain temperature ($n^\circ\text{C}$), K is temperature dependency constant, while $\text{temp}_{\text{actual}}$ is the simulated ammonia fluxes. K varied between 1.02-1.12 indicating that some pools are more temperature dependent than others. The only forcing function was the temperature. Afterward the simulated fluxes becomes numerical integrated so the yearly internal loading, due to the flux dynamics, for the pools becomes simulated.

Calculations of the ammonia mass efflux (kg N) were also performed assuming that the mean efflux rates in July could represent a mean value for spring and summer, and that the mean efflux rates in November could represent a mean value for fall and winter. In the main channel it was assumed that there is no significant seasonal variation.

Results

During the life span of the pools temperature increased dramatically during the day (Fig. 2) and, as we should expect, mean water temperature increased seasonally, reaching a maximum value of 32 °C, in July and a minimum of 10 °C in November. Oxygen concentration decreased during the night, (always less than 5 mg O₂ l⁻¹), when primary production was unable to compensate for heterotrophic oxygen consumption. This situation was more accentuated in July when temperature was about to 20 °C, (1 mg O₂ l⁻¹). So, pools become anoxic, for short periods. Salinity varied seasonally as a function of the freshwater discharges from the Pranto River (Fig. 1) (Marques et al. 2002). The lowest salinities were recorded in November, always less than 5 and the highest in July, reaching 36.

Fig. 2. Seasonal variation (July and November) of water pools temperature (°C). Salinity and Oxygen (mg O₂ l⁻¹): a) *Spartina maritima* covered pool, b) Muddy bare bottom pool, c) Sandy bare bottom pool.

Ammonia dynamics

Seasonal differences were observed concerning the initial total amount of ammonium in the pools (Fig. 3). In July 97 the initial values varied between 8 and 12 mg m⁻² (579 and 724 µmol m⁻²) in the *Spartina* covered pool, 8 and 12 mg m⁻² (547 and 742 µmol m⁻²) in the muddy bare bottom pool and 0.7 mg m⁻² (49 µmol m⁻²) in the sandy bare bottom pool (Fig 3). In November, we quantified 46 mg m⁻² (3275 µmol m⁻²) in the *Spartina* covered pool, 14 and 21 mg m⁻² (1009 and 1513 µmol m⁻²) in the muddy bare bottom pool, and 8 and 11 mg m⁻² (566 and 812 µmol m⁻²) in the sandy bare bottom pool (fig 3). The total amount of ammonia was therefore much higher in November. Moreover, the total amount of ammonia in *Spartina* covered pool was much more dynamic in November, which may be a reflection of plants annual dynamics.

Fig. 3. Seasonal variation of ammonia total amount (mg NH₄-N m⁻²) during the life span of the low water pools.

Ammonia formation is close related to mineralisation, and therefore plotted against oxygen consumption.

Results show that in all three pools, higher ammonia efflux rates occurred during the night period when heterotrophic respiration was not compensated by primary production (Fig 4). Considering separately each type of pool, results showed that the

Fig. 4. Seasonal variation of ammonia efflux and uptake rates vs. oxygen efflux and uptake rates: a) *Spartina maritima* covered pool, b) Muddy bare bottom pool, c) Sandy bare bottom pool.

efflux rates of ammonia in the *Spartina maritima* covered pool was very dependent on plant dynamics. In July there was ammonia influx during the day and an efflux during the night (Fig. 4.1A). Furthermore, although oxygen depletion during night was higher in July, ammonia efflux was much higher in November and occurred even during the day (Fig. 4.2A). In the muddy bare bottom pool, there were always effluxes of ammonia (day and night), and oxygen concentration decreased along the life span of the pool during day and night periods (Fig. 4.1B, 4.2B). Oxygen depletion was similar in July and November. In the sandy bare bottom pool, although, efflux rates during the day were lower in November, it also seemed to increase during night (Fig. 4.1C, 4.2C).

Considering the mean daily efflux rates (day and night) from each type of pool there was a clear seasonal variation of the efflux rates, with higher N-effluxes during fall and lower during summer (Fig. 5). This seasonal variation is particularly clear in the *Spartina* covered pool. The efflux rates measured in the main channel are comparatively very low (Fig. 5).

As a preliminary approach, we simulate the internal loading of ammonia based on a temperature-dependent model (Fig. 6). Despite cautions in interpreting this simulation, due to the lack of points for the calibration of the model, it express a decrease of ammonia efflux during the warmer periods (spring and summer) and an increase during fall and winter, especially in the *Spartina* covered pools.

Fig. 5. Seasonal variation of phosphate mean efflux rates ($\text{mg NH}_4\text{-N m}^{-2} \text{h}^{-1}$) at each pool type and the main channel.

Fig. 6. Simulation of the yearly variation in ammonia fluxes, in pools located in the bare bottom muddy areas, sandy areas and in *Spartina maritima* covered areas of the estuary.

Fig. 7. Yearly net efflux of ammonia (kg N) for each type of pool and the main channel.

Calculations of the ammonia net effluxes (kg N), based on the simulated values, suggest a strong contribution of the bare bottom muddy pool to the whole system internal ammonia loading (Fig 7), and especially during fall and winter. The *Spartina*

covered pool and the sandy covered pool had a similar yearly contribution to the system internal ammonia loading, but higher than the channel (Fig. 7). Although, sandy pools did not show a clear seasonal variation.

Phosphate dynamics

The initial total amount of phosphate in the pools decreased from July to November in *Spartina* covered pool and in the bare bottom muddy pool, but increased slightly in the bare bottom sandy pool (Fig. 8). In July 97, the initial values varied between 2 and 3 mg m⁻² (115 and 126 $\mu\text{mol m}^{-2}$) in the *Spartina maritima* covered pool, 1 and 4 mg m⁻² (52 and 310 $\mu\text{mol m}^{-2}$) in the muddy bare bottom pool and 0.8 mg m⁻² (55 $\mu\text{mol m}^{-2}$) in the sandy bare bottom pool (Fig 8). In November, we quantified 4 mg m⁻² (268 $\mu\text{mol m}^{-2}$) in the *Spartina maritima* covered pool, 1 mg m⁻² (75 $\mu\text{mol m}^{-2}$) in the muddy bare bottom pool, and 1 and 1.5 mg m⁻² (85 and 107 $\mu\text{mol m}^{-2}$) in the sandy bare bottom pool (fig 8). The phosphate total amount was therefore higher in July. Moreover, the total amount of phosphate in *Spartina* covered pool was much more dynamic in November, which may also reflect the plants annual dynamics.

Fig 8. Seasonal variation of phosphorus total amount (mg PO₄-P m⁻²) during the life span of the low water pools.

The comparison of phosphate and oxygen efflux rates ($\mu\text{mol m}^{-2} \text{h}^{-1}$) in July and November shows that for the same type of pool, the process varies seasonally (Fig. 9), with lower efflux rates in November. Efflux rates were always higher within the first hour after pool formation. Considering each type of pool, *Spartina* covered pool phosphate dynamics seems much dependent on plant dynamics. In fact efflux rates are much lower during the day and increase during the night, in July (Fig. 9.1A). On the other hand, in November, although efflux rates are lower (Fig. 9.2A), higher total amount of phosphate occurred during the day and under oxic conditions (Fig. 8). In the muddy bare bottom pool there was always efflux of phosphate (Fig. 9.1B, 9.2B). In the sandy bare bottom pools, values found for the phosphate efflux were lower than in the other two types of pools, but showing the same tendency as in the bare bottom muddy pool (Fig. 9.1C, 9.2C).

Fig. 9. Seasonal variation of phosphate efflux and uptake rates vs. oxygen efflux and uptake rates: a) *Spartina maritima* covered pool, b) Muddy bare bottom pool, c) Sandy bare bottom pool.

Considering the mean daily efflux rates (day and night) from the muddy bare bottom pool there was a clear seasonal variation of the efflux rates, with higher P-effluxes during Spring/Summer and lower during Fall/Winter (Fig. 10). This seasonal variation is not visible in the *Spartina* covered pool, or in the sandy pools. The efflux rates from the main channel are comparatively low (Fig. 10).

Fig 10. Seasonal variation of phosphate mean efflux rates ($\text{mg PO}_4\text{-P m}^{-2} \text{d}^{-1}$) at each pool type and the main channel.

Discussion

Ammonia dynamics

Results clearly show the influence of plant coverage, especially with higher temperatures and during the day. In fact, rooted macrophytes keep nutrients cycling close coupled to the rhizosphere, and it is known that rooted vegetation potentially enhances denitrification (Valiela 1995, Flindt et al. 1999). Moreover, ammonia can be removed from water by plants uptake, algae and bacteria, which may also explain the depressed fluxes, meaning uptake of ammonia from the water column in the *Spartina maritima* covered pool. Therefore, we might expect a different nutrient dynamics between *Spartina maritima* covered intertidal pools and bare bottom pools (muddy and sandy sediments). Furthermore, the organic content in sediments also reflects the availability of nutrients, and results from a previous study (Flindt et al. 2002) showed that in *S. maritima* covered pools the organic content was about 8 % in the sediment upper layer, decreasing to about 5% at 12.5 cm's depth. Comparatively, muddy sediments presented an organic content of about 5 % in the upper layer, decreasing to about 2 % while sandy bare bottom sediments exhibited the lowest organic content with approximately 0.95 % without any variations as a function of depth. All these facts may explain the higher effluxes of ammonia in bare bottom muddy pools, followed by *Spartina maritima* covered pools and sandy bare bottom pools. Comparatively with a study performed in the River Colne estuary, efflux rates of ammonia are in the same range ($\mu\text{mol NH}_4^+ \text{-N m}^{-2} \text{h}^{-1}$) respectively for sandy sediments and muddy sediments, probably as a function of organic matter content and higher ammonification rates in the sediment (Ogilvie et al. 1997). On the other hand, if we consider a day/night cycle, ammonia efflux rates are higher during the night, which is in agreement with an experimental study performed by Kristensen (1993). Furthermore, anoxic conditions occurred during the night, especially in July, so it may also be responsible for the higher ammonia efflux (Pettine 1983). On a

seasonal scale, ammonia effluxes were always higher during winter in the muddy sediment pools (bare bottom and *Spartina* covered). These results are in agreement with results from Ogilvie and others (1997), which could just find this seasonal variation in a muddy sediment site with higher organic matter content, although Kristensen (1993) could not find a significant seasonal pattern. In the sandy sediment there was no clear seasonal variation in ammonia efflux, which is in agreement with results from Ria Formosa (Portugal), (Falcão and Vale, 1998), where the NH_4^+ flux in sandy sediments was production-rate controlled, and the flushing from intertidal sediments was similar in February in June.

Calculations of the ammonia net effluxes (kg N), express not only the N-efflux seasonal variation in *Spartina* and muddy pools, but evidences the importance of intertidal low water pools to the internal nitrogen loading, as well the clear contribution of the bare bottom covered pools to the whole system internal nitrogen loading.

Most probably differences in ammonia fluxes between the muddy bare bottom pool and the *Spartina* pool is due to plant associated process. Based on this difference in the temporally integrated ammonia mass, we were able to calculate the plant production in nitrogen units. Taking into account, field data from Lillebø (2000) of aboveground mean C/N-ratio (35.5) for the *Spartina maritima*, we calculated the local plant production to a mean yearly net primary production of $503 \text{ g C m}^{-2} \text{ y}^{-1}$, which corresponds to a mean aboveground biomass of 1200 g m^{-2} . This result is in agreement with literature values for *Spartina maritima* production: $670 \text{ g C m}^{-2} \text{ y}^{-1}$ for 200-2500 g m^{-2} aboveground biomass, in the Tagus estuary (Catarino 1981), and $296 \text{ g C m}^{-2} \text{ y}^{-1}$ for a mean aboveground biomass of 628 g m^{-2} , in Bay of Biscay (Benito and Onaindia 1991).

Phosphate dynamics

In the Mondego estuary, the highest phosphate effluxes occurred in summer, which is in agreement with previous studies (Sfriso 1988, Valiela 1995, Falcão and Vale 1998, Pardal 1998, Asmus et al. 2000) showing that phosphate regeneration, into the dissolved form is dependent on temperature and redox-state of the sediment. This is also evident if we compare day and night ebb tides. These effluxes are more evident in muddy bare bottom pools, followed by *Spartina maritima* covered pools, and by the sandy bare bottom pools. In July 97 *Spartina maritima* covered pool was the only one where night efflux is greater than day. This is most probably related to sediment properties such as, sediment organic content, sediment pore-water profiles of phosphate, and phosphorus adsorption capacity of the sediment (Valiela 1995, Flindt et al. 1999, Flindt et al. 2002, Martins et al. 2002). Although, as described before, the organic matter content in the sediment was higher in *Spartina maritima* covered pools, muddy *Spartina* rhizosphere had also the highest adsorption capacity, $4.5 \text{ } \mu\text{mol PO}_4\text{-P g}^{-1} \text{ dwt. sediment}$. While the bare muddy area in the same sediment depth had a capacity of $2.6 \text{ } \mu\text{mol PO}_4\text{-P g}^{-1} \text{ dwt. sediment}$ and the sandy sediment showed a capacity of only $0.97 \text{ } \mu\text{mol PO}_4\text{-P g}^{-1} \text{ dwt. sediment}$. Furthermore, several authors (Carignan and Kalff 1980, Smart and Barko 1985, Chambers et al. 1989) have shown

that rooted submersed macrophytes are able to uptake almost all needed phosphorus from the sediments. So, most probably, during the night ebb, when primary production does not occur and oxygen concentration in the pool decreases to anoxic levels, phosphate efflux takes place. In fact, other studies have focussed this close relation between phosphorous and oxygen concentration suggesting that under anoxic conditions sediments may turn from sinks to sources of phosphorous (Sfriso 1988, Perttilä et al. 1995).

Considering the fact that sediments constitute a major source of phosphorous supply for submersed macrophytes, during the day there might be a certain competition between two processes: *Spartina maritima* facilitated adsorption capacity and the efflux from the sediment, expressing the efficiency of the plants in oxidizing reduced compounds of the sediment (Caçador and Vale 2000). On the other hand this situation did not occur in November, probably as a result of decrease temperature and decrease plant activity. So, mean efflux rates were closer among the muddy pools at this time of the year. The efflux rates in the sandy pool were in the same range as recorded by Asmus et al. (2000) for Ria Formosa lagoon (Portugal) sandy sediments ($63 - 8.8 \mu\text{mol m}^{-2} \text{h}^{-1}$), with less than 1 m water depths and similar temperature range. The efflux rates in the muddy bare bottom pool were lower but in the same range as recorded by Forja et al. (1994 in Asmus et al. 2000) for muddy sediments in the Bay of Cadiz (Spain) ($20.8 - 379.2 \mu\text{mol m}^{-2} \text{h}^{-1}$).

This study shows that macrophytes such as *Spartina maritima* controls phosphate efflux from the sediment, but in absence of enough light for primary production to take place, during night, high temperatures and anoxic conditions promote phosphate efflux to the overlaying water column. On a seasonal scale, results suggest that phosphate efflux is temperature dependent and that in *Spartina maritima* covered pools it is also dependent on plant dynamics. Moreover, the highest mean phosphate effluxes occurred in summer with a clear contribution of the bare bottom muddy areas, which may be explained by the organic matter content, and the P-sorption capacity of the plants (Flindt et al. 2002).

Taking into account these results, we hypothesised that the efflux of phosphorus in these intertidal pools could constitute an important contribution to the system internal loading of phosphorus (See Lillebø et al. 2002).

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