

Effects of macrofauna excretion and turbulence on inorganic nitrogenous exchanges at the water-sediment interface. Experimental approach in microcosms.

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Résumé : L'influence de l'excrétion ammoniacale de la macrofaune et celle d'une turbulence simulant une tempête sur les flux d' NH_4^+ et de NO_3^- à l'interface eau-sédiment d'un parc à huîtres ont été respectivement étudiées. Les expériences ont été réalisées dans des microcosms en conditions hivernales et estivales.

Une forte augmentation de la teneur en NH_4^+ de la colonne d'eau et de l'eau interstitielle est observée en présence de crabes (*Carcinus maenas*) ou d'huîtres (*Crassostrea gigas*). L'excrétion de la macrofaune accroît le flux d' NH_4^+ d'un ordre de grandeur en hiver et de 2 ordres de grandeur en été.

Une forte agitation de la colonne d'eau (simulation de tempête) conduit également à une importante augmentation de la teneur en NH_4^+ de la colonne d'eau mais à une diminution de la teneur en NH_4^+ de l'eau interstitielle. Les échanges de nutriments observés pendant cette turbulence semblent davantage liés à un processus physique qu'à un processus biologique.

En revanche, la teneur en nitrate de la colonne d'eau et de l'eau interstitielle n'est que peu affectée par l'excrétion de la macrofaune ou la turbulence.

La turbulence perturbe l'activité métabolique de la macrofaune, évaluée ici par le taux d'excrétion ; selon la saison et l'espèce considérée, cette perturbation est plus ou moins importante.

Abstract : The respective effects of macrofauna excretion and turbulence on the NH_4^+ and NO_3^- fluxes occurring at the water-sediment interface of an oyster-bed were studied, using marine microcosms in which this interface was reconstituted. Experiments were performed in winter and summer conditions.

In the presence of macrofauna (*Carcinus maenas* or *Crassostrea gigas*), the NH_4^+ content of the water column and of pore-water markedly increased. NH_4^+ fluxes at the water-sediment interface were 1 and 2 orders of magnitude greater than in the absence of macrofauna, in winter and summer respectively.

A turbulence simulation in the water column leads simultaneously to a marked increase of the water column NH_4^+ content and a decrease of the pore-water NH_4^+ content. According to the duration of the experimental turbulence, these nutrient exchanges likely resulted from a physical process (sediment resuspension) rather than biological processes.

In contrast to NH_4^+ , the NO_3^- content of pore-water and water column was not significantly modified by either turbulence or macrofauna.

Ammonia excretion rate of crab and oyster was to a various extent influenced by turbulence according to the season and the considered species.

INTRODUCTION

Various parameters are involved in the nutrient dynamics at the water-sediment interface of marine ecosystems, and among these, activity of benthic macrofauna has to be considered. The effects of this activity have been investigated along several lines: 1) sediment reworking and, consequently, enhancement of

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diffusive fluxes and oxygenation of the sediment (Rhoads, 1974; Mc Caffrey *et al.*, 1980; Gust & Harrison, 1981; Hines & Lyons, 1982; Grant, 1983; Aller *et al.*, 1983; Asmus, 1986) - 2) changes in nitrification-denitrification processes occurring in sediment (Henriksen *et al.*, 1980; Sayama & Kurihara, 1983; Kristensen *et al.*, 1985; Enoksson & Samuelsson, 1987) - 3) increase of NH_4^+ content of pore-water and water column as a result of ammonia excretion of macrofauna. Although attention has already focused on this last line (Nixon *et al.*, 1976), only specific studies have attempted to evaluate the contribution of ammonia excretion of benthic fauna to the nitrogen exchanges between sediment and the overlying water (Henriksen *et al.*, 1983; Koike & Mukai, 1983; Kristensen, 1984; Vetter & Hopkinson, 1985; Regnault, 1986; Boucher & Boucher-Rodoni, 1988). Therefore, this aspect of bioturbation will be mainly considered in the present study.

In addition to disturbances caused by the benthic fauna, physical factors such as storms, wind or tidal currents, induce various types of modifications, especially in estuarine and coastal systems; among these modifications are mixing and oxygenation of shallow water masses (Hopkinson *et al.*, 1985), resuspension of the bottom sediment (Gordon, 1975; Ward *et al.*, 1984; Ward, 1985), changes in nutrient fluxes at the water sediment interface (Davies, 1975; Oviatt *et al.*, 1981; Boucher & Boucher-Rodoni, 1985), changes in heterotrophic activity of planktonic and epibenthic bacteria (Hubbard & Chrzanowski, 1986; Plummer *et al.*, 1987) and changes in phytoplankton populations (Riaux-Gobin, 1987; De Jonge & Van den Bergs, 1987; Estrada *et al.*, 1987). Thus, it would be interesting to compare the respective effects of these two kinds of natural disturbances and to study any possible interaction between them.

An estuarine oyster-bed, in the Roscoff area (N-Brittany), regularly submitted to these disturbances was studied. In this approach, the water sediment interface of the oyster-bed was reconstituted in experimental enclosures (25 l. microcosms) in order to determine both the effect of ammonia excretion of some species (oysters and crabs) and the effect of a physical turbulence on the NH_4^+ and NO_3^- exchanges between the sediment and the water column. In addition, we have looked for an answer to the following question: is the activity of macrofauna affected by a storm event? This study is part of a comparative approach using: 1) core tubes in which the nutrient fluxes at the undisturbed water-sediment interface were measured during short-term incubations (Lerat *et al.*, in press); 2) microcosms (this study) in which the effect of ammonia excreted by macrofauna on nitrogen exchanges was observed before and after a brief turbulence; 3) mesocosms (2m^3 enclosures anchored in a flooding dock) in which the effect of turbulence on sediment resuspension and on nutrient, pigment and oxygen content of the water column was studied (Lasserre *et al.*; in prep.). The aim of this comparative approach was to determine at different time scales the effects of different physical mixing schedules on nutrient fluxes in a marine shallow-water ecosystem submitted to a macrotidal regime.

MATERIAL AND METHODS

The sediment was collected at low tide at the surface of an oyster-bed located in the Bay of Morlaix (north coast of Brittany). The characteristics of the study site and the in situ nitrogen fluxes at the water-sediment interface have previously been reported (Boucher & Boucher-Rodoni, 1988). The sediment, a muddy (2,6 % silt content) sand of 200 μm medium grain size, was 2-mm sieved, in order to remove macrofauna, shells and algal residues, before its utilization in microcosms.

Microcosms

Each microcosm consisted of a bicompartiment PVC box: in the A compartment ($L=30$ cm, $l=13.5$ cm; $h=42$ cm) a plastic grid covered with a nylon net (100 μm mesh size) was fixed at 6 cm from the bottom; the B compartment ($L=30$ cm, $l=16.5$ cm, $h=42$ cm) had a regular bottom; a communication between both compartments was managed in their lower part. Sediment (around 4 liters) was spread over the double-bottom of the A compartment to obtain a sediment layer of 400 cm^2 surface and 10 cm height. Microcosms were equally filled with sea-water (25 l. per microcosm) collected at high tide on the oyster-bed. The depth of the water column overlying sediment was 15-20 cm. Sea-water circulated in these closed systems from B (water reserve) to A, through an air-lift pump fixed in B, and passed through the sediment layer by gravity. These microcosms have previously been figured (Regnault, 1986). A batch of 8 microcosms was used for each experiment. The microcosms were kept in a culture room under natural photoperiod. They were set up in these conditions 2 weeks prior to the first experiment. During this delay, the condition of the sediment layer was followed through the frontal plexiglas wall of compartment A. In a few days ($< 4\text{d.}$), the sediment looked fairly oxygenated and was naturally bioturbated by small polychaetes and gastropods. Two weeks were estimated to be sufficient for the recovery of the sediment layer and the SW-sediment interface.

Experiment

During each experiment (7h) the water circulation was stopped in the microcosms, the water level was equilibrated in both compartments, then macrofauna was introduced into A. Thus, ammonia excreted by the macrofauna accumulated in the water column (5 ± 0.1 l.) overlying the sediment. This procedure avoided the dilution of excreted ammonia in the whole volume (25 l) and more especially, its progressive loss when passing through the sediment as a result of adsorption and/or nitrification processes. In addition to the 4 microcosms with macrofauna, 2 or 4 other microcosms without added macrofauna (controls) indicated the NH_4^+ and NO_3^- exchanges between sediment and water column when water was not circulating.

Following the introduction of macrofauna (time: T_0) in some microcosms, the water column overlying the sediment of all microcosms was regularly sampled (2 x 4 ml samples) for NH_4^+ and NO_3^- ($\text{NO}_2^- + \text{NO}_3^-$) analysis. Before sampling, the water column was gently mixed with the sampling syringe to avoid ammonia stratification, even though the natural activity of the macrofauna (oyster filtration and crab movement) and the water depth did not favour it. After 4h (T_4), the water column overlying the sediment was submitted to a strong turbulence caused by a swing paddle (horizontal moving, $30 \text{ cm} \cdot \text{sec}^{-1}$ velocity) operated for 2 min. Turbulence was simulated individually in each microcosm and water was sampled immediately thereafter and again $1\frac{1}{2}$ h and 3h later. Samples of surface sediment (5 ml) were taken (horizontal cores between -5 mm and -15 mm depth) using 1 cm diameter tubes, immediately before and after turbulence; they were centrifuged to collect the sediment pore-water, which was immediately diluted and analysed as the water-column samples for NH_4^+ and NO_3^- content, using a Technicon auto-analyser (Le Corre & Treguer's method, 1976). The oxygen content of the water column was measured (O_2 electrode, OXY-550 WTW) immediately before and after turbulence, then again at the end of experiment. At the end of the experiment (T_7), macrofauna was removed and water circulation was re-established in the microcosms. These were left for 2 or 3 days in these conditions before the next experiment. Five or six experiments were performed over a 2-week period. Experiments were run in January ($t = 11^\circ\text{C}$, winter conditions) and Aug-Sept. ($t = 16\text{--}18^\circ\text{C}$, summer conditions) using sediment collected on the oyster-bed at the respective periods.

Macrofauna

Two species characteristic of the studied oyster-bed were selected: a filter-feeder, the oyster *Crassostrea gigas* and a scavenger, the crab *Carcinus maenas*. They were kept in individual aquaria and running sea-water for a week before being tested; crabs were fed daily, except for the 12h prior to experiment; oysters did not receive any food complement.

Macrofauna was introduced into the microcosms (2 crabs or 2 oysters/400 cm^2 sediment surface) for the experiment duration (7h), then removed and weighed. Each experiment was performed with a new batch of animals. The mean fresh weight of a whole crab was $31.4 \pm 2.12 \text{ g}$ ($n=10$) in winter and $24.7 \pm 0.80 \text{ g}$ ($n=10$) in summer; the mean fresh weight of an oyster (excluding shell) was $8.06 \pm 0.46 \text{ g}$ ($n=10$) and $15.14 \pm 2.76 \text{ g}$ ($n=10$), respectively.

Calculation of flux and excretion rate

Each experiment represented 4 distinct time periods: 1) the "stress period" (1h) from T_0 to T_1 , where the excretion rate of the animals was affected by being handled; 2) the period before turbulence (3h) from T_1 to T_4 (=BT); 3) the turbulent period (2 min.) from T_4 to T_4' (T); 4) the post-turbulence period (3h) from T_4' to T_7 (=PT).

NH_4^+ or NO_3^- fluxes were calculated from the changes in nutrient concentration (μM) of the water column overlying the sediment, that were observed during each of the 4 periods defined above. Diffusive flux calculation from sediment pore-water and water column concentrations was not made as the value would be influenced by the presence of macrofauna (Simon, 1988). NH_4^+ and NO_3^- fluxes were expressed as $\mu\text{mol.m}^{-2}.\text{h}^{-1}$ except for the turbulence period where they were expressed as $\mu\text{mol.m}^{-2}.\text{min}^{-1}$. A negative value represented a nutrient loss from the water column and, conversely, a positive value represented a nutrient gain.

Ammonia excreted by macrofauna during each of the 4 above periods was obtained by subtracting the change in ammonia amount in the water column of control microcosms (mean value of 4 microcosms) from the change in ammonia amount observed during this time in the water column of the microcosms containing either crabs or oysters. Ammonia excretion rate was expressed as $\mu\text{mol NH}_3.\text{g}^{-1} \text{ fresh weight}.\text{h}^{-1}$.

Turbulence

In order to evaluate the turbulence intensity in our microcosms, the kinetics of sediment resuspension and redeposition was determined in the course of a separate experiment without macrofauna. Water samples (100 ml) were collected, by siphoning, at 1 cm above the water-sediment interface just before and after turbulence, and then at regular time intervals during 8 hours. The samples were filtered through preweighed Whatman GF/C glass-fibre filters, which were dried in an oven (60°C) to a constant dry weight.

Before turbulence the suspended particulate matter (SPM) was $0.22 \pm 0.008 \text{ g dry W}^{-1}$. Immediately after turbulence, it was considerably greater ($5.19 \pm 0.7 \text{ g.l}^{-1}$); this steep increase was in agreement with that reported by De Jonge and Van den Bergs (1987) under similar conditions. In the present case, redeposition was observed to be completed in less than 1h (Fig. 1). According to Plummer *et al* (1987), the SPM loading of the water column before turbulence corresponded to the permanently suspended particles (PSP) when two particle populations (PSP + resuspended sediment, RSS) were present after turbulence.

RESULTS

NH_4^+ and NO_3^- content of the water column and flux rate

Changes in the NH_4^+ and NO_3^- content of the water column overlying the sediment during the 7h-experiments run in winter conditions (mean value of 5 runs from Jan. 24 to Feb. 2) and in summer conditions (mean value of 5 runs from Aug. 28 to Sept. 12) are shown in Fig. 2.

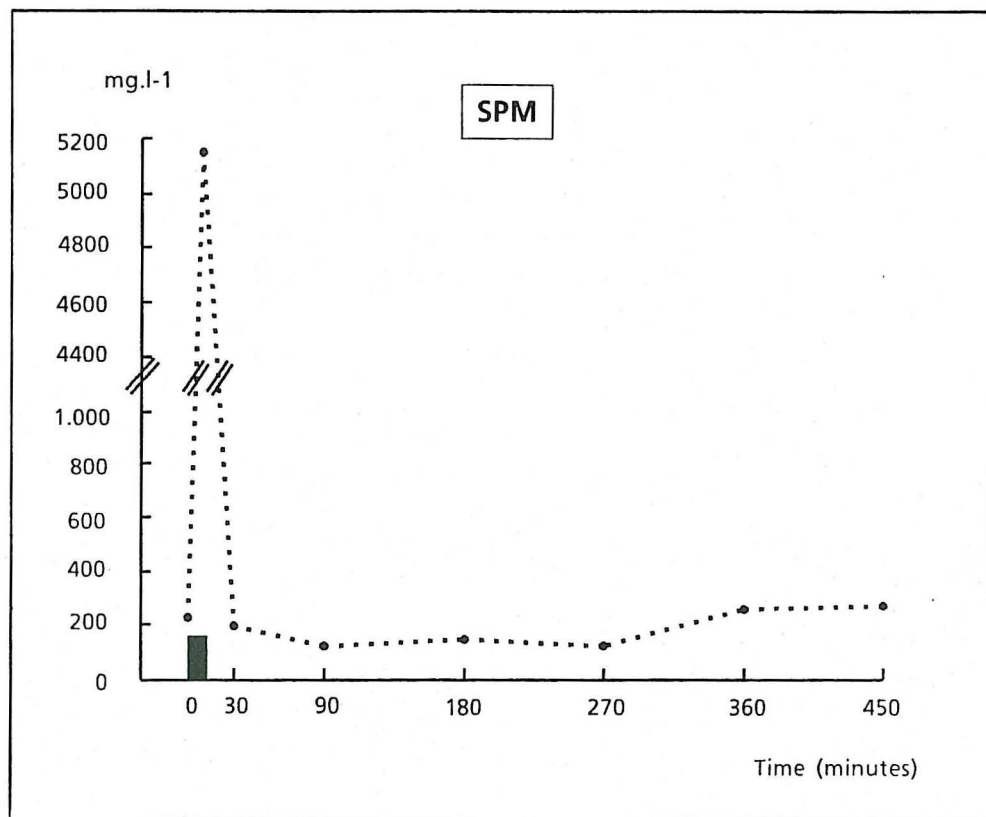


Fig. 1 - Effect of turbulence on the suspended particulate matter (SPM) (mg dry weight.l⁻¹) of the water column overlying the sediment ; Turbulence (2 min) is indicated by the black solid bar.

In the calm period before turbulence, slight and opposite changes in nutrient content (NH_4^+ increase, NO_3^- decrease) were observed in the water column of control microcosms (S). Low NH_4^+ flux rates, $6.07 \pm 3.52 \mu\text{mol.m}^{-2}.\text{h}^{-1}$ ($n = 17$) and $7.48 \pm 2.02 \mu\text{mol}$ ($n = 18$), were observed in winter and summer respectively; the NH_4^+ flux always was from sediment into the water column (Tables 1-2). The initial NO_3^- content of the water column was high (40 and 52 μM in winter and summer respectively) and the NO_3^- exchanges at the water-sediment interface ($-70 \pm 59 \mu\text{mol.m}^{-2}.\text{h}^{-1}$ in winter and $-140 \pm 152 \mu\text{mol}$ in summer) were greater than the NH_4^+ exchanges; however, the direction of the NO_3^- flux was extremely variable from one to another S microcosm. In contrast, marked changes in nutrient content occurred before turbulence in the water column of microcosms with crabs (C) and microcosms with oysters (H). The NH_4^+ content rapidly increased during the first hour as a result of the handling stress effect on the ammonia excretion rate of macrofauna (see below). In the BT period,

a regular and slower increase of the NH_4^+ content of the water column was observed in these microcosms. NH_4^+ flux rates of $88.90 \pm 23.02 \mu\text{mol.m}^{-2}.\text{h}^{-1}$ ($n = 9$) in C and $71.21 \pm 12.02 \mu\text{mol}$ ($n = 10$) in H, thus of 1 order of magnitude greater than the NH_4^+ flux rate in S, were observed in winter conditions. Even higher NH_4^+ flux rates ($376 \pm 96 \mu\text{mol.m}^{-2}.\text{h}^{-1}$ in C and $101 \pm 30 \mu\text{mol}$ in H) were obtained in summer. The NO_3^- content of the water column was much less influenced by macrofauna than the NH_4^+ content during the preturbulence period. Changes in NO_3^- content were mainly observed in the course of each set (2 weeks) of experiments. At T_0 of the first experiment, the NO_3^- content of the water column in H and C ranged from 40 to 44 μM in winter and from 55 to 58 μM in summer; these values were not significantly different from the value respectively observed in S. However, an increase of the NO_3^- content with time was noted in all microcosms, especially in C, as shown in Fig. 3 (summer period) and in Figs 2B and 2D by the mean T_0 value. This likely resulted from the excreted ammonia recycling between 2 successive experiments when water circulation was re-established. This nevertheless did not explain the NO_3^- increase with time observed in the microcosms without macrofauna (Fig. 3). During the BT period, the NO_3^- content of the water column slightly increased in C and H, whereas it decreased in S. A mean NO_3^- flux rate of $24 \pm 21 \mu\text{mol.m}^{-2}.\text{h}^{-1}$ was obtained in winter, whereas a NO_3^- flux extremely variable in direction and intensity was observed in summer (Table 2).

The effect of the experimental turbulence was a significant increase in inorganic nitrogen content of the water column (Tables 1-2). In control microcosms (S), the NH_4^+ content increased from 0.90 μM to 1.57 μM in winter and from 1.20 μM to 1.93 μM in summer. Mean NH_4^+ fluxes ($80 \mu\text{mol.m}^{-2}.\text{min}^{-1}$) from sediment into the water column were observed at both seasons. The NO_3^- content increased very slightly in winter but noticeably in summer. The total inorganic nitrogen input into the water column as a result of turbulence corresponded to a flux rate of 130 $\mu\text{mol N.m}^{-2}.\text{min}^{-1}$ in winter and 845 $\mu\text{mol.m}^{-2}$ in summer when nitrate contributed by 90 % to the total flux. In presence of crabs and oysters, NH_4^+ fluxes twice as high as those observed in absence of macrofauna were obtained during turbulence in winter conditions; at the same time, the mean NO_3^- flux was $445 \pm 195 \mu\text{mol.m}^{-2}.\text{min}^{-1}$. In winter, the turbulence effect was a total inorganic nitrogen flux of 620 $\mu\text{mol N.m}^{-2}.\text{min}^{-1}$, thus a flux around 5 times greater than in absence of macrofauna. In summer conditions, the inorganic nitrogen exchanges in C and H during turbulence were, as in S, mainly depending on the NO_3^- exchanges. As an opposite mean NO_3^- flux was observed in presence of crabs and oysters, the turbulence effect was a nitrogen enrichment of the water column in C microcosms and a nitrogen loss in H microcosms. Another general effect of turbulence was a decrease of the $\text{NO}_3^- : \text{NH}_4^+$ ratio in the water column of all microcosms, except for C in summer.

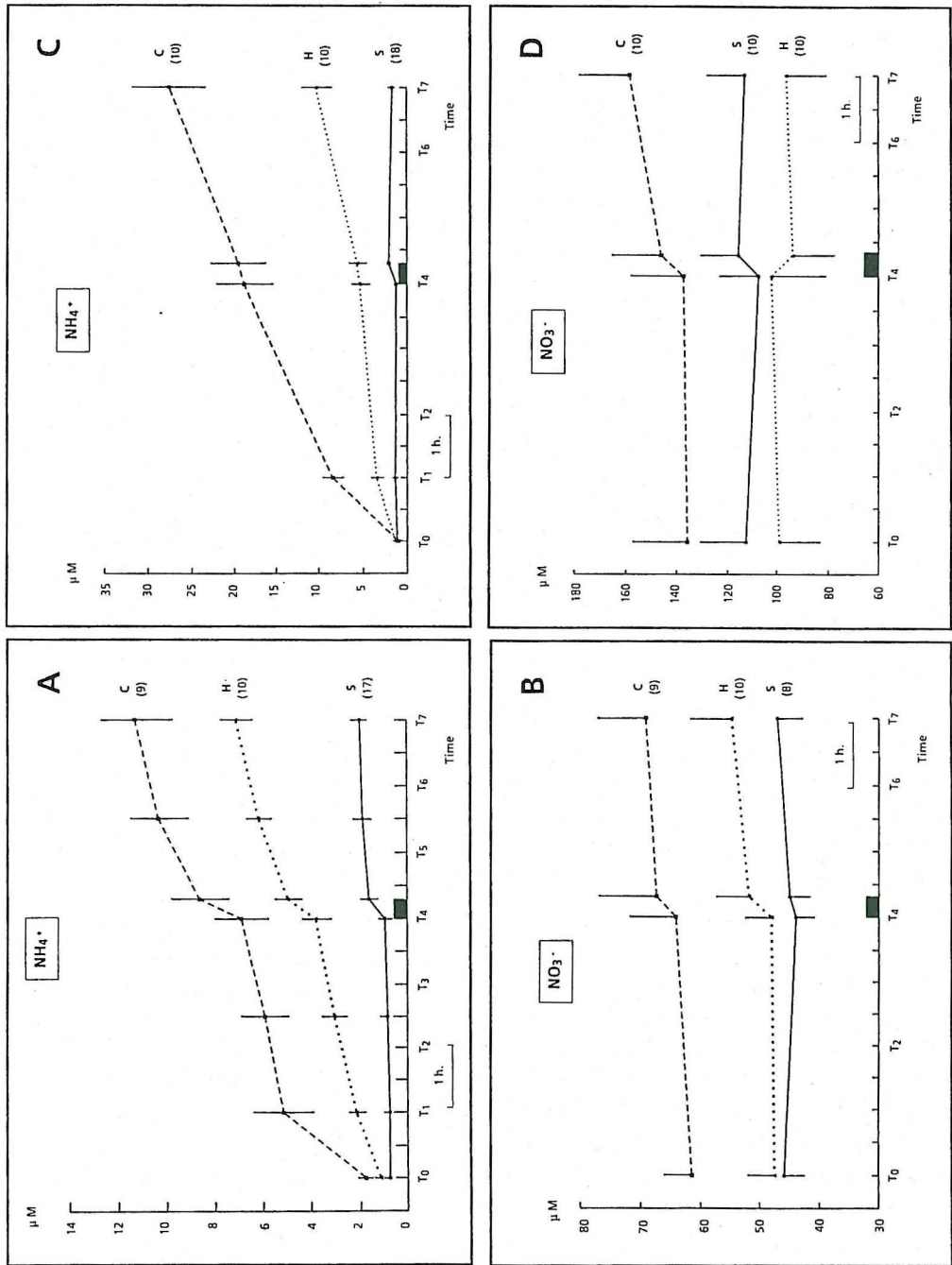


Fig. 2 - NH_4^+ and NO_3^- concentration in the water column of microcosms during experiments run under winter conditions (A and B) and summer conditions (C and D). Mean value ($\bar{X} \pm \sigma/\sqrt{n}$) of 5 experiments; n = number of microcosms (in brackets). S = microcosms without macrofauna = controls; C = microcosms with crabs; H = microcosms with oysters. Turbulence (2 min) is indicated by the black solid bar.

In the hours following turbulence (PT period), the inorganic nitrogen content of the water column of control microcosms showed opposite changes according to the experimental period (Tables 1-2). It increased in winter and the NH_4^+ and NO_3^- fluxes were significantly greater than those observed during the BT period. In contrast, the NH_4^+ and NO_3^- content decreased in summer and a negative flux, a great as in the BT period for NH_4^+ and half as great as in BT for NO_3^- , was observed. In any cases, fluxes of same order of magnitude were obtained before and after turbulence. A slight residual effect of turbulence (increased release of NH_4^+ and NO_3^- into the water column during PT) could be noted in winter but not in summer. In presence of macrofauna, the NH_4^+ fluxes observed during PT did not significantly differ from those observed during BT, except in summer in presence of oysters. The NO_3^- flux in C and H always was greater during the PT period than during BT and this supported the hypothesis of an enhanced nitrification in the hours following turbulence.

Effect of turbulence on the NH_4^+ and NO_3^- content of pore-water and on the O_2 content of water column.

Pore-water in the sub-surface sediment (horizontal cores between -5 mm and -15 mm depth) was analysed for its NH_4^+ and NO_3^- content, immediately before and after the turbulence. From data obtained in winter conditions (Table 1), the NH_4^+ concentration was around 8 times higher in the sediment pore-water than in the water column of control microcosms before turbulence; the mean NH_4^+ flux observed at this time was along the concentration gradient. The mean NO_3^- concentration was also higher (around 4 times) in the sediment pore-water than in the water column, but the mean NO_3^- flux, which was calculated from the changes observed in the water column content, was against the NO_3^- concentration gradient. A significant decrease of pore-water NH_4^+ content but no significant change in its NO_3^- content were observed as a result of the turbulence. On the other hand, the inorganic N content of the sediment pore-water was not significantly influenced by the presence of crabs or oysters.

The oxygen content of the water column was recorded at T_4 , T_4 and T_7 (Tables 1-2). Before the experimental turbulence, the water column was fully oxygenated in control microcosms (93 % and 97 % saturation in winter and summer respectively), whereas an oxygen depletion due to animal respiration was noted in microcosms with macrofauna; O_2 saturation of the water column was 70 % and 66 % in winter and summer respectively in presence of crabs, 86 % and 81 % in presence of oysters. In no case, the turbulence had a significant effect on the O_2 content of the water column. This indicated that the upper layer of sediment was as oxygenated as the water column.

TABLE 1

Characteristics of microcosms in winter conditions (11°C). (C) : microcosms with *Carcinus maenas* (n = 9); (H) : microcosms with *Crassostrea gigas* (n = 10). Two crabs or two oysters per microcosm ; T4, T4' and T7 : sampling time ; BT : before turbulence period ; T : turbulence period ; PT : post-turbulence period.

winter conditions	Control microcosms (S)			Microcosms with macrofauna (C) & (H)		
	T4	T4'	T7	T4	T4'	T7
water column						
NH ₄ ⁺ (μM)	0.90 ± 0.27 (n = 17)	1.57 ± 0.31	2.00 ± 0.34	(C) 6.95 ± 1.15 (H) 3.77 ± 0.60	8.67 ± 1.17 4.97 ± 0.58	11.30 ± 1.49 7.16 ± 0.67
NO ₃ ⁻ (μM)	44.0 ± 3.2 (n = 8)	44.94 ± 3.33	47.08 ± 4.20	(C) 64.21 ± 7.73 (H) 48.02 ± 3.16	67.57 ± 9.62 51.90 ± 5.04	69.26 ± 8.10 54.96 ± 7.04
O ₂ (mg.l ⁻¹)	8.29 ± 0.15 (n = 10)	8.15 ± 0.05	7.98 ± 0.06	(C) 6.18 ± 0.17 (H) 7.71 ± 0.14	6.13 ± 0.13 7.56 ± 0.13	4.41 ± 0.34 7.06 ± 0.11
pore water						
NH ₄ ⁺ (μM)	7.44 ± 1.50 (n = 15)	4.98 ± 1.02	-	9.68 ± 1.53 (n = 18)	6.28 ± 1.1	
NO ₃ ⁻ (μM)	237.50 ± 6.30 (n = 10)	237.6 ± 5.3	-	243.7 ± 5.9 (n = 10)	246.6 ± 7.4	
	BT (3 h)	T	PT (3 h)	BT (3 h)	T	PT (3 h)
fluxes (μmol.m ⁻² .h ⁻¹)						
	h ⁻¹	2 min ⁻¹	h ⁻¹	h ⁻¹	2 min ⁻¹	h ⁻¹
NH ₄ ⁺	6.07 ± 3.52 (n = 17)	79.9 ± 12.0	16.5 ± 3.6	(C) 89 ± 23 (H) 71 ± 12	196 ± 24 150 ± 38	95 ± 31 84 ± 11
NO ₃ ⁻	-70 ± 59 (n = 8)	51 ± 52	75 ± 50	24 ± 21 (n = 15)	445 ± 195	92 ± 62

TABLE 2

Characteristics of microcosms in summer conditions (16-18°C).

(C) : microcosms with *Carcinus maenas* (n = 10) ; (H) : microcosms with *Crassostrea gigas* (n = 10) ; same legends as Table 1.

summer conditions	Control microcosms (S)			Microcosms with macrofauna (C) & (H)		
	T4	T4'	T7	T4	T4'	T7
water column						
NH ₄ ⁺ (μM)	1.20 ± 0.19 (n = 18)	1.93 ± 0.18	1.69 ± 0.21	(C) 18.8 ± 3.3 (H) 5.3 ± 1.1	19.5 ± 3.2 5.6 ± 1.0	27.6 ± 4.2 10.4 ± 1.7
NO ₃ ⁻ (μM)	107.0 ± 15.0 (n = 10)	115.0 ± 15.2	113.0 ± 15.5	(C) 137.4 ± 20.7 (H) 102.2 ± 20.8	146.3 ± 19.5 93.5 ± 15.9	158.9 ± 19.8 96.1 ± 15.3
O ₂ (mg.l ⁻¹)	7.62 ± 0.04 (n = 16)	7.40 ± 0.05	7.32 ± 0.05	(C) 5.20 ± 0.24 (H) 6.40 ± 0.23	5.19 ± 0.22 6.38 ± 0.20	3.39 ± 0.29 4.92 ± 0.31
pore water						
NH ₄ ⁺ (μM)	-	-	-	-	-	-
NO ₃ ⁻ (μM)	197.6 ± 10.7 (n = 8)	188.4 ± 12.7	-	-	-	-
	BT (3 h)	T	PT (3 h)	BT (3 h)	T	PT (3 h)
fluxes (μmol.m ⁻² .h ⁻¹)	h ⁻¹	2 min ⁻¹	h ⁻¹	h ⁻¹	2 min ⁻¹	h ⁻¹
NH ₄ ⁺	7.48 ± 2.02 (n = 18)	79.2 ± 10.3	- 8.4 ± 3.4	(C) 376 ± 96 (H) 101 ± 30	83 ± 45 42 ± 15	257 ± 41 165 ± 31
NO ₃ ⁻	- 140 ± 152 (n = 10)	765 ± 409	- 71 ± 102	(C) 32 ± 161 (H) 72 ± 286	850 ± 408 - 715 ± 945	156 ± 149 140 ± 165

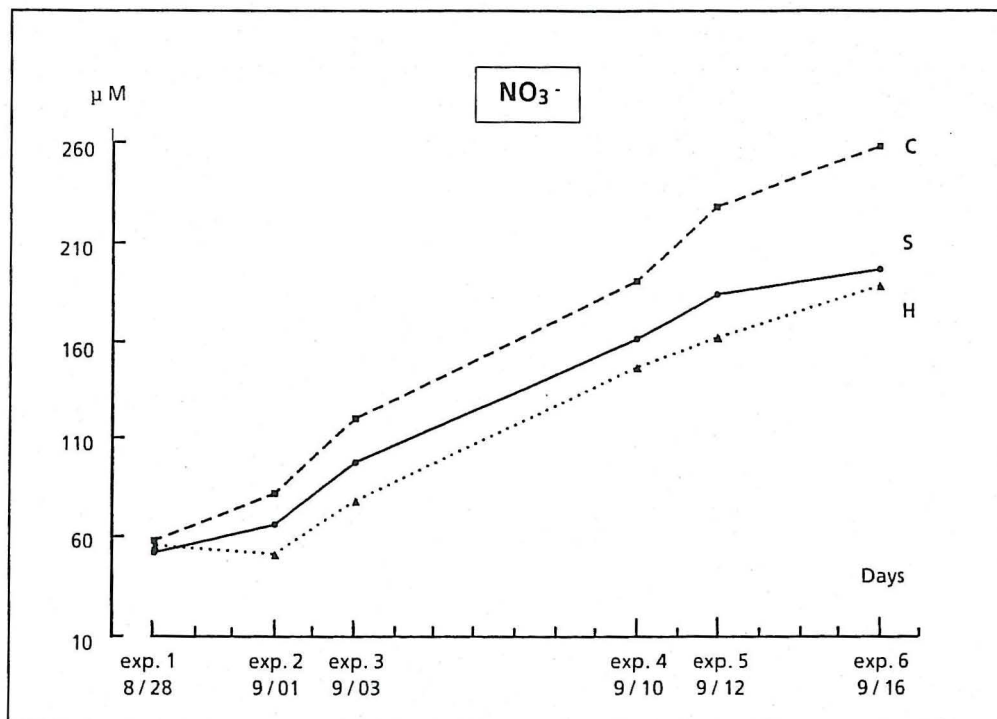


Fig. 3 - Changes in the water column NO_3^- content as a function of time during the all experimental summer period. Mean T_0 value observed in microcosms at the beginning of experiments run from Aug. 28 to Sept. 16.

S = microcosms without macrofauna; C = microcosms with crabs; H = microcosms with oysters.

Macrofauna excretion rate

During the preturbulence period (from T_1 to T_4), the mean ammonia excretion rate of crabs was $0.050 \pm 0.020 \mu\text{mol NH}_3 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ in winter and $0.276 \pm 0.075 \mu\text{mol NH}_3 \cdot \text{g}^{-1}$ fresh meat $\cdot \text{h}^{-1}$ in summer and the mean excretion rate of oysters was $0.132 \pm 0.026 \mu\text{mol NH}_3 \cdot \text{g}^{-1}$ fresh meat $\cdot \text{h}^{-1}$ and $0.114 \pm 0.037 \mu\text{mol}$ respectively (see Methods for calculation). Higher excretion rates were observed in the first hour (from T_0 to T_1) as a result of the handling stress imposed to animals (Table 3); this was more pronounced for crabs than for oysters. During turbulence, a steep increase of the excretion rates of both species was observed in winter. These rates, even though they could be observed for 2 minutes, appeared very high. They were nevertheless, as far as crabs were concerned, still lower than those previously measured in "excited" and fed *Carcinus maenas* ($3.69 \pm 0.34 \mu\text{mol NH}_3 \cdot \text{g}^{-1}$ wet W $\cdot \text{h}^{-1}$, Regnault & Aldrich, 1988). In summer, turbulence had an opposite effect as the excretion of crabs was noticeably reduced and no excretion could be detected in oysters during this period. The excretion rates observed in the 3 hours following

turbulence show that both species recovered from the turbulence effect more rapidly in winter than in summer. In the last case, the crabs had an excretion rate which was less depleted than during turbulence but still low as compared to their rate in BT, whereas the oysters had a high excretion rate which was similar to that observed as a result of the handling stress.

In the experimental conditions, the ammonia excretion of crabs and oysters contributed by 90 %-92 % in the BT period and by 80 %-82 % in the PT period to the NH_4^+ flux respectively observed in these microcosms in winter. This contribution was higher, especially during the PT period, in summer. During turbulence, macrofauna excretion only contributed by 60 % (crabs) and 45 % (oysters) to the NH_4^+ flux observed at this time in winter when both species presented the highest excretion rates. In contrast, the contribution of macrofauna to the NH_4^+ flux observed during turbulence in summer was negligible.

TABLE 3

Ammonia excretion rates ($\mu\text{ mol NH}_3\cdot\text{g}^{-1}$, wet $\text{W}\cdot\text{h}^{-1}$) of *Carcinus maenas* and *Crassostrea gigas* in winter and summer, according to the experimental conditions.

EXPERIMENTAL PERIODS	WINTER CONTITIONS		SUMMER CONDITIONS	
	<i>Carcinus maenas</i>	<i>Crassostrea gigas</i>	<i>Carcinus maenas</i>	<i>Crassostrea gigas</i>
from T_0 to T_1 (handling stress)	0.231 ± 0.108	0.198 ± 0.059	0.633 ± 0.125	0.278 ± 0.079
from T_1 to T_4 (pre-turbulence)	0.050 ± 0.020	0.132 ± 0.026	0.276 ± 0.075	0.114 ± 0.037
from T_4 to T_4' (turbulence)	2.05 ± 0.45	4.87 ± 2.74	0.091 ± 0.052	0
from T_4' to T_7 (post-turbulence)	0.046 ± 0.020	0.156 ± 0.027	0.195 ± 0.033	0.202 ± 0.038

DISCUSSION

The present study shows that the epibenthic macrofauna has a permanent and noticeable effect on the NH_4^+ content of the water column and on the NH_4^+ exchanges between sediment and overlying water, as these were increased by one or two orders of magnitude. At the same density ($50\text{ animals}\cdot\text{m}^{-2}$), crabs (*Carcinus maenas*) were observed to have a stronger effect than oysters (*Crassostrea gigas*), especially under stress and summer conditions, according to their metabolic rates (Table 3). Such a response of the ammonia excretion rate to these conditions is well known in Crustacea (Regnault, 1986 ; 1987 ; Regnault & Aldrich, 1988). In the

experimental conditions, the ammonia excretion of each of the 2 species always contributed by more than 80 % to the net NH_4^+ flux observed in the nonturbulent periods. At the same time, the excretion rates nevertheless appeared low in comparison with the rates previously observed in *C. maenas* (Regnault & Aldrich, 1988) and with those estimated in *C. gigas* from *in situ* measurements (Boucher & Boucher-Rodoni, 1988); higher rates were also reported by Nixon *et al.* (1976) for *Callinectes sapidus* and *Modiolus demissus* in a salt-marsh. Since an enhanced nitrification was observed in the microcosms with macrofauna (see below), this would suggest that ammonia excretion of macrofauna was underestimated and consequently, an even greater ammonia input resulting from macrofauna excretion could be expected in *in situ* conditions. On the other hand, the episodic presence of unfed oysters in microcosms did not favour the production of feces and pseudofeces, which are a noticeable source of ammonification in an oyster-bed. Therefore, the net NH_4^+ flux measured in H microcosms might also be underestimated. A NH_4^+ flux similar to that observed in H in winter, but twice as great in summer, was measured *in situ* in presence of an equivalent oyster biomass (80 g dry W.m^{-2}). In contrast, much greater NH_4^+ fluxes (from 4 to 5.5 $\text{mmol.m}^{-2}.\text{h}^{-1}$) were estimated for a mussel-bed (Nixon *et al.*, 1976) and were measured on an undisturbed oyster reef having a biomass of 200 g dry W.m^{-2} (Dame *et al.*, 1985). These high values probably resulted from the environment conditions (balance between ammonification and nitrification/denitrification processes) rather than from the biomass or the production of feces. The actual contribution of crabs and oysters to the net NH_4^+ flux was in fact dependent on the conditions prevailing *in situ*.

In the microcosms, nitrification was important as shown by the high initial NO_3^- content (40-55 μM) of the water column and by its regular increase with time (Fig. 3). *In situ*, the highest NO_3^- content (22 μM) of the water column was observed in winter (Boucher & Boucher-Rodoni, 1988). According to Mevel and Chamroux (1981), this type of experimental marine closed system, in which a high oxygen level was maintained, strongly favoured the nitrification process. In the present study, the water column and the surface sediment of microcosms were fully oxygenated (Tables 1-2). Such a situation, which was presently due to the experimental enclosures, could nevertheless be observed in shallow estuaries when favourable O_2 and NH_4^+ conditions are encountered (Helder & De Vries, 1986). This nitrification process was enhanced in the C microcosms in which crabs were periodically introduced; it was also mainly observed between the experiments when water was circulating and macrofauna were absent. In contrast, only slight changes in NO_3^- content and NO_3^- flux could be attributed to macrofauna when it was present, at least during the preturbulence period. It is usually observed that epibenthic macrofauna is associated with ammonia regeneration in the water column of coastal ecosystems but has no discernible effect on other nutrients (Vetter & Hopkinson, 1985; Regnault, 1986), whereas benthic infauna, through the microbial activity which is associated with its specific microenvironment, stimulates either

mineralisation or nitrification/denitrification, according to the organic matter and oxygen availability (Blackburn & Henriksen, 1983 ; Henriksen *et al.*, 1983 ; Koike & Mukai, 1983 ; Kristensen, 1985 ; Enoksson & Samuelsson, 1987).

The specific effect of a brief disturbance of the water column caused by mechanical stirring could be observed in absence of macrofauna. It markedly increased the ammonium content (a 75 % and 60 % increase in winter and summer, respectively) of the water column, but not its nitrate content. At the same time, the pore-water NH_4^+ content was reduced to 2/3 of its initial value. A net NH_4^+ flux of the same intensity ($80 \mu\text{mol. m}^{-2}.\text{h}^{-1}$) was observed in both experimental periods (winter and summer) ; this flux was approximately 40 times greater in winter and only 4 times greater in summer than the NH_4^+ fluxes respectively observed as a result of macrofauna excretion. The sudden input of ammonia in the water column might be explained by resuspension of surface sediment and the consequent release of pore-water which was richer than the water column. This would presuppose similar changes in nitrate content of both pore-water and water column, especially as the NO_3^- concentration gradient was high. In fact, nitrate was apparently little influenced by turbulence in winter and the strong NO_3^- release into the water column in summer could not be solely justified by the NO_3^- loss of the pore-water. However, a noticeable increase of the NH_4^+ flux without an accompanying change in the NO_3^- flux was also recorded *in situ* in spring-tide at the water-sediment interface of the present oyster-bed (Boucher & Boucher-Rodoni, 1985). According to these authors, increase of the NH_4^+ flux in comparison to that observed in neap-tide resulted from a stronger tidal current and resuspension of the bottom sediment.

The most remarkable observation was the short duration of the turbulence effect. The suspended particle matter redeposited in less than 1h. and the NH_4^+ and NO_3^- flux rates were, in the 3h. following turbulence, very similar to the rates observed before turbulence ; a slight residual effect on these could only be noted in winter. As nutrient fluxes rapidly decreased as soon as the turbulence was stopped, it might be suggested that NH_4^+ and NO_3^- were adsorbed on the suspended particles and redeposited with them. Our results show that this hypothesis could be envisaged in summer but not in winter. A sporadic release of large amounts of nutrients into the overlying water column has been reported as a result of either a natural wind-driven turbulence (Davies, 1975 ; Ward, 1985) or a simulated storm in mesocosms (Lasserre *et al.*, in prep.) ; in each case, it was explained by resuspension of bottom sediment. In contrast, Oviatt *et al.* (1981), by simulating a storm event in mesocosms (MERL), argued that increase of the NH_4^+ content of the overlying water column was a result of either enhanced benthic mineralisation or reduction of primary productivity by turbidity rather than a result of sediment resuspension. In the present study, such biological processes could not have been important, due to the short duration (2 min.) of turbulence ; changes observed in nutrient fluxes were thus very likely caused by a physical process.

One purpose of this study was to examine any possible interaction between bioturbation as understood in this study and turbulence. In all cases, the ammonia excretion rate of crabs and oysters was influenced by turbulence. Hopkinson *et al.* (1985) showed that mixing of shallow-water masses during a thunderstorm caused a marked oxygenation of bottom water that stimulated aerobic respiration and benthic metabolism. However, various responses according to the season and species were observed in the microcosms. In winter conditions, the initial low excretion rate of both species was considerably enhanced during turbulence and its increase was more important than that resulting from the handling stress (Table. 3). In summer conditions, both species presented a high excretion rate before turbulence; this rate was reduced to a third in *Carcinus maenas* and to a negligible value in *Crassostrea gigas* during turbulence. Consequently, excretion rate of *C. gigas* was higher after than before turbulence, as the oysters had to eliminate the ammonia that built-up while their shell was closed. The impact of bioturbation was thus modified not only during turbulence but also, for some species, in the hours following it. Although the effect of turbulence was much stronger than the effect of bioturbation, it must be kept in mind that the turbulence has a sporadic and short-term effect whereas bioturbation has a permanent and long-term effect.

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