Measurement of benthic nutrient fluxes in Mediterranean shellfish farms : a methodological approach

Benthic heterogeneity
Nutrient fluxes
Enclosure methods

Hétérogénéité benthique
Flux de nutriments
Méthode des chambres benthiques

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ABSTRACT

Benthic chambers were used to investigate fluctuations in nutrient fluxes and oxygen changes when comparing two benthic biota. Muddy sediments located under mussel-cultivation ropes were compared with a station outside the mussels' influence. Biodeposition from the mussels results in a drastic increase in the nutrient fluxes. A standard procedure is established in order to give reliable comparisons between stations and seasons: as a rule it should include six-hour periods of incubation with sampling intervals of two hours and at least four benthic chambers - preferably six - at each station.

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RÉSUMÉ

Mesures de flux de nutriments à l'interface eau-sédiment dans une zone conchylicole méditerranéenne : une approche méthodologique

Des chambres benthiques sont utilisées pour étudier les fluctuations des flux de nutriments et d'oxygène à l'interface eau-sédiment de différents biotopes benthiques. Des sédiments vaseux situés sous des tables à moules, sont comparés à des stations situées hors de l'influence des cultures de moules. La biodéposition due aux moules provoque, en général, un accroissement des flux de nutriments, des sédiments vers l'eau. Un protocole standard peut être établi, afin de permettre des comparaisons valables entre stations et saisons. Notre étude nous conduit à préconiser des périodes d'incubation de six heures, avec des intervalles de mesures de deux heures et l'utilisation d'au moins quatre chambres benthiques -six de préférence- à chaque station.

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INTRODUCTION

The role of benthic nutrient fluxes in water chemistry has been the subject of much speculation and, more recently, of an impressive number of experimental works (Boynton *et al.*, 1980; Zeitschel, 1980; Nowicki and Nixon, 1985; Keizer *et al.*, 1989).

In shallow waters, such as coastal waters, sediment-water exchanges strongly influence nutrient chemistry and, consequently, productivity of the overlying water column (Nixon, 1981; Hopkinson, 1987). The overlying water in sediment cores has been used to describe chemical processes by means of direct analysis of samples or the use of sensors (Henriksen, 1980; Enoksson and Rudenberg, 1983). Such measurements in the laboratory are not reliable because of disturbances induced by confinement and transport of cores. Detailed processes may also be studied on pore waters extracted from cores but this method severely affects living organisms.

In order to avoid such phenomena, benthic chambers in field experiments have often been used. Since the early experiments by Bervald (1939) who simply turned an aquarium upside down to follow oxygen changes in the

sediment-water boundary layer, many authors have applied the bell-jar methods with *in situ* chambers pressed into the sediment. An extensive review of the use of such chambers (agitation, black or light conditions...) is given by Pamatmat (1977). Water can be allowed to circulate (Pamatmat, 1965) or not (Odum, 1957). The number of chambers monitored for one experiment rarely exceeds two. Their volume, as well as the duration of experiments, may vary considerably according to the purposes of the studies (*see* review by Hall, 1984; Nowicki and Nixon, 1985; Charpy-Roubaud, 1988). In the specific fields of shellfish farms, some studies of respiratory metabolism, and nitrogen exchanges at the sediment boundary layer have been conducted *in situ* by Boucher and Boucher-Rodoni (1988). These authors used bell jars, 50 cm diameter, covering 0.2 m^2. Jars with and without oysters were compared and another was isolated from the bottom with a dark base. Water in the jar was recirculated continuously for four hours. A one litre-sample was collected every hour by means of a syringe used by a scuba diver. We wanted to investigate nutrient fluxes in rope-cultivation mussel farms, in which large quantities of organic matter were removed from the surrounding waters, leading to high sedimentation rates (Dahlbäck and Gunnarsson, 1981; Rosenberg and Loo, 1983; Rodhouse and Roden, 1987; Kaspar *et al.*, 1985). In order to assess the importance and role of this biodeposition in sediment biochemistry, we used a device similar to that of Boucher and Boucher-Rodoni. As pointed out by Nixon *et al.* (1980), fluxes measured at stations within a few metres of each other on the bottom exhibit large variance. Heterogeneity of spatial distribution of macro- and microorganisms on and in the sediment is a well-known feature. It has been investigated by Plante *et al.* (1986) for chlorophyll pigments on sediments similar to those studied here. When giving estimates of the chlorophyll content in a given station, five to seven "blocks" (each of which approximatively corresponds to a bell jar size) are required to achieve a precision level of the mean of ± 10 % at a significance level (α) of 5 % (Reys *et al.*, 1987).

Preliminary studies for working out a suitable strategy for such measurements are reported here. They deal with incubation duration related to confinement effects, and the number of bell jars to be used to obtain significant results.

MATERIALS AND METHODS

Study area

The bay of Carteau is located in the Gulf of Fos (West Mediterranean Sea, southern coast of France). When our experiment was started, the locality contained about 76 mussel tables, each carrying 1 000 to 1 500 mussel ropes (each of wich in turn was bearing about 50-70 kg wet weight). In order to study nutrient exchanges between sediments and water, two stations were selected: one under a recently settled table where sedimentation rate is high (UMT), the other in a part of the bay unaffected by mussel biodeposition (OMT).

Originally, at both stations, sediments might be classified as muddy sand (average water concentration: 33 % in the 0-1 cm layer with 25 to 50 % of grain size less than 63 µm). Installation of mussel tables induces changes in the top layer: average water concentration 50 % with 75 % of grain size less than 63 µm. Under these conditions (UMT) the redox break line is observed within only 2 mm from the surface of sediment, whereas in OMT, redox break line depth is more than 5 mm. Organic matter concentrations (Grenz, 1989), expressed as organic C in percent of dry matter, reach 1.89 ± 0.26 in UMT sediment and 0.73 ± 0.46 in OMT sediment only in the first 2 cm. In the 2-5 cm layer the concentrations remain similar in both stations, decreasing from 0.78 to 0.35 %.

Differences between sediments are then related to biodeposits as they only appear in the superficial layer. Both stations are 6 m deep. Light intensity at the bottom is about 10 - 20 % of surface layer intensity.

Polyacrylic bell jars

The bell jars used consist of polyacrylic hemispheres with a diameter of 39.5 cm covering 0.1225 m^2 (Fig. 1). Isolated water volume is about 17 l. Six jars were used in each station. Several hours before beginning the experiments, very often the day before, 6 PVC embasements per station were pushed randomly into the sediment. Sediment structure could then be restored during the night. On the following day, bell jars were clipped onto the embasements. A tight-fitting lid on top of the chamber included a magnetic stirring bar. A waterproof housing, with battery and stirrer, is located at the level of the stirring bar (Fig. 1). When this housing is set up, the stirring bar rotates (500 rpm ca.). CdNi batteries provide an autonomy of at least 24 h.

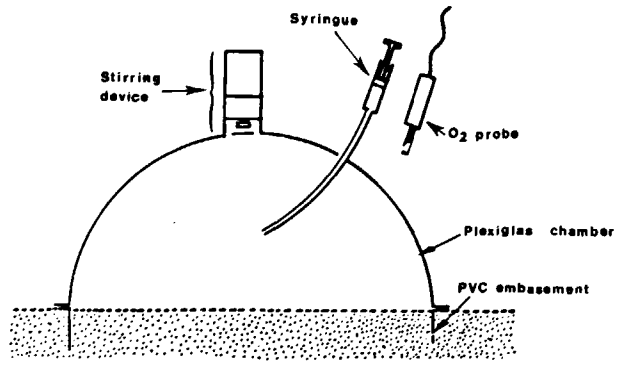

Figure 1

Measurement enclosure (17 l, Ø = 39.5 cm)
Enceintes de mesures de flux.

Preliminary tests with dyes have shown that this stirring was efficient in continuously homogeneizing the bell volume. Water sampling and oxygen measurements were made in the chamber, through a hole fitted with a soft rubber stopper. At fixed intervals, stoppers were removed for syringe sampling or the O_2 sensor set up.

As an exploratory study and in some experiments, simple techniques were used to test the role played by the various biological compartments in processes which take place at the sea - sediment interface. A base made of PVC isolates chamber water and enabled us to determine the importance and role of phenomena which are exclusively linked to the water column. Furthermore, chambers were darkened, by means of a black plastic cover, in order to estimate the intensity of processes when light is lacking. All experiments were monitored by divers.

Sampling and analysis

Temperature, O_2 content expressed as $mg.l^{-1}$, were measured at fixed hours (oxymeter Orbisphère - model 2609 - Sensor with stirrer n° 2112, cell n° 2120, with automatic correction for salinity and temperature, calibration in water saturated air). Salinity was estimated using an American Optical Refractometer.

Syringe samples (100 ml x 4) were immediately frozen after filtering on 0.45 µm filters, except ammonium samples which were treated according to Koroleff (1976). Water volume removed during sampling was replaced by allowing outside seawater to enter, in order to avoid contamination with sediment pore water.

Analyses for dissolved nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and silicate ($Si.(OH)_4^-$) were performed on a Technicon-Analyser II, using the method of Treguer and Le Corre (1975). All values are expressed in $\mu mol\ l^{-1}$.

Fluxes were estimated from the fluctuations in nutrient content against time. They are expressed as mg O_2 or $\mu mol\ m^{-2}h^{-1}$. The variation in nutrient contents outside the jars during the same period was estimated by sampling sea water at the same depth.

RESULTS

Evolution of nutrient fluxes

DETERMINATION OF INCUBATION DURATION

An experiment was performed on a long time scale (20 h) in September 1987, using 3 benthic chambers at the UMT station (- 6 m).Sampling and measurements (O_2) were made every four hours, from 5 to 1 pm, the next day (night lasts from 6 pm to 5.5 am). The aim of the survey was to understand the fluctuations in nutrient contents in the course of the experiment and possibly to detect confinement phenomena (asphyxiation).We decided to start at dawn in order to minimize the influence of oxygen production by microphytes on other parameters, as well as nutrient consumption by these sediment organisms.

Figure 2 shows results of the experiment for one bell jar. Some variations appeared between nutrient fluctuations in the three chambers, but the pattern of variations was quite comparable. Nutrient contents evolved in different ways: NH_4^+, $Si\ (OH)_4^-$, PO_4^{3-} content increased steadily; NO_2^- content was stable whereas NO_3^- decreased slightly. O_2 concentration decreased quickly and regularly during the night, but increased in the morning, as this parameter is obviously linked to photosynthesis. The evolution of parameters such as ammonia, silicates, phosphates, is obviously linear for the first 8 hours (NH_4^+

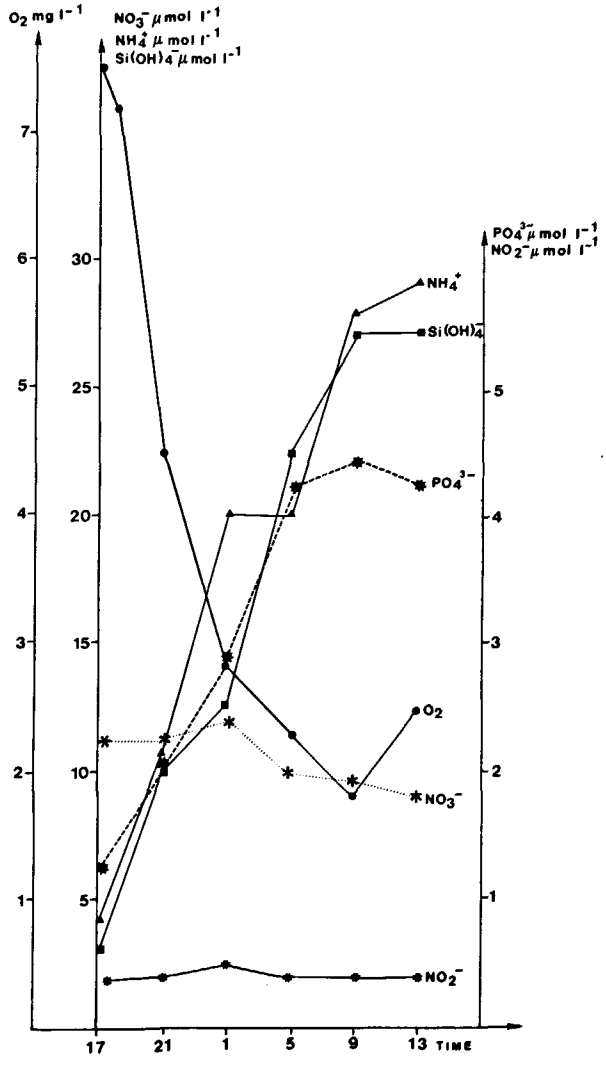

Figure 2

Changes in nutrient concentrations in a bell jar (20 hours duration). Évolution des concentrations en nutriments dans une cloche au cours d'une période de 20 heures.

release and O_2 consumption) or 12-16 h ($Si(OH)_4$; PO_4^{3-} release).

The slope of the curve is different in the last hours of the experiment. This might suggest the beginning of a confinement phenomenon. Therefore, in order to approach the natural conditions of the environment as closely as possible, incubation time had to be reduced.

CONSEQUENCES

If varying parameters (Fig. 3) like A (release or production) or B (uptake or consumption) are to be estimated, the linear part of the curve is only taken in account for flux calculation as instantaneous rates. In this case, incubation duration would be less than eight hours. We decided to choose an incubation time of six hours, which is in the linear part of the curve and included a fair number of samples (every two hours). This incubation time seems to be sufficient to give evidence of fluxes. It is short enough to avoid inflexion points on the curves, according to seasons, nature of sediments, and so on.

As we were primarily concerned with heterogeneity between individual estimates of overall fluxes, we decided to perform all experiments during day time as it is easy to approach night conditions using blackened bell jars. Fluxes were calculated directly from nutrient concentration versus time curves (least square regression), and expressed as mg or µmol $m^{-2}h^{-1}$.

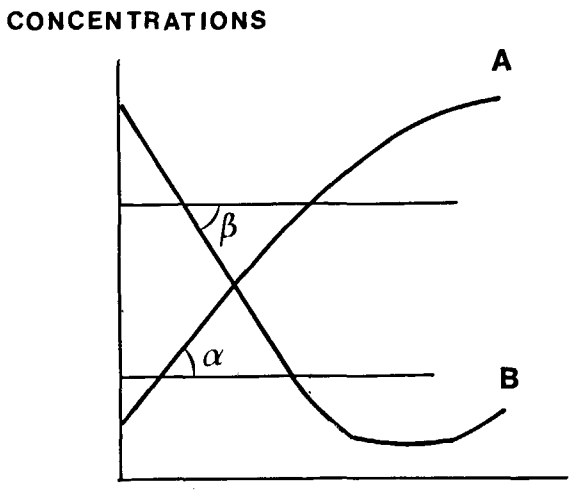

Figure 3

Diagram of benthic fluxes: A Release or production - B Uptake or consumption.
Schéma des flux benthiques : A : relargage ou production ; B : assimilation ou consommation.

Statistics and choice of the number of observations

Regarding flux estimations, our first experiments using two or three bell jars showed high variability in the results. It was then necessary to increase the number of observations to obtain reasonable confidence limits for our measurements. We made an attempt to reconcile the time required to obtain full observations for a sampling step of 2 hours and the number of bell jars used in the

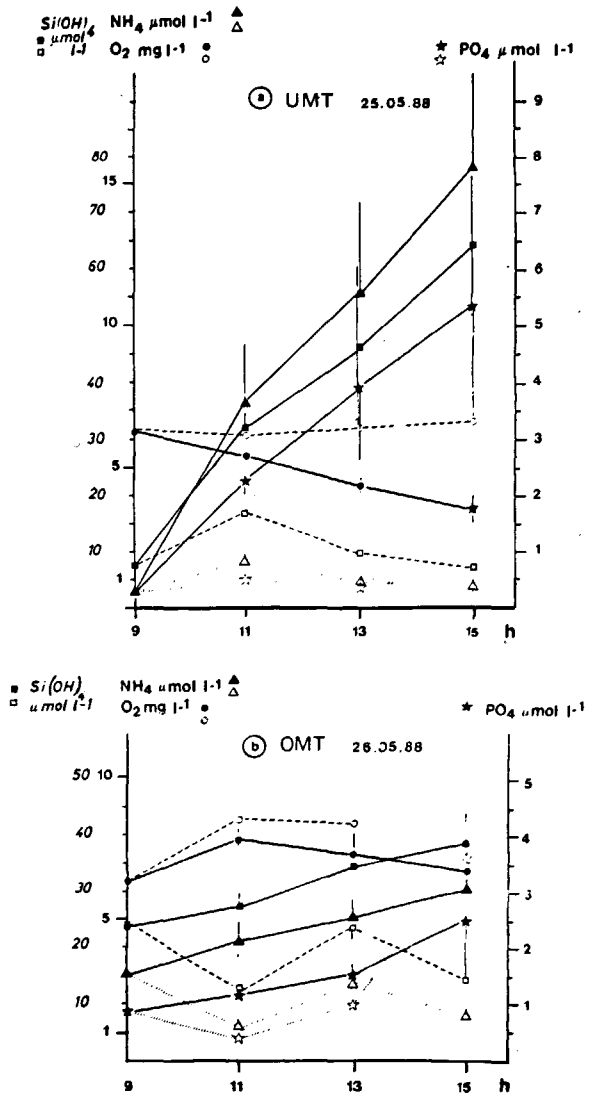

Figure 4

Changes in nutrient and oxygen concentrations: full symbols = mean value (±IC = interval of confidence) from 6 bell jars; open symbols = external water - a) UMT = Under Mussel Table station. b) OMT = Outside Mussel Table station.
Évolution des concentrations en nutriments et en oxygène ; symbole plein : valeur moyenne (±IC = intervalle de confiance) de six cloches ; symbole vide : eau extérieure - a) UMT : station sous les tables à moules ; b) OMT : station hors des tables à moules.

investigations. Experiments were consequently performed with 6 benthic chambers (experiments of May 1988).
Figure 4 shows that this strategy enabled us to obtain significant differences between the two stations (UMT and OMT), while nutrient concentrations in external water at both stations are quite stable. This indicates that differences between stations are actually due to the location of bell jars and not to climatic changes between experimental days.

The following statistical procedure, described in Dagnélie (1975), was used to test the difference between stations and lead us to choose the minimal number of replicates to be used in this case. In our procedure, we consider all the sampling units (*i.e.* concentrations)

measured in the 6 bell jars, corresponding to a total sample size of N = 24 (6 bell jars x 4 samples). In this way it is possible to improve precision by increasing the degrees of freedom, whereas N decreases to 6 only when considering every calculated flux as a sampling unit. Before testing the difference between stations, we have to test firstly the homogeneity of variances between each chamber, and secondly similitude between each slope (*i.e.* chambers) to calculate a mean slope.

HOMOGENEITY OF SLOPES

Before comparing the slopes (*i.e.* fluxes) under each separate situation and for each parameter, the homogeneity of variances calculated for each chamber is tested by a Hartley's test which consists in comparing the ratio between the highest and smallest variances (H_{obs}) with a theoretical value (H_{theo}).

For each parameter, H_{obs} in both stations was calculated as follows:

$$H_{obs} = \frac{SS_{y.x\,max}}{SS_{y.x\,min}}$$ where $SS_{y.x}$ = residual sum of squares

In our case H_{theo} is 62.0 when p=6 (bell-jars) and k = 3 degrees of freedom

	$NH4^+$		$PO4^{3-}$		Si (OH) 4	
	UMT	OMT	UMT	OMT	UMT	OMT
$SS_{y.x}$ max	5.29	0.21	1.98	1.89	152.68	49.11
$SS_{y.x}$ min	0.44	0.06	0.01	0.08	16.02	1.89
H_{obs}	12.02	3.50	198.00	23.63	9.53	25.98
(6,3 DF)	ns	ns	**	ns	ns	ns

ns: not significant; **: significant at alpha = 1 %.
Except in one case (PO_4^{3-} - UMT) homogeneity could not be rejected.

As far as PO_4^{3-} (UMT) is concerned, we reject H_0. This could be caused by inaccurate differences between variances due to erroneous observations. Elimination of such data from the pool of observations must be performed in this particular case. For the other stations and nutrients, similitude tests could be performed. Further on, we only consider NH_4 data.

SIMILITUDE TEST

In order to test null hypothesis (H_0: $a_1 = a_2 = ...a_6 = a$) which means that all slopes (a_i) are similar, we performed a variance ratio test (F test): for both stations, the calculated values (UMT: 3.19; OMT: 1.21) of F_{obs} are compared to F_{theo} (5,12 DF) = 8.89 (p=0.999).
The observed F value enables us to accept the hypothesis of identity between regression coefficients for each station. Consequently a mean slope (a) can be estimated for each station.

COMPARISON OF STATIONS

First, homogeneity of variances between the two stations was tested (F test):

$$F_{obs} = \frac{SS_{y.x}\,a_{UMT}}{SS_{y.x}\,a_{OMT}}$$ where $SS_{y.x}a$ = residual sum of squares

$F_{obs} = 5.08 < F_{theo} = 7.00$ (12,12 DF , p=0.999).

Stations UMT and OMT were compared, taking into account the six slopes in each station, when estimating the residual sum of squares:

$$t_{obs} = d\,\frac{\sqrt{(\Sigma\,SSx_i)}\ \sqrt{(N - 2p)}}{\sqrt{(SS_{y.x}\,{}^aUMT + SS_{y.x}\,{}^aOMT)}} \qquad (1)$$

where p : number of bell jars
N : total number of observations .
$\Sigma\,SSx_i$: sum of individual SS for x
$d = a_{UMT} - a_{OMT}$: flux difference expressed in µmol 0.1225 m^{-2} h^{-1}

$NH4^+$	a	$SS_{y.x}$	d = (^{a}UMT)-(^{a}OMT)	t_{obs}
a_{UMT}	2.456	102.036	1.925	6.61
a_{OMT}	0.531	20.100		

therefore, for 12 degrees of freedom H_0 ($^aUMT = {}^aOMT$) is rejected ($p < 0.001$). For NH_4 data, fluxes between UMT and OMT stations are significantly different.

CRITICAL VALUE

From equation (1), d_{crit} can be estimated:

$$d_{crit} = t_{95\%}\,\frac{\sqrt{(SS_{y.x}\,a_{UMT} + SS_{y.x}\,a_{OMT})}}{\sqrt{(N - 2p)}\ \sqrt{(\Sigma\,SSx_i)}}$$

d_{crit} is the smallest detectable difference which is required between slopes to distinguish between two stations. d_{crit} (µmol 0.1225 m^{-2} h^{-1}) changes as a function of p, the number of bell jars to be used:

p	$\Sigma\,SSxi$	t95%	N-2p	d_{crit} $NH4^+$
2	40	2.776	4	2.4254
3	60	2.447	6	1.4253
4	80	2.306	8	1.0074
5	100	2.228	10	0.7486
6	120	2.179	12	0.6346

In order to give the best possible security, d_{crit} was calculated from the sum of residual mean square when using: p = 6. d_{crit} tends to stabilize when $p \geq 4$ (Fig. 5), a value which is then to be considered as a minimal number of bell jars when differences between stations are to be evidenced.

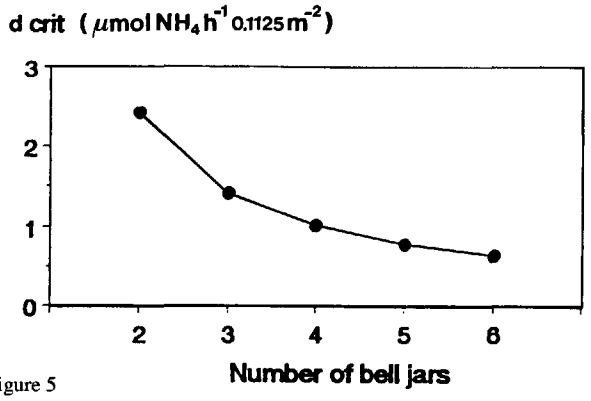

Figure 5

Changes in dcrit (smallest detectable difference between nutrient fluxes) as a function of the number of benthic chambers used.
Évolution de dcrit (plus petite différence détectable entre flux de nutriments) en fonction du nombre de chambres benthiques utilisées.

DISCUSSION AND CONCLUSION

There are two methods for obtaining data on nutrient flux from sediments: first, the calculation of flux using concentration gradients of constituents of the interstitial water and second, measuring flux directly by *in situ* or laboratory experiments. Both methods present similar, unavoidable limitations. Whatever the technique used in direct measurements, the system to be measured undergoes unavoidable perturbations. For instance, by placing a chamber on the sea floor, we block the currents and eddies which could alter the boundary layer resistance and flux we seek to measure (Santschi *et al.*, 1983). Artificial simulation of flow with a stirrer, as a compromise, can overcome this problem. Most often, the lack of information about fine-scale vertical profiles of sediment nutrient content as well as biogenic disturbances and physical stress (*e.g.* turbulence) represent serious shortcomings for attempting benthic flux estimations through diagenic models (Hall *et al.*, 1989), despite recent efforts devoted to the determination of biophysico-chemical processes involved in benthic fluxes, for example diffusivity and/or biological processes (*e.g.* bioturbation or kinetics of bacteriological processes; Aller, 1980; Vanderborght and Billen, 1975; Van Raaphorst *et al.*, 1988).

Since our concern was to determine whether mussel cultivation through biodeposition induces modification of benthic fluxes, the bell jar technique seemed suitable. Similarly, as we did not want to study specific processes inducing benthic fluxes, but were mostly interested in the comparison of instantaneous rates, we favoured incubations of brief duration. Long lasting incubation or asphyxiation technique as proposed by Rutgers van der Loeff *et al.* (1984) could be considered as a method for distinguishing between molecular diffusion and biologically mediated transport at the sediment-water interface. The lack of oxygen causes the activity of benthic infauna to cease and thereby limit solute transport to that accomplished by molecular diffusion. Because of the continuous large input of organic matter on the bottom at UMT stations, and the necessity of being in the linear phase of the release kinetic of benthic flux, 6 hours incubation with a 2 hours sampling step seems appropriate, avoiding modification of metabolism due to confinement phenomena as pointed out by Propp *et al.* (1980). In this case, flux calculation represents instantaneous rates, occurring as a result of biological, chemical and physical processes (photosynthesis of microphytes with oxygen production, respiration of the community, animal excretion, bacterial nutrient production or consumption, biochemical or purely physical diffusion, for example).

As indicated in the Table, variances reported by various authors in different areas, indicate the great disparity in replicate flux measurements. This emphasizes the problem concerning wide confidence intervals when differences between stations are to be evidenced. In order to include low abundant macrofauna in a representative way, Balzer *et al.* (1983) used a large bell jar enclosing 2094 l of bottom seawater over 3.14 m^2. On the other hand, changes in concentration in large volumes require long lasting incubations, often on a time scale that is greater than the pattern to be investigated (diurnal, for example). As a compromise, overcoming these problems, by using relatively small volume (17 l) systems deployed in replicates reduces significantly confidence limits and incidentally, increases the statistical significance when comparisons between stations are to be evidenced.

Our statistical demonstration can be considered as being of general value; it might be used as a general framework when intercomparisons are required between

Table
Average coefficient of variation in replicate benthic flux measurements reported by various authors.
Valeurs moyennes du coefficient de variation du flux benthique d'après plusieurs auteurs.

	NH4 fluxes		
Author	number of replicates	coefficient of variation(%)	location (depth)
Hale (1974)*	4	25	Narragansett Bay (Rhode Island) (< 10 m)
Rowe *et al.* (1975)*	3	79	New York bight (35,5 m)
Hartwig (1976)*	3	37	La Jolla Bight (California) (18 m)
Nixon *et al.* (1980)	3	45	Narragansett Bay (6 m)
Elderfield *et al.* (1981)	4	43	Narragansett Bay (upper bay, nearshore sediment)
Pregnall and Miller (1988)			Nahaut Bay (Massachusetts, USA)
	3	70	Little Nahaut North (surf zone)
	8	25	Nahaut baycenter (14 m)
Present study			Golfe de Fos (France)
	6	11	Shellfish farm sediment (6 m)
	6	22	muddy sand (6 m)

* data are presented in Nixon *et al.* (1980).

different areas and different methods of benthic flux measurements. We suggest that sets of at least 4 chambers per station are required. Such a conclusion strictly applies to our own experiment sites. Nevertheless, we did cover two situations: a station with high organic matter content and another with a lower one, grain size of the surficial layer being different in each cases. Therefore, a generalization may be extended to sheltered fine sediments of the infralittoral zone which are most often the typical environments of shellfish cultures.

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