

A luminophore tracer technique for bioturbation studies

Marie-Laure MAHAUT *, Gerhard GRAF

Institut für Meereskunde, Düsternbrooker Weg 20, D 2300, Kiel, FRG.

* Present address: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), DERO/EP, BP 337, F 29273 Brest Cedex, France.

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ABSTRACT

The qualitative and quantitative evaluation of bioturbation is important in understanding the impact of the latter on marine ecosystems. Luminophores, colored sand grains, visible under UV light, were used as tracers for a bioturbation study in the Baltic Sea. Distribution of luminophores added to sediment cores was followed over a period of one month, the cores being kept in a laboratory temperature bath in order to evaluate particle bioturbation rates. Reconstituted cores, populated artificially with single animal species, were also used to identify important qualitative differences in sediment displacement by a deposit feeding bivalve (*Macoma calcaria*) and a carnivorous wandering polychaete (*Nephtys caeca*). Use of luminophores as tracers appears to be an effective technique, very simple in principle, whose applications could be greatly extended if an automated method for counting of tracers could be developed.

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RÉSUMÉ

Technique de marquage par les luminophores pour l'étude de la bioturbation

La bioturbation consiste en un remaniement d'origine biologique des composés du sol qui favorise des échanges entre l'eau et les particules sédimentaires. Parvenir à évaluer les modalités et l'intensité de ce phénomène permettrait de mieux cerner son impact dans l'écosystème marin. Des luminophores, grains de sable colorés, visibles sous lumière UV, ont été utilisés comme traceurs pour l'étude de la bioturbation en littoral. Leur répartition observée hebdomadairement pendant un mois dans des carottiers prélevés en hiver à une station d'étude de la Mer Baltique et conservés dans des bains thermostatés, a permis d'évaluer des vitesses de bioturbation. D'autre part, la reconstitution de carottes, peuplées artificiellement d'une seule espèce d'animaux, a permis de vérifier des différences importantes dans la manière de déplacer le sédiment chez des bivalves filtreurs (*Macoma calcaria*) et chez des polychètes carnivores errantes (*Nephtys caeca*). L'utilisation des luminophores en tant que traceurs s'est avérée être une technique efficace, très simple dans son principe, mais dont les applications pourraient être multipliées si une méthode automatique pour le comptage de ces traceurs était mise au point.

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INTRODUCTION

Bioturbation is defined as a biological reworking of particles and pore water (Rhoads, 1974; Schink, Guinasso, 1977; Yingst, Rhoads, 1980). The effect of a benthic organism on sediment structure and chemistry depends on its mode of feeding, locomotion and relation to the substrate (Lee, Swartz, 1980).

To date, several methods have been tested for the study of sediment particle displacement caused by epifaunal and infaunal bioturbation. For example, photographs and radiographs of sediment profiles have been made through panes of plexiglas [Perpex (Rhoads, Germano, 1982; Rhoads *et al.*, 1986)], permitting a qualitative analysis of bioturbation.

Only the use of tracers reveals sediment particle movement in a more quantitative way. Natural tracers such as ^{14}C (Erlenkeuser, 1980), ^{210}Pb (Demaster, Cochran, 1977), ^{32}Si (Demaster, 1979), ^{234}Th (Aller, Cochran, 1976; Aller *et al.*, 1980) permit general observation of long-term and short-term sediment reworking, but in order to observe displacement of specific particles it is necessary to add artificial tracers.

This paper describes an evaluation of luminophores, tracers previously used only in coastal engineering studies of sediment displacement from harbours on dykes (Ruck, 1977), for their usefulness in the study of bioturbation. These tracers consist of sand grains, marked by a small amount of a fluorescent dye deposited in grain fractures and crevices. This dye lasts for several years and does not alter the physical properties of the treated sand grains. Information on the chemical treatment of sand grains during coloration is at present protected by patent. Luminophores are available in six colours and fluoresce under ultraviolet illumination (Ruck, 1972; 1977). This latter property allows a nearly quantitative recovery of the added tracers.

In the present study we examined whether luminophores could be used to record the timecourse of particle displacement, and whether particle selection could be distinguished by tracing movement of particles differentially coloured according to size.

MATERIAL AND METHODS

Study area and sediment collection

The study area is situated in the southwestern Kiel-Bight (Baltic Sea) and is known as the "Hausgarten area" ($54^{\circ}32'\text{N}$; $10^{\circ}03'\text{E}$). Sediments from a silty sand bottom at 17 m depth were collected with a boxcorer (20×30 cm) in January and May 1985.

Subcores of 10 cm inner diameter (78.5 cm^2) were taken to a depth of 15 to 20 cm sediment with 10 to 15 cm overlying water.

Similar experiments were conducted with subcores from the January and May samples. Selected examples are reported here to illustrate particular aspects of this technique.

Treatment of cores

The cores were kept for up to four weeks in the laboratory in a water bath at 2°C during January (*in situ* temperature = -0.1°C), and in May at the *in situ* temperature of 3°C . On alternate days the overlying water was carefully replaced by oxygen saturated seawater collected at the study site. Salinity was 13.3 during the first experiment in January and 16.8 during the second period in May.

Artificial cores

For some experiments, macrofauna was removed. Oxidized and reduced sediments were then separated and restored in their original proportions. Reduced sedi-

ments were exposed to air for less than five minutes. The cores were then repopulated with *Macoma calcarea* (Bivalvia) and *Nephtys caeca* (Polychaeta), sampled at the study site.

Luminophores

Two different categories of the tracer were used during the study:

1) Luminophores were produced through staining of unsieved sand. These varied in size from 63 to 900 μm in diameter. A particle count yielded a density of approximately 30 000 luminophores/g. This mixture of luminophores was used in the experiments with natural sediment and for the time-series experiments.

2) Sand was sieved in two distinct size fractions before staining. Particles of 250-315 μm diameter were separated from those of 500-800 μm diameter. Luminophore densities for these fractions were, respectively, 37 670/g (red) and 3 550/g (yellow).

These tracers were used for observation of specific grain size dependents effects of bioturbation by isolated species on artificial sediments.

Staining with the different dyes for each fraction of sand grains was conducted by the *Bundesanstalt für Wasserbau* (2300 Kiel, Schleuseninsel, FRG). We are especially indebted to Dr. W. Peetz, who made luminophores available for our study. Details of the coloration process are protected by patent.

Luminophores were added to the cores only the day after sampling, to allow for the adaptation of the fauna to the experimental conditions. A weighed quantity of luminophores was wetted by shaking it with sea water in plastic vials, in order to prevent problems of surface tension. The vials were opened under the water surface of each core and moved slowly in a circular pattern to ensure an even distribution of luminophores on the sediment surface.

Recovery of luminophores

Overlying water was carefully siphoned off the cores, except for a thin layer on the sediment surface which was left to avoid a loss of luminophores. The sediment was then carefully extruded from the core with a piston apparatus. The superficial layer on the surface sediment (1 to 2 mm) was removed and dried at 70°C before weighing. Two grams of this dried surface sediment were set aside for luminophore counting. The volume and the thickness of this layer were calculated using previously determined values for the water content of the sediment.

The remainder of the core was cut into a series of horizontal sections at about 5 mm intervals down to the zone where traces of macrofauna were no longer visible.

Macrobenthos located within each sediment layer were isolated and identified. Each layer was then thoroughly mixed to homogenize luminophores and sediment as completely as possible. Three subsamples (6 cm^3 each) were removed from these homogenates. The volume of the remainder was measured before discarding. Thus,

the total volume of each layer was known and a correction for the thickness of the layer was calculated. Any particularly voluminous animals, empty shells or stones found in the section were taken into account and the volume corrected accordingly.

Three subsamples of each layer were oven dried for about 24 h. The dried sediment had to be broken carefully to prevent fractioning of luminophores.

Counting of luminophores

Luminophores counts were conducted under an ultra-violet light source (20 watts). Visual counting was necessary because siliceous skeletons of bryozoans and shell fragments of molluscs also show some fluorescence (Nachtigall, 1964; Ruck, 1972; 1977).

We also conducted counting trials using available digital image processing facilities at Siegen University (FRG). The image analyser could clearly distinguish luminophore size and color in photographs of sample preparations. However sample treatment prior to counting and analyses of resulting data proved to be too time-consuming and this approach was abandoned in favour of the manual technique.

RESULTS AND DISCUSSION

Control core and errors

To investigate effects caused by the luminophore addition and core extrusion procedures, an artificial core was run without macrofauna. Added to this core were 11 250 luminophores of the small size fraction and 3 750 of the large fraction.

Theoretically, tracers should have remained at the surface of the control core, since luminophores have the same density as sand grains excluding spontaneous

dispersion due to gravity. The sediment, although sandy, contained some silt. Despite cautious handling some very fine particles may have been resuspended during core manipulation and their subsequent resettling could have resulted in burial of the luminophores. This effect was examined in the control core by removing the top 3 mm of sediment.

The analysis of the control core resulted in a recovery of added luminophores of 98.1% for the small luminophores and 99.3% for the large ones. Of these 97.6% of the recovered small luminophores (96.6% for the large ones) were found in the top 3 mm of the sediment, an additional 2.25% (3.4%) was found between 3 and 12.5 mm depth, and 0.15% of the small luminophores had penetrated the layer between 12.5 and 20.5 mm.

These results indicate that handling effects did not result in an important downward displacement of luminophores below 3 mm.

Occasionally thinner surface layers were successfully removed from experimental cores, but this was not consistently possible as in the case of the control core. Thus the exact importance of the fauna in the displacement of luminophores in the first 3 mm of sediment cannot be evaluated.

A second possible artifact of the experimental procedure is that luminophores at the periphery of the core surface may have been carried down the walls of the core tube during the extrusion procedure, a phenomenon that could be observed with the naked eye. As reported above the maximum error caused by this "wall effect" was 2.4% for small and 3.4% for large luminophores below 3 mm sediment depth. For the reported experiments, these outer portions of the sediment sections were discarded, so that this source of error could be ignored.

In most cores, 90 to 100% of the added tracers were recovered. Occasionally, however, total counts yielded <80% or >100% of those initially added. Because the

Table

Analysis of one of the natural cores sampled in January 1985, labelled with unsieved luminophores (15000). Incubation time was two weeks. The thickness of each layer was calculated as described in methods. The entire surface layer (0-0.1 cm) was first weighed, then an aliquot of 2 g was counted.

Analyse d'une des carottes naturelles prélevées en janvier 1985 et marquées avec des luminophores non tamisés (15000). La période d'incubation a duré ici deux semaines. L'épaisseur de chaque couche a été calculée comme décrit dans « Methods ». De la couche superficielle (0-0,1 cm) préalablement pesée, seuls les traceurs présents dans un échantillon de 2 g ont été comptés.

Thickness (cm)	Luminophores		in percent of		Macrofauna
	in subsamples	per layer	introduced	found	
0.00-0.10	1 100 (in 2 g sediment)	5 775	38.50	44.93	<i>Diastylis rathkei</i> <i>2 Ophuira albida</i>
0.10-0.85	per 6 cm ³ 545 566 555	5.368	35.78	38.89	7 <i>Pectinaria koreni</i> 2 <i>Abra alba</i> (6 mm; 9 mm)
0.85-1.40	195 150 195	1 490	8.60	10.04	1 <i>Arctica island</i> (21 mm)
1.40-1.90	20 28 43	192	1.28	1.49	1 <i>Macoma calcar</i> (15 mm)
1.90-2.65	12 25 22	190	1.27	1.48	1 <i>Nephtys caeca</i> (40 mm)
2.65-3.40	6 3 3	39	0.26	0.30	1 <i>Mya truncata</i> (15 mm)
3.40-4.00	— — —	—	—	—	
		12 854	85.69	100.00	

number of luminophores (15000) in the above cases was estimated by weighing, some variation in numbers was unavoidable. Standard deviation of weighing is 50 luminophores. Since luminophore counts were made from subsamples only (cf. Tab.), inadequate homogenization of the sediment before subsampling is presumably the main reason for these findings. No relationship between incubation period and percentage recovery of added luminophores was apparent. Thus observable concentration, with time, of luminophores in the outer, discarded portion of the cores did not occur.

Example analysis of an experiment with a natural core

One natural sediment core from January 1985 was incubated for two weeks with luminophores of unsieved sand. After two weeks, 45% of the luminophores were still located in the surface layer and 39% in the 0.1-0.9 cm layer (Tab.). Only 13% had been transported into deeper layers down to 3.4 cm. Recovery of added tracers was relatively low (85.69%). The calculated thickness of the analysed sediment layer (1st column Tab.) varied from 5 to 8 mm, demonstrating that the correction by volume is essential.

From the macrofauna present, *Diastylis rathkei* and *Ophura albida* would have influenced only the sediment surface layer. The seven *Pectinaria koreni* represent a dense population of this species, but are too small to have caused a deeper bioturbation effect. Only *Nephtys caeca* and *Mya truncata* could have been responsible for a deeper transport of luminophores.

Study of bioturbation rate

Eight natural cores taken during January 1985 were marked with luminophores of unsieved sand as described in the example above and run in two parallels for a period of four weeks. Macrofauna species were the same as in the Table, but, the species composition and individual numbers were different in every core as a result of their natural patchiness. The percentage of luminophores recovered as a function of depth was determined for each core (Fig. 1). The figure reveals that with time the luminophores are incorporated progressively into the sediment.

It is, however, easier to follow the luminophore displacement by defining standard amounts of tracer which have reached a certain sediment depth. Figure 2 shows the depths above which 50, 70, 90, 98 and 100% of the recovered tracers were distributed in each core, demonstrating the downward displacement (net transport) of luminophores over time and the variation between the two cores. The slope of the curves in Figure 2 can be used to estimate the rate of net transport of various proportions of the introduced luminophores. For example, in the first week, 50% of the tracers are buried at a maximal rate of 0.14 mm/d, 20% at 0.49 mm/d, 20% at 1.06 mm/d, 8% at 1.43 mm/d and 2% at 4.93 mm/d. The following week, downward displacement decreased at all depths. This may be explained by the decrease in both macrofauna and luminophores with increasing sediment depth; this of course reduced the frequency of faunal/luminophore

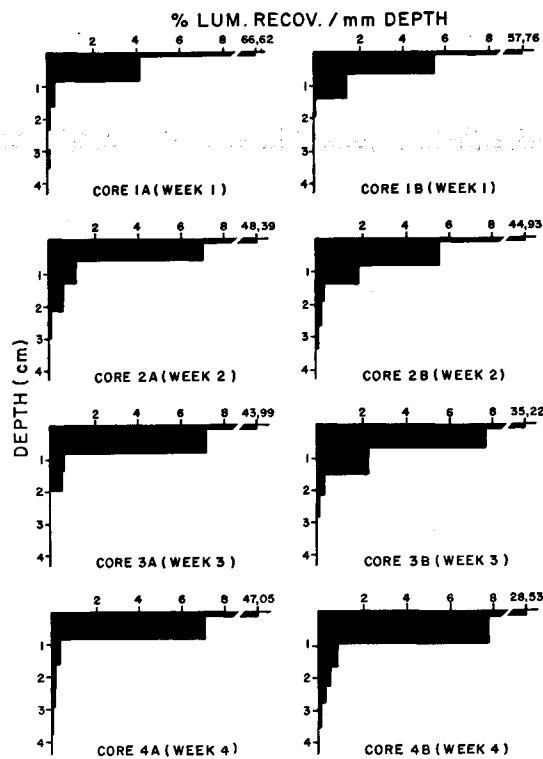


Figure 1
Depth distribution of luminophores found in duplicate cores sampled in January and analysed at weekly intervals over one month.

Distribution des luminophores en fonction de la profondeur, observée dans les carottes prélevées en janvier et analysées chaque semaine pendant un mois.

encounters. During the third and fourth weeks, little further downward displacement of luminophores was apparent. Figure 2 reveals that nearly all luminophores were dispersed within the upper 2 cm of sediment. This was the zone where most of the macrofauna was found. At our study site, the anoxic zone (Eh < +100 mV)

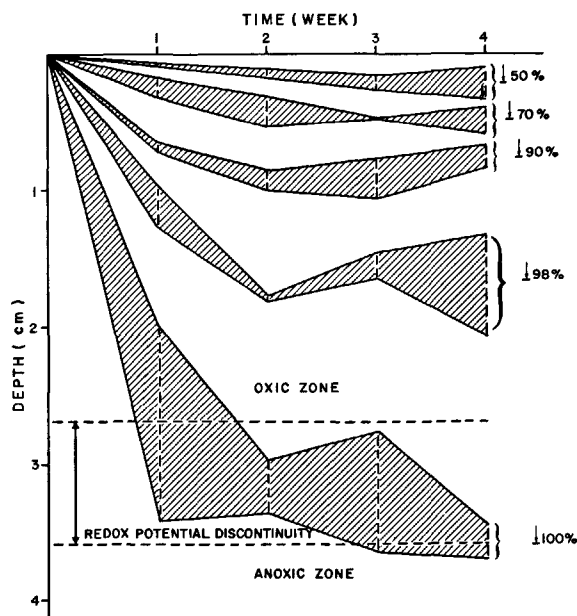


Figure 2
Rate of luminophore displacement of the recovered tracers calculated from Figure 1.

Vitesse de déplacement des traceurs retrouvés, calculée d'après la figure 1.

commenced below 27 to 36 mm sediment depth. Obviously this physico-chemical barrier constitutes an additional reason why only a small minority of tracers (2%) descended quickly to the upper part of the anoxic zone but then seemed to be abruptly prevented from further penetrating deeper anoxic sediments.

The bioturbation rates of up to several millimetres per day are comparable to results revealed by other methods (Lee, Swartz, 1980; Czytrich *et al.*, in press), but considering the low temperature they are surprisingly high.

Example for the study of particle selection

In this experiment, the surface of artificial cores were labelled with a mixture of 11 250 small (250-315 μm) and 3 750 large luminophores (600-800 μm). These proportions were estimated to result in an animal having the same probability of encountering a small or a large luminophore, taking into account the different surface areas of the two particle sizes. These initial proportions were set to correspond to a reference rate of 1:1. Any later deviation from this ratio would indicate size selection during the process of bioturbation. Figure 3

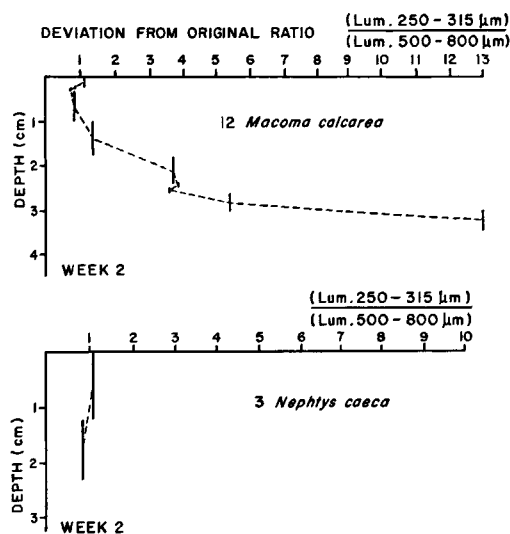


Figure 3
Ratio of small/large recovered luminophores as a function of depth after two weeks incubation time.

Rapport en fonction de la profondeur des petits/gros luminophores retrouvés après deux semaines d'incubation.

depicts the effect of 12 *Macoma calcarea* and 3 *Nephtys caeca* on this ratio after 2 weeks. The strong increase of the ratio with increasing depth demonstrates a biogenic effect on particle distribution. Observation of the behavior of *Macoma calcarea* explains the distribution of the tracers. The long in-siphon allows this bivalve to live several centimetres below the sediment surface. When the animal is feeding, it preferentially ingests finer particles from the sediment surface with its in-siphon; with its short out-siphon, it ejects faeces into

the sediment slightly above the shell, at 2 to 3 cm sediment depth. *Nephtys caeca*, a carnivorous polychaete that selects its food before ingestion, will not ingest many tracers. The downward transport of the luminophores is then an indirect effect of the movement of the polychaetes in the sediment. Therefore, an equal distribution of the two tracer sizes is visible in the sediment selections where tracers were found.

CONCLUSIONS

The idea of using coloured sand to study bioturbation is certainly not new. Jacobsen, for example, stained sand with Sudan III to study bioturbation and feeding of the lugworm *Arenicola marina* (Jacobsen, 1966). The disadvantage of histological colorants such as Sudan III is that they bind only to lipid or other organic films on sediment particles (Gabe, 1968). This colorant can be lost through digestive activity of fauna and microorganisms as well as by physical or dissolutive deterioration of colored organic films. The advantages of the luminophores are the long-lasting nature of the colorant and its fluorescent properties. This offers the possibility for, respectively short and long-term studies without loss of coloration and adaptation to automated counting techniques. A problem of compatibility between this experiment procedure and available image processing technology will have to be resolved before automated luminophore counting can be profitable. A protocol of integrated experimentation and automated analysis such as that developed by Rhoads *et al.* (1986) would greatly expand the application of luminophores in bioturbation studies. This would allow multiplication of replicates and analysis of more sediment layers, and thus increase the scope and accuracy of studies of bioturbation.

Both examples give only a first impression of how the luminophore technique may be applied. The method appears to be well suited to the evaluation of mathematical models of particle displacement (Goreau, 1977) or investigation of the effect of particle selection by a certain species or population (Whitlatch, Weinberg, 1982). The preliminary results for *Macoma calcarea* reveal that surprisingly short experimental periods can yield definitive information.

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