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Effects of food availability on sediment reworking in *Abra ovata* and *A. nitida**

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ABSTRACT: We used a new experimental approach involving thin aquaria, luminophores, time lapse photography and image analysis to assess sediment reworking in 2 closely related bivalves, *Abra ovata* and *A. nitida*. The method proved efficient based on the highly significant correlation between the concentrations of luminophores assessed using image analysis and direct counting of sediment slices. *A. ovata* and *A. nitida* exhibited different sediment reworking behaviours. *A. ovata* remained immobile within the sediment and transferred luminophores within the sediment through its siphonal activity, which resulted in the creation of typical inverse conical structures. *A. nitida* moved within the sediment and reworked a thinner sediment layer. Both *A. ovata* and *A. nitida* were characterised as biodiffusers. Biodiffusion coefficients (D_b) were maximal at intermediate food concentration in *A. ovata* and at high food concentration in *A. nitida*. This new approach allowed assessment of the effects of spatial scale and vertical grid size on the computation of D_b. In both species D_b decreased with spatial scale up to 3.750 cm and then remained constant. It is suggested that this pattern partly resulted from heterogeneity linked to: (1) the mode of sediment reworking (*A. ovata*) and (2) the relative proportion of reworked sediment surface (*A. nitida*). Vertical grid size >0.250 cm resulted in a significant overestimation of D_b in *A. nitida*, due to the low thickness of the sediment layer reworked by this species. The implications of these results on the main characteristics (duration, spatial scale, vertical grid size) of classical luminophore experiments are discussed.

KEY WORDS: Sediment reworking · Image analysis · Food availability · Scaling · Luminophores · *Abra ovata* · *Abra nitida*

INTRODUCTION

Benthic infaunal organisms, through their activity of burrowing, feeding, defecation and locomotion induce sediment reworking that affects the physical, chemical and geotechnical properties of the sediment column (Gray 1974, Rhoads 1974, Aller 1982, Rhoads & Boyer 1982, Meadows & Meadows 1991, Gilbert et al. 1995, Rowden et al. 1998, Lohrer et al. 2004). Biogenic sediment reworking affects, in particular, the fluxes of oxygen, nutrients, contaminants and pollutants and, more generally, the transfer and the mineralisation of organic matter at the water–sediment interface (Lee & Swartz 1980, Aller 1982, 1994, Aller & Yingst 1985, Andersen & Kristensen 1991, Furukawa et al. 2001, Mermillod-Blondin et al. 2005). Analysis and quantification of sediment reworking processes are considered key points in understanding benthic ecosystem functioning (Biles et al. 2002, Solan et al. 2004a). A better understanding of sediment reworking requires the study of dominant species that are liable to induce particular sediment reworking patterns depending on their behaviour and response to changes in biotic and abiotic environmental factors (Gérino 1990, Biles et al. 2002, Ouellette et al. 2004).

Several experimental techniques involving natural or artificial tracers (e.g. radionuclides, glass beads,

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metal-doped sediment, chlorophyll *a*, luminophores) have been used to estimate sediment reworking by benthic fauna (Robbins 1982, Robbins et al. 1984, Krezoski et al. 1984, Smith et al. 1986, White et al. 1987, Sharma et al. 1987, Sun et al. 1991, Wheatcroft 1992, Wheatcroft et al. 1994, Olmez et al. 1994, Gérino et al. 1998). These tracers are indicative of sediment reworking occurring at different timescales. Luminophores (i.e. fluorescent particles) have been widely used in laboratory (Mahaut & Graf 1987, Gérino et al. 1998, Ouellette et al. 2004, Mermillod-Blondin et al. 2005) and *in situ* experiments (Mahaut & Graf 1987, Gérino 1990, Gérino et al. 1994, 1998, Biles et al. 2002, Mugnai et al. 2003, Solan et al. 2004b). Experiments classically consist of spreading luminophores at the surface of experimental sediment cores containing test organisms at time 0 and then incubating those cores for a known amount of time. At the end of the experiments, the cores are sliced horizontally and the vertical profiles of luminophore concentration assessed. The profiles are fitted to mathematical models that allow computation of coefficients characterising the intensity of sediment reworking. The main potential drawbacks of those approaches are: (1) the lack of a dynamic view of sediment reworking, since the cores are destroyed for the assessment of luminophore profiles, (2) the vertical resolution of the luminophore profile, which is limited by the minimal thickness of the sliced sediment layer (i.e. roughly 0.5 cm), (3) the impossibility of assessing sediment reworking rates at a spatial scale smaller than the experimental core, (4) the time requested to carry out the processing of the cores and the counting of the luminophores and (5) the possible mixing of luminophores between adjacent sediment layers during slicing, which may lead to an overestimation of sediment reworking (Berg et al. 2001).

To overcome these drawbacks, time-lapse photography, thin aquaria, or sediment profiling, allowing the monitoring of a vertical plan of the sediment column, and automated image analysis technique have recently been introduced. Gilbert et al. (2003) used a combination of these techniques to study sediment reworking induced by benthic communities originating from the Gullmarsfjord and the Skagerrak. Solan et al. (2004b) used time-lapse recording to assess *in situ* sediment reworking rates in the Gullmarsfjord. However, to our knowledge, such an approach has not been used either to assess sediment reworking rates of individual species or to assess the impact of food availability on sediment reworking rates. During the last 10 yr considerable expertise in imaging marine organisms and populations has been developed for assessing, e.g. larval behaviour (Duchêne & Nozais 1994, Duchêne et al. 2000, Duchêne & Queiroga 2001, Duchêne 2004) and, more recently, feeding behaviour of both suspension (Jordana et al. 2000) and deposit feeders (Hollertz & Duchêne 2001, Grémare et al. 2004).

The aims of the present study were thus: (1) to combine the use of thin aquaria, luminophores and automated image analysis techniques to infer temporal changes in sediment reworking rates, (2) to describe sediment reworking in *Abra ovata* and *A. nitida*, 2 closely related bivalves with different feeding ethology and functional responses, (3) to assess the effects of changes in organic matter availability on sediment reworking rates and (4) to assess the effects of spatial and temporal scales on the computation of sediment reworking rates.

MATERIALS AND METHODS

Bivalve collection and maintenance. *Abra ovata* and *A. nitida* are deposit-feeding bivalves that live buried a few centimetres below the sediment and feed at the water–sediment interface using their inhalant siphon (Guelorget & Mayere 1981, Wikander 1981).

Abra ovata is a typical species of sandy mud in NW Mediterranean lagoons, where it can be found in densities of up to several thousand individuals per square metre (Guelorget & Mayere 1981). *A. ovata* were hand collected in July 2004, at depths of <1 m on the north side of the Lapalme Lagoon (NW Mediterranean). Water temperature was 20°C and water salinity 6 PSU. Bivalves were kept for 15 d at the Observatoire Océanologique de Banyuls sur Mer (France) in natural sediment and well-aerated lagoon seawater. Salinity was then progressively increased to 20 PSU. At the end of July, animals were packed in sealed, refrigerated boxes filled with O_2 saturated water and brought to the Kristineberg Marine Research Station (Sweden), where they were kept in tanks with flow-through surface seawater (20°C, 24 PSU) and natural sediment from the Gullmarsfjord (Sweden).

Abra nitida is abundant in muddy sediments in the Skagerrak and occurs in densities of up to several thousand individuals per square metre (Josefson 1982). The bivalves were collected in August 2004 in the Gullmarsfjord between 80 m (58° 14′ 38″ N, 11° 31′ 00″ E) and 109 m (58° 17′ 18″ N, 11° 30′ 39″ E) using a Waren dredge. The bivalves were kept at the Kristineberg Marine Research Station in tanks containing natural sediment flushed with deep seawater (8°C, 35 PSU). In addition, a batch of bivalves was progressively adapted to a temperature of 15°C. The 2 species were fed every other day with crushed Tetramin fish food. Before each experiment, specimens were measured to the nearest millimetre. The total lengths of the shells of *A. ovata* and *A. nitida* used during the experiments were between 12 and 13 mm.

Sediment reworking experiments. The same sediment was used for both species to better account for differences in sediment reworking by *Abra ovata* and *A. nitida*, since a similar approach has already been used by Grémare et al. (2004) to assess differences in feeding activity between these 2 species. Visual observations showed that after their deposit at the sediment surface *A. ovata* burrowed quickly into the Gullmarsfjord sediment and then featured a feeding behaviour similar to the one observed during preliminary studies performed with Lapalme Lagoon sediment (Maire pers. obs.). We filled 45 thin aquaria (33 × 17 × 1.2 cm) with 15 cm of muddy sediment (median diameter: 9.50 µm; organic carbon: 2.85% DW [dry weight]; nitrogen: 0.34% DW) collected in the Gullmarsfjord with a 0.05 m^2 Olausson sediment corer at 83 m depth (58° 17′ 4″ N, 11° 30′ 7″ E). The sediment was previously sieved through a 1 mm mesh to remove macrofauna. All aquaria with sediment were kept in a thermoregulated room under running seawater for a few days.

Sediment reworking was quantified by using luminophores (Mahaut & Graf 1987). Luminophores are natural sediment particles covered by a thin layer of paint that fluoresces under ultraviolet (UV) light. The luminophores used during the present study were between 100 and 160 µm in size. Preliminary studies (Maire pers. obs.) showed that both species of *Abra* were able to ingest luminophores. Three bivalves (corresponding to a density of 1470 ind. m^{-2}) were gently deposited on the sediment surface. They usually buried themselves within a few minutes, but were replaced if they did not do so within 1 h. After 24 h of acclimation, which is sufficient to insure the constancy of subsequent feeding behaviours in both species according to Grémare et al. (2004), 3 g of luminophores were homogeneously and gently spread on the sediment surface of each aquarium with a Pasteur pipette. Then, 2.86 mg C m^{-2} of phytodetritus (*Tetraselmis* 3600 Premium Fresh, Reed marine culture) was simultaneously added to a first batch of 5 aquaria (Treatment A) and 28.6 mg C m^{-2} of *Tetraselmis* was added to a second batch of 5 aquaria (Treatment B); 5 other aquaria did not receive any food addition (Treatment T). *Tetraselmis* has already been used as a surrogate of sedimenting phytodetritus during several experimental studies (Hassler 1998, Grémare et al. 2004). The 2 concentrations used for food additions corresponded to maximal and intermediate values of the 5 concentrations used by Grémare et al. (2004) based on the gross sedimentation rates reported by Lindahl (1988) for the Gullmarsfjord and by Grémare et al. (1997) for the NW Mediterranean. These 2 concentrations were selected because they corresponded to maximal feeding activity in *A. ovata* and *A. nitida*, respectively (Grémare et al. 2004). Immediately after the luminophore input, the aquaria were placed in a stand in front of a digital camera (Olympus Camedia E10) and the 2 sides were photographed under UV light. This operation was repeated after 3, 6, 12, 24 and 48 h. The UV light was produced by 2 black bulbs located in a reflective semi-cylinder placed about 10 cm in front of the aquarium. Camera settings were adjusted for adequate fluorescent detection, and the photographic field (15 × 17 cm) resulted in a resolution superior to the luminophore size (1 pixel = 75 × 75 µm). The aquaria were flushed with flow-through seawater after 3 h and kept in darkness during the entire experiment. Three experiments were run: 1 with *Abra ovata* (20°C) and 2 with *A. nitida* (8 and 15°C). In addition, we ran 3 controls without bivalves and without food addition (1 at 8°C and 2 at 20°C).

Image analysis. Images were saved in jpg format and red-green-blue (R-G-B) colour, with a size of 2240 × 1680 pixels, and were later assembled into an AVI film. Further analyses were performed directly from the film. Image treatments were carried out with the CVABimage software developed at the Laboratoire Océanologique de Banyuls (Duchêne & Nozais 1994, Duchêne et al. 2000). On each image, the water–sediment interface was manually drawn. This line represented the initial reference used to calculate luminophore penetration depths. It was then flattened and transferred to the first pixel row of each pixel column. After this operation, the pixel *y*-position in the picture thus corresponded directly to its depth within the sediment. The second step consisted of assessing the proportion of each of the 3 colours (R-G-B) of the luminophore pixels and recording all corresponding points. Images were then thresholded and transformed to a binary matrix, where luminophore pixels were assigned a value of 1 and sediment pixels a value of 0. Luminophore pixels were finally summed for each pixel line (i.e. depth), and vertical profiles of luminophore distribution were generated.

The vertical distributions of luminophores were also assessed directly in 5 aquaria selected at random. At the end of the experiments, these aquaria were frozen (–20°C) and their sediment column was sliced (in 0.5 cm layers between 0 and 5 cm depth and in 1 cm layers between 5 and 10 cm depth). Sediment samples were frozen, freeze dried, dispersed over a Petri dish under UV light and photographed. Images were processed and luminophore pixels counted using the CVABimage software. The luminophore profiles thus obtained were then compared with those derived from the analysis of the photograph of the walls of the corresponding aquaria.

Quantification of sediment reworking. We used 2 crude indices of sediment reworking: (1) the maximum luminophore penetration depth (MPD), mea-

sured as the distance between the sediment surface and the deepest luminophore pixel, and (2) the proportion of the total sediment surface that had been reworked by the bivalves (PRS). The assessment of PRS was based on the quantification of the proportion of the aquaria surface devoid of luminophores by the bivalves. These 2 parameters were directly assessed using CVABimage software.

Biodiffusion coefficients (D_b) were computed using a biodiffusive model. Based on the luminophore distribution observed at each time, from time = 3 h to time = 48 h, D_b was computed with the 1-dimensional model given by Crank (1975):

$$\frac{\partial^2 C}{\partial t} = D_b \frac{\partial^2 C}{\partial z^2} \quad (1)$$

A solution to Eq. (1) is:

$$C(z,t) = \frac{M}{\sqrt{\pi D_b t}} \exp\left(\frac{-z^2}{4 D_b t}\right) \quad (2)$$

Eq. (2) (Crank 1975) describes the spreading by diffusion of an amount of tracer M deposited at $z = 0$ and at time = 0, assuming that D_b and M remain constant and that the luminophores can move from the surface only toward depth (impermeable boundary at $z = 0$).

The boundary conditions are:

1. Upper boundary condition: $\frac{\partial C}{\partial z} = 0$ at $z = 0$
2. Lower boundary condition: $C(z \to +\infty, t) = 0$

D_b was estimated by convergent iterations and weighted least-squares regression of observed luminophores profiles on predicted tracer concentrations (François et al. 2002):

$$r = \sum_{i=1}^{n} \frac{(\mathrm{obs}_i - \mathrm{pred}_i)^2}{\mathrm{obs}_i + 1}$$

where r is the sum of the residuals and i is the number of sediment layers.

For each combination of aquarium and incubation duration, D_b was averaged for both sides of a given aquarium.

Effect of spatial scale on the computation of D_b. The effect of horizontal spatial scale on the computation of D_b was assessed using a bootstrap procedure. For each photograph recorded after 48 h, D_b values were computed based on 100 randomly selected areas of increasing size (corresponding to rectangles delimited by the vertical length of the whole aquarium and by horizontal linear segments of 0.075, 0.375, 0.750, 1.500, 3.750, 7.500, 11.250, 13.500 and 17.000 cm). When biogenic structures resulting from sediment reworking were apparent (i.e. during the experiments with *Abra ovata*), we also ran a direct comparison of D_b values computed based on the vertical distributions of luminophores within: (1) the whole aquarium and (2) a single biogenic structure.

The effect of vertical grid size was also assessed by computing D_b based on the photograph of the whole aquarium shot after 48 h of experiments. We used several vertical grid sizes: 0.052, 0.105, 0.247, 0.502, 0.750 and 0.997 cm and computed the corresponding luminophore profiles, which were then fitted as described above to derive D_b.

Statistical analysis. The directly measured luminophore concentrations and those derived from the photographs of the walls of the corresponding aquaria were compared using simple linear regression models. In *Abra ovata*, differences in MPD, PRS and D_b values between experiment durations and food treatments were assessed using 2-way ANOVAs (analysis of variance). Differences in: (1) MPD, PRS and D_b values in *A. nitida* between experiment durations, food treatments and temperatures and (2) MPD, PRS and D_b values between *A. ovata* and *A. nitida* were assessed using 3-way ANOVAs. Three-way ANOVAs were also used to assess the effects of horizontal scale and vertical grid size on D_b. Data were square-root transformed to homogenise variances. Whenever appropri-

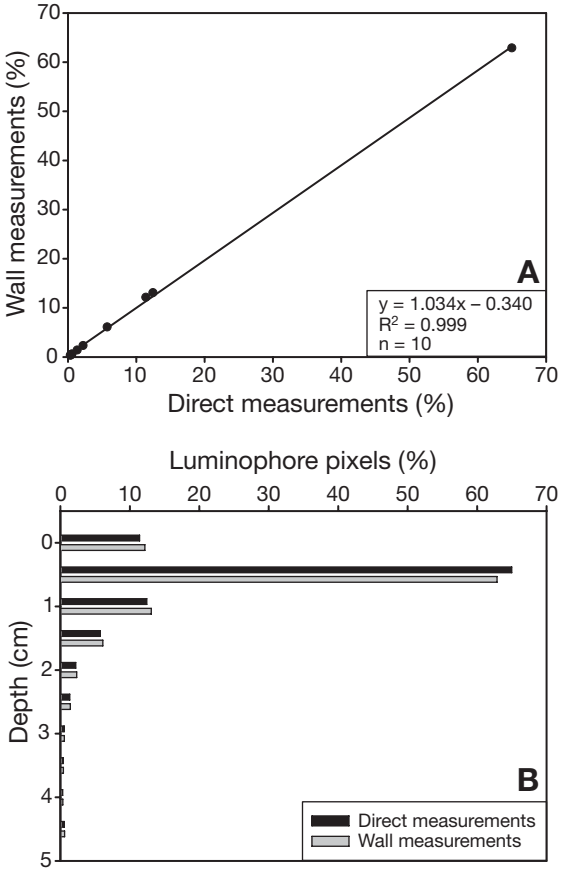

Fig. 1. Relationship between luminophore concentrations directly assessed and derived from the analysis of the photographs of the walls of the same aquarium (A), and comparison of the corresponding luminophore profiles (B)

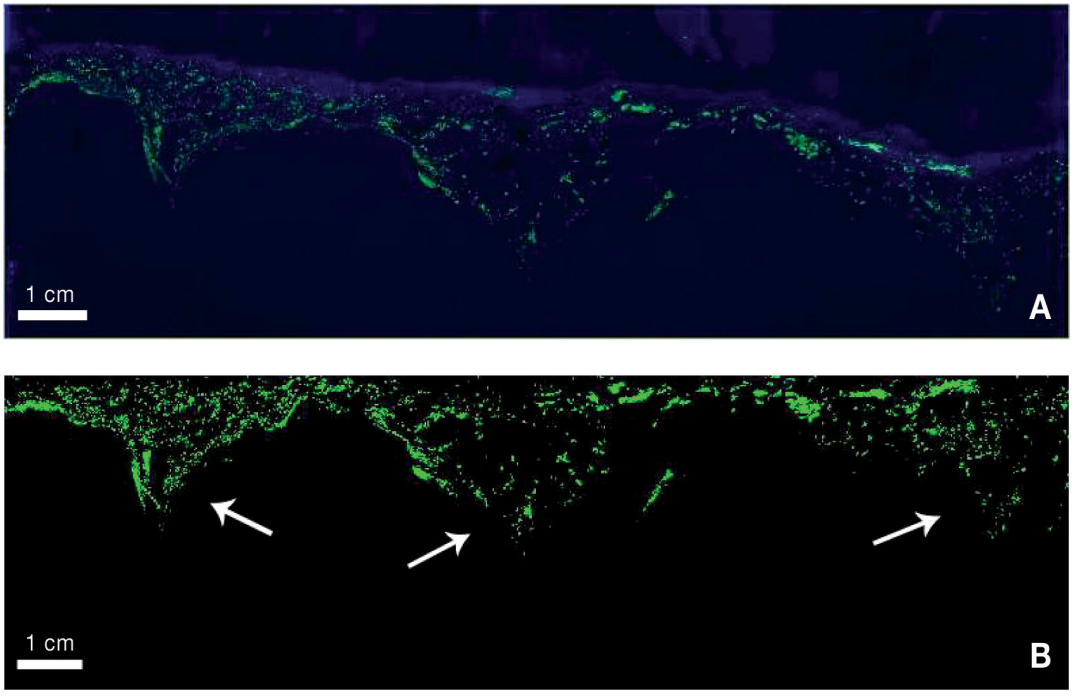

Fig. 2. *Abra ovata*. (A) Example of an original image with the sediment appearing in dark and luminophores in green. (B) Corresponding thresholded image with a flattened sediment–water interface. Note the occurrence of the 3 inverse conical structures characteristic of sediment reworking by individual *A. ovata* (white arrows)

ate, *a posteriori* least significant difference (LSD) tests were used to assess differences among treatments, species and temperatures.

RESULTS

Comparison between the 2 methods for assessing luminophore profiles

In all 5 tested aquaria, there was a highly significant correlation between luminophore relative concentrations based on: (1) direct measurements and (2) the analysis of the photographs of the walls of the aquaria (Fig. 1A; $n = 10$, $r^2 > 0.837$, $p < 0.001$ in all cases). The vertical luminophore profiles assessed by these 2 approaches were thus almost identical (Fig. 1B).

Abra ovata

The final distribution of luminophores in aquaria containing bivalves showed significant sediment reworking. Conversely, in control aquaria, luminophores remained at the water–sediment interface during the whole experiment. Visual observations carried out throughout the experiment revealed that after their initial burying, bivalves did not change position within the sediment. Initially, the inhalant siphon was erected vertically above the shell and later it began exploring the sediment surface. During this process the inhalant siphon periodically retracted and then re-emerged a few millimetres away, which progressively extended the search area around the first siphonal channel. This behaviour resulted in the formation of a reworked area with a typical inverse conical shape (Fig. 2). The recording of the position of individual bivalves at the end of the experiments confirmed their localisation below the summit of the cones.

The deepest occurring luminophores were located at the summit of the cones. MPD increased significantly with experiment durations (2-way ANOVA, $p < 0.001$; Fig. 3A). At time 0, the deeper fluorescent particles were, on average, located 1.09 cm below the surface, as they were buried in the large galleries initially formed by *Abra ovata*. During the first 6 h, luminophores were transported downwards in the sediment at a mean rate of 0.13 cm h^{-1}, and MPDs were similar between all food treatments. After 6 h, MPD increased more slowly (mean rate of 0.02 cm h^{-1}), and greater differences appeared between treatments. After 48 h, MPDs were between 1.93 and 4.90 cm, with a mean of 3.25 cm in Treatment T, between 1.56 and 4.25 cm, with a mean of 3.18 cm in Treatment B, and between 1.42 and 7.21 cm, with a mean of 4.04 cm in Treatment A. Overall, MPDs seemed greater for Treat-

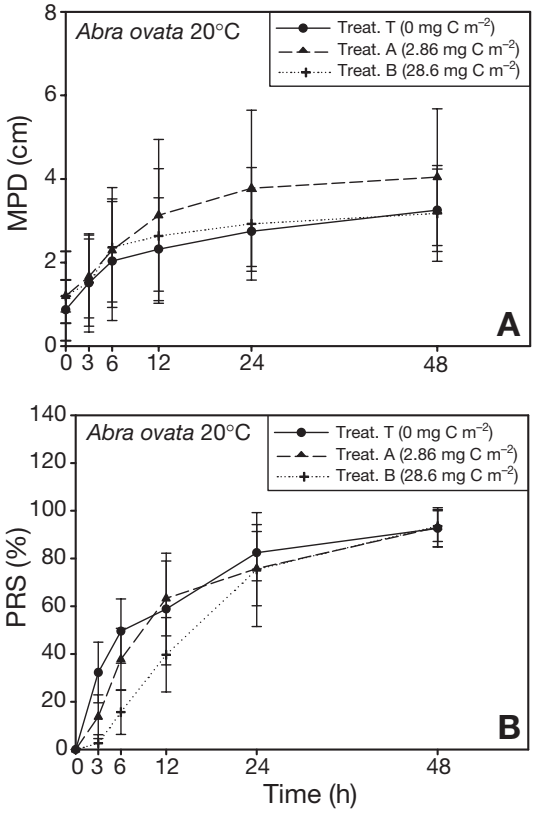

Fig. 3. *Abra ovata*. Temporal changes in (A) MPD (maximum luminophore penetration depth) and (B) PRS (proportion of reworked sediment surface) for the 3 food treatments. Vertical bars are standard deviations

ment A than for Treatments T and B. However, these differences were not statistically significant (2-way ANOVA, $p = 0.054$) due to the high variability within each treatment.

PRSs increased significantly with experiment durations (2-way ANOVA, $p < 0.001$) and were significantly affected by food treatments (2-way ANOVA, $p < 0.001$; Fig. 3B), with a significant interaction between experiment duration and food treatment ($p < 0.001$). During the first 24 h, PRSs were higher for Treatments T and A. After 48 h, PRS averaged 93% for all 3 food treatments.

Three main types of vertical luminophore profiles were observed (Fig. 4). Type 1 consisted of an exponential decrease of tracer concentration with depth. Type 2 consisted of an exponential decrease with a subsurface secondary peak. Type 3 was characterised by a subsurface peak with surficial layers almost completely devoid of fluorescent particles. Temporal changes in the proportions of these profiles are presented in Table 1. When considering the whole aquarium, luminophore profiles were mostly of Types 1 and 2 during the first 24 h, whereas Type 3 was most frequent after 48 h. The same pattern, with an even higher proportion of profiles of Type 3 after 24 h, was observed when the analysis was restricted to characteristic conical areas (see below).

An example of a vertical luminophore profile together with the corresponding fitting of the biodiffusion model is presented in Fig. 5A. D_b values were first

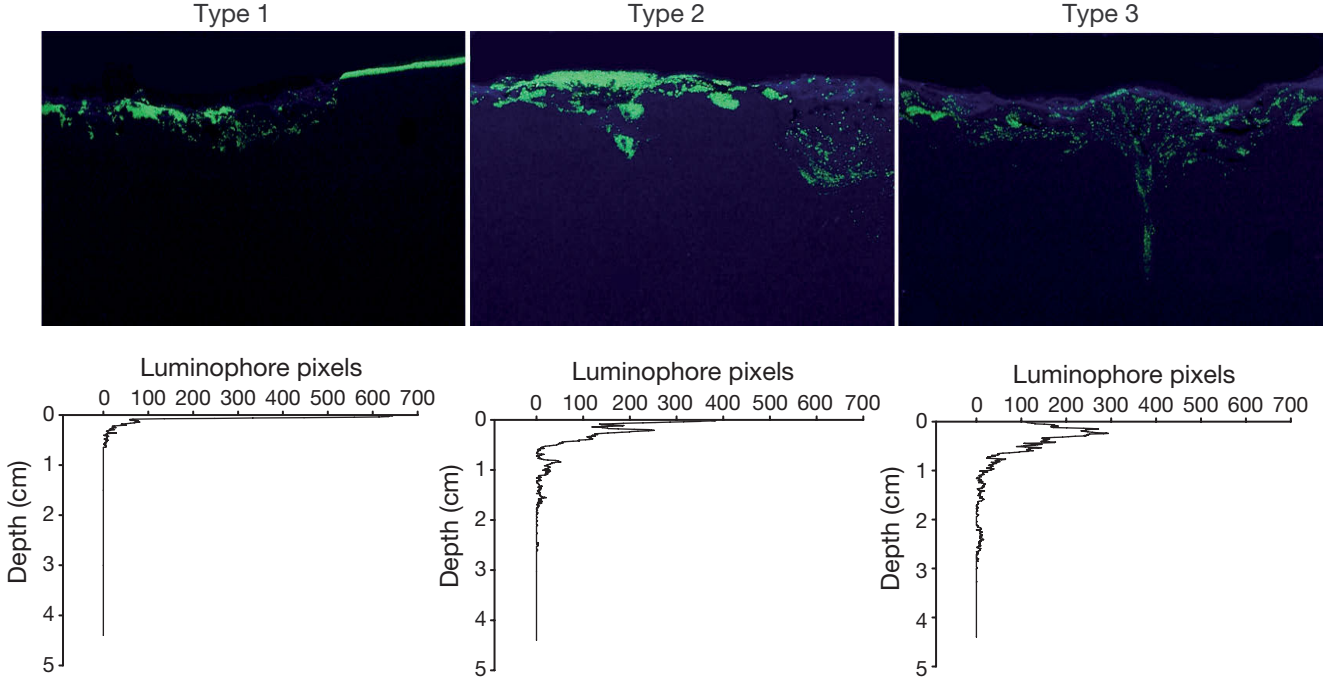

Fig. 4. *Abra ovata*. Presentation of the 3 main types of luminophore vertical profiles: upper panels are original images; lower panels are the corresponding vertical profiles

computed by fitting to profiles of whole aquaria. D_b values increased significantly with experiment durations (2-way ANOVA, $p < 0.001$) and were significantly affected by food treatments as well (2-way ANOVA, $p < 0.001$), with no significant interaction between these 2 factors ($p = 0.615$; Fig. 6). Temporal changes mostly corresponded to an increase of D_b with experiment durations in Treatment B and to a lesser extent in Treatments A and T. Treatment A resulted in the highest D_b values (LSD-test, $p < 0.05$). Corresponding D_b values were between 4.38 and 95.80 cm^2 yr^{-1}, with an average of 42.51 cm^2 yr^{-1}. Treatment T resulted in intermediate D_b values (between 2.47 and 41.30 cm^2 yr^{-1}, with an average of 22.71 cm^2 yr^{-1}). Treatment B resulted in the lowest D_b values (LSD-test, $p < 0.05$). Corresponding D_b values were between 1.26 and 49.38 cm^2 yr^{-1}, with an average of 11.21 cm^2 yr^{-1}.

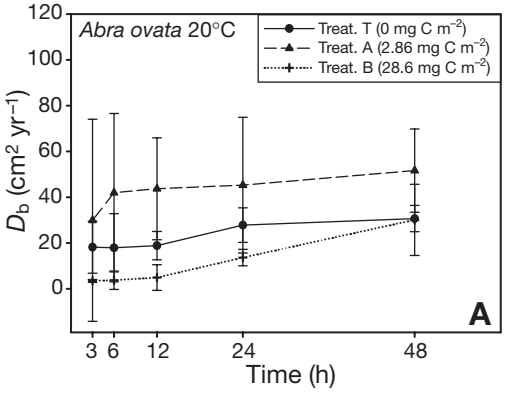

Fig. 6. *Abra ovata*. Temporal changes in average D_b when computed based on whole aquaria. Vertical bars are standard deviations

Abra nitida

Patterns of sediment reworking differed in *A. nitida* and in *A. ovata*. As opposed to what was observed for *A. ovata*, *A. nitida* sometimes moved within the sediment. Moreover, in *A. nitida*, the inhalant siphon tended to 'randomly' prospect the sediment surface, and the sediment area located right above the shell did not constitute a favoured foraging area. This resulted in a reworking of surficial sediment without particular structures, in contrast to those observed for *A. ovata* (Fig. 7).

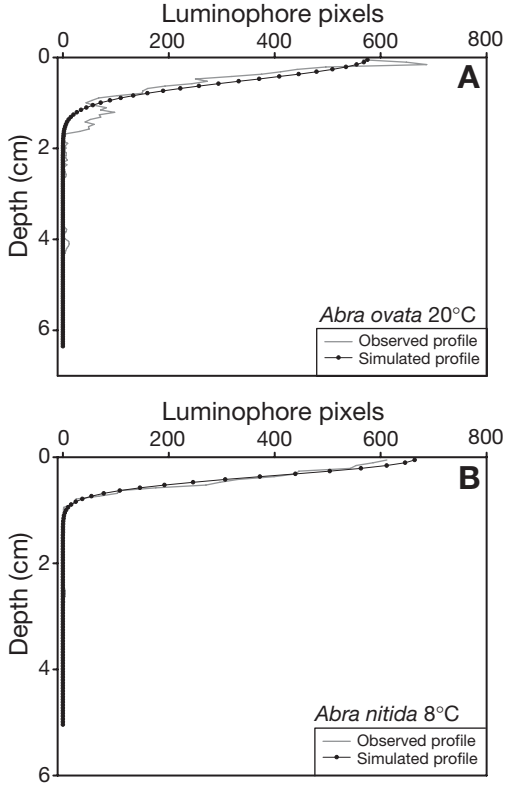

Fig. 5. *Abra* spp. Examples of the fitting of the biodiffusion model to vertical luminophore profiles: (A) *A. ovata* (20°C) and (B) *A. nitida* (8°C)

MPDs were significantly affected by experiment durations, food treatments and temperature (3-way ANOVAs, $p < 0.001$, $p = 0.014$ and $p = 0.045$, respectively), with no significant interaction between these 3 factors. MPDs increased with experiment durations (Fig. 8A, D). At time 0, the average MPDs were 0.85 cm. During the first 12 h, luminophores penetrated quickly into the sediment at a mean rate of 0.06 cm h^{-1}. After 12 h, MPDs increased much more slowly (mean rate of 0.009 cm h^{-1}). MPDs were maximal in Treatment B. The highest MPDs were measured in Treatment B (between 0.81 and 3.82 cm, with an average of 2.05 cm at 8°C, and between 0.86 and 4.74 cm, with an average of 2.85 cm at 15°C after 48 h). MPDs thus tended to be higher at 15 than at 8°C.

PRSs were also significantly affected by experiment durations, food treatments and temperature (3-way ANOVAs, $p < 0.001$, $p < 0.001$ and $p = 0.035$, respectively), with significant interaction between experiment durations and food treatments and between food treatments and temperature ($p < 0.001$ in both cases; Fig. 8B, E). PRSs increased with experiment durations; however, this increase occurred much earlier and was steeper in Treatments A and B than in Treatment T. PRSs were higher when food was added (Treatments A and B). At 8°C PRSs were higher in Treatment B than in Treatment A, whereas PRSs associated with these 2 treatments were almost similar at 15°C. The significant effect of temperature mostly reflected higher values of PRSs in Treatment A at 15°C.

An example of a luminophore profile for *Abra nitida* is presented in Fig. 5B. The same 3 types of vertical luminophore profiles were observed as in *A. ovata*.

However, their proportions were different (Table 1), as profiles of Types 1 and 2 were always dominant. D_b values were significantly affected by experiment durations and food treatments, but not by temperature (3-way ANOVAs, $p < 0.001$, $p < 0.001$ and $p = 0.975$, respectively), with a significant interaction between experiment durations and food treatments ($p = 0.001$; Fig. 8C, F). D_b tended to decrease with experiment duration. D_b values were highest in Treatment B, intermediate in Treatment A and lowest in Treatment T. The interaction between experiment durations and food treatments mostly reflected the fact that differences in D_b between food treatments decreased with experiment duration. The most important differences between treatments were observed after 3 and 6 h. After 12 h, there was only little difference in D_b among treatments.

Comparison between *Abra ovata* and *A. nitida*

The comparison between *A. nitida* and *A. ovata* was based on the results of experiments performed at 8 and 20°C, respectively (i.e. at the 2 seawater temperatures during field collection). MPDs were significantly affected by experiment durations, food treatments and species (3-way ANOVAs, $p < 0.001$, $p = 0.037$ and $p < 0.001$, respectively), with a significant interaction between experiment durations and species ($p = 0.014$). MPD increased with experiment duration. MPDs were almost similar for both tested species at the beginning of the experiments and then became higher for *A. ovata*. Despite the lack of significant interaction between food treatments and species ($p = 0.083$), MPDs were clearly higher in Treatment A in *A. ovata* and in Treatment B in *A. nitida*.

PRSs were significantly affected by experiment durations, food treatments and species (3-way ANOVAs, $p < 0.001$ in all cases), with significant interactions between experiment durations and species and between food treatments and species ($p < 0.001$ and $p < 0.001$, respectively). PRS increased with experiment duration. This increase was much steeper in *Abra nitida* than in *A. ovata*, which accounted for the significant interaction between experiment durations and species. As stated above, PRSs in *A. nitida* were always highest in Treatment B, intermediate in Treatment A and lowest in Treatment T. Conversely, PRS in *A. ovata* tended to be higher in Treatments T and A and lower in Treatment B. This discrepancy accounted for the significant interaction between food treatments and species. PRS clearly differed between species. Values first tended to be higher in *A. nitida* (Food Treatments B and A) and then became higher in *A. ovata* after 48 h.

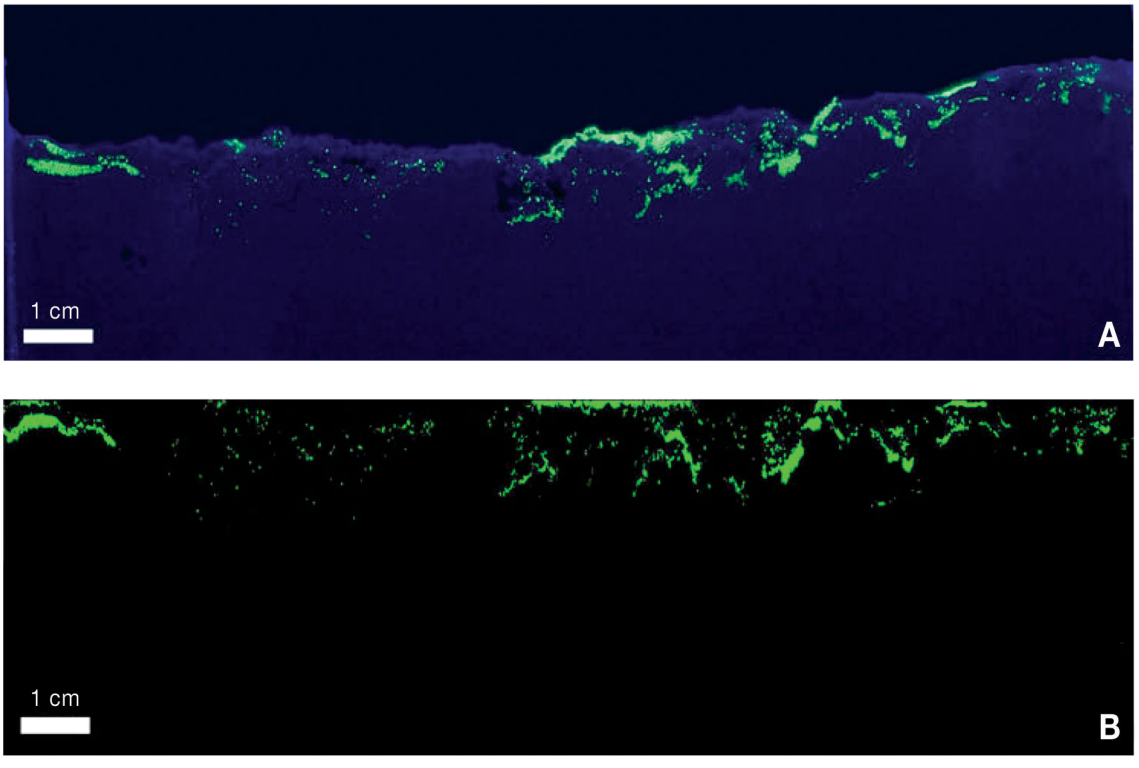

Fig. 7. *Abra nitida*. (A) Example of an original image with the sediment appearing in dark and luminophores in green. (B) Corresponding thresholded image with a flattened sediment–water interface. Note the lack of inverse conical structures

D_b significantly differed between experiment durations and food treatments, but not between species (3-way ANOVAs, p = 0.004, p = 0.001 and p = 0.225, respectively), with significant interactions between experiment durations and species and between food treatments and species ($p < 0.001$ and $p < 0.001$, respectively). D_b in *Abra ovata* tended to increase with experiment duration, whereas D_b in *A. nitida* clearly decreased with experiment duration. D_b values in *A. ovata* were highest in Treatment A, intermediate in Treatment T and lowest in Treatment B. Conversely, D_b values in *A. nitida* were highest in Treatment B, intermediate in Treatment A and lowest in Treatment T. In *A. nitida* differences in D_b between food treatments decreased with experiment duration. D_b first tended to be higher in *A. nitida* (especially in Food Treatments B and A) and then became higher in *A. ovata* after 48 h.

Effect of spatial scale on the computation of D_b

Besides through food treatments and species, D_b values were also significantly affected by spatial scale (3-way ANOVA, $p < 0.001$; Fig. 9). There was a significant interaction between spatial scale and experiment (i.e. *Abra ovata* 20°C, *A. nitida* 8°C and *A. nitida* 15°C), but not between spatial scale and food treatment (3-way ANOVAs, p = 0.002 and p = 0.065, respectively). Overall, D_b values computed at small spatial scales tended to be higher and also much more variable than those computed at large spatial scales. For the 3 experiments, average values of D_b became closer to the value computed for the whole aquarium for linear sections >3.750 cm. The interaction between spatial scale and experiments mostly resulted from the occurrence of a peak in D_b at intermediate spatial scale (i.e. 0.037 cm) during the *A. nitida* experiment carried out at 8°C. The results of this experiment also suggest that the effect of spatial scale on D_b was higher for Treatments A and B (i.e. when D_b values were higher).

In *Abra ovata*, D_b values computed for characteristic conical biogenic structures were significantly higher than those com-

Table 1. *Abra ovata* and *A. nitida*. Temporal changes in the proportions of the 3 main types of luminophore vertical profiles (T1, T2 and T3)

Species, temperatures	Food treatments (mg C m^{-2})	Time 0 h			Time 3 h			Time 6 h			Time 12 h			Time 24 h			Time 48 h		
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
A. ovata, 20°C	0	100	0	0	100	0	0	100	0	0	80	20	0	20	30	50	0	40	60
	2.86	90	10	0	80	20	0	80	20	0	40	50	10	0	80	20	0	60	40
	28.6	100	0	0	100	0	0	100	0	0	100	0	0	70	20	10	0	40	60
	All food treatments	96.7	3.3	0	93.3	6.7	0	93.3	6.7	0	73.3	23.3	3.3	30	43.3	26.7	0	46.7	53.3
A. ovata, conical area, 20°C	0	60	40	0	50	40	10	50	30	20	30	40	30	0	40	60	0	0	100
	2.86	80	20	0	80	20	0	40	50	10	10	40	50	0	20	80	0	0	100
	28.6	80	20	0	80	20	0	80	20	0	70	30	0	30	20	50	0	10	90
	All food treatments	73.3	26.7	0	70	26.7	3.3	56.7	33.3	10	36.7	36,7	26.7	10	26.7	63.3	0	3.3	96.7
A. nitida, 8°C	0	80	20	0	80	20	0	70	30	0	60	40	0	70	30	0	40	60	0
	2.86	100	0	0	70	30	0	70	30	0	60	40	0	60	40	0	40	50	10
	28.6	100	0	0	40	50	10	40	60	0	40	60	0	30	70	0	30	70	0
	All food treatments	93.3	6.7	0	63.3	33.3	3.3	60	40	0	53.3	46.7	0	53.3	46.7	0	36.7	60	3.3
A. nitida, 15°C	0	80	20	0	80	20	0	80	20	0	80	20	0	70	30	0	40	60	0
	2.86	90	10	0	70	30	0	70	30	0	50	50	0	40	50	10	20	50	30
	28.6	100	0	0	30	70	0	40	60	0	40	60	0	40	60	0	40	50	10
	All food treatments	90	10	0	60	40	0	63.3	36.7	0	56.7	43.3	0	50	46.7	3.3	33.3	53.3	13.3

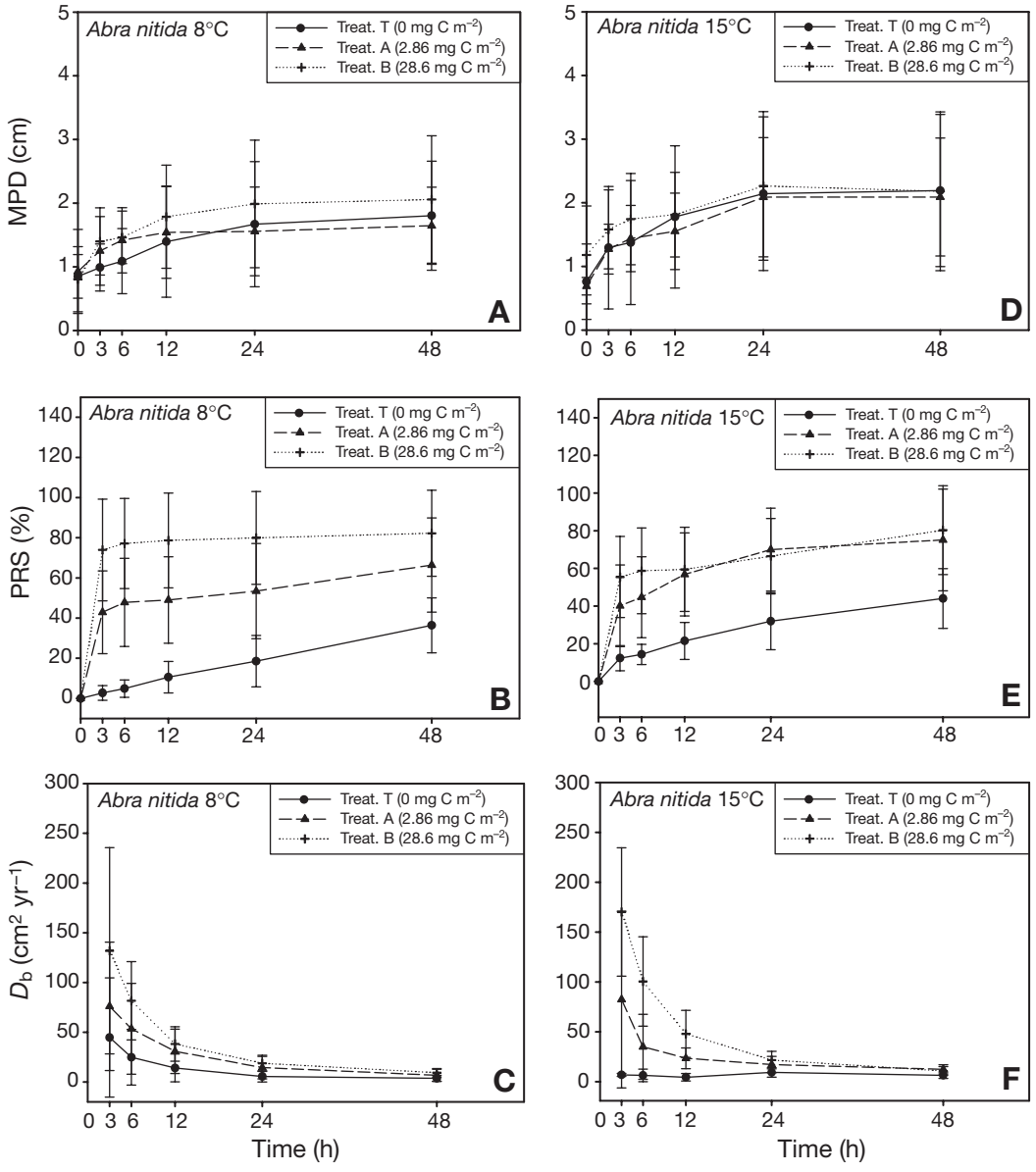

Fig. 8. *Abra nitida*. Temporal changes in average (A, D) MPDs, (B, E) PRSs and (C, F) D_b recorded during the (A to C) 8 and (D to F) 15°C experiments. Vertical bars are standard deviations

puted based on the whole aquarium (3-way ANOVA, $p < 0.001$; Fig. 10). Here again, average D_b increased significantly with experiment duration and significantly differed between treatments (3-way ANOVAs, $p < 0.001$ in both cases). There was significant interaction between the surface used for the computation of D_b and experiment duration, but not with food treatments (3-way ANOVAs, $p < 0.005$ and $p = 0.933$). The most important differences between treatments were recorded between 6 and 24 h, when Treatment A resulted in higher D_b than Treatments T and B. After 48 h, D_b values were similar in all 3 treatments (mean D_b of 97.07 cm^2 yr^{-1} for Treatment T, 90.93 cm^2 yr^{-1} for Treatment A and 98.32 cm^2 yr^{-1} for Treatment B).

The size of the vertical grid significantly affected D_b (3-way ANOVA, $p < 0.001$; Fig. 11), with a significant interaction between grid size and experiments, but not between grid size and food treatments (3-way ANOVAs, $p = 0.003$ and $p = 0.963$, respectively). The effect of grid size was much less pronounced in *Abra ovata* than in *A. nitida*. In *A. ovata*, D_b increased only slightly with vertical grid size (e.g. from 30.68 to 41.77 cm^2 yr^{-1} for grid sizes 0.052 and 0.998 cm, respectively, during Treatment T; Fig. 11). This increase was much more pronounced in *A. nitida*, especially for grid sizes >0.247 cm (e.g. from 3.41 to 3.97 and 18.77 cm^2 yr^{-1} for grid sizes of 0.052, 0.247 and 0.998 cm, respectively, during Treatment T at 8°C).

DISCUSSION

Description of sediment reworking in *Abra ovata* and *A. nitida*

In control aquaria without bivalves, luminophores remained at the sediment surface during the whole duration of the experiments and there was no temporal change in luminophore vertical profiles. Thus, sediment reworking by small infaunal organisms (<1 mm) and/or physical disturbance (e.g. caused by running seawater arrival) were negligible, and the observed patterns of sediment reworking clearly resulted from the activity of the 2 tested bivalves.

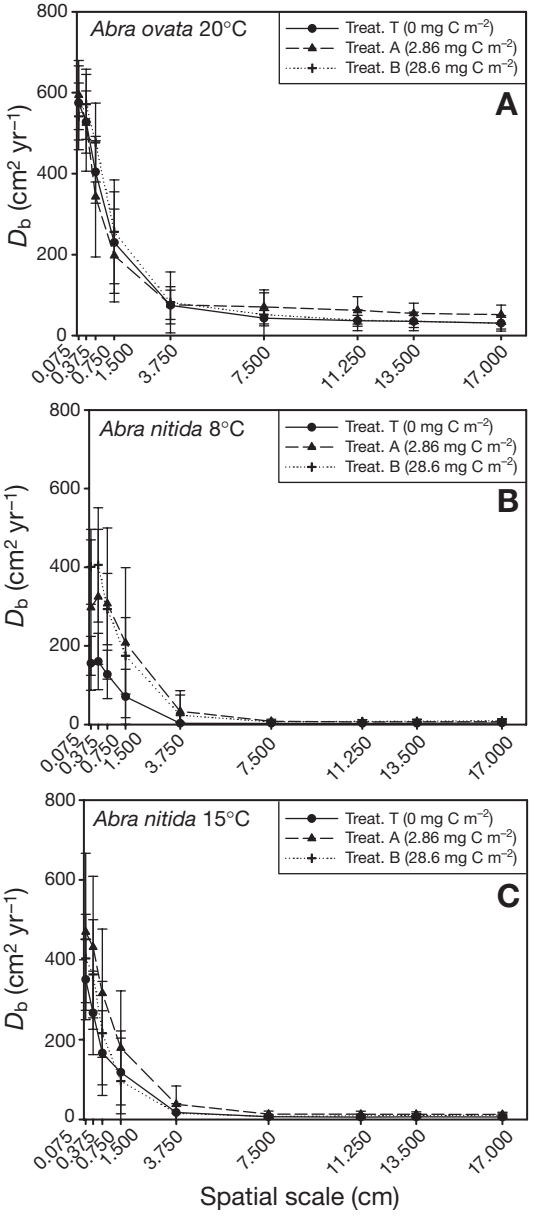

Fig. 9. *Abra* spp. Changes in average D_b computed at 48 h for increasing horizontal spatial scales: (A) *A. ovata* (20°C), (B) *A. nitida* (8°C) and (C) *A. nitida* (15°C). Vertical bars are standard deviations

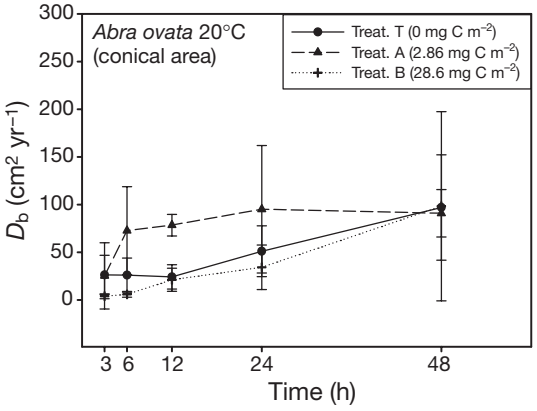

Fig. 10. *Abra ovata*. Temporal changes in average D_b when computed based on inverse conical areas (see 'Results'). Vertical bars are standard deviations

Abra ovata and *A. nitida* clearly exhibited 2 distinct patterns of sediment reworking corresponding to their feeding behaviour and, more specifically, to their motility and the functioning of their inhalant siphons. In *A. ovata*, distinct biogenic structures associated with sediment reworking by individual bivalves were observed at the end of all experiments. These structures consisted of inverse cones enriched in luminophores relative to the surrounding sediment. They coincided with the network of siphonal channels created by *A. ovata* from the subsurface chamber where it is located in the sediment. The tip of the cone was located right above this chamber, and the base of the cone corresponded to a section of the area explored by the inhalant siphon. We observed that *A. ovata* explored the sediment by: (1) extending the tip of its inhalant siphon from the aperture of a siphonal channel and (2) the creation of new siphonal channels. In this species, the first siphonal channel was always located almost vertically above the subsurface chamber. Two consecutive channels were always very close to one another, and the bivalves increased the surface of the explored area by progressively increasing the angle of their siphonal channels from the vertical. Due to the stationary position of *A. ovata* in the sediment this resulted in a network of galleries with a typical inverse conical shape. According to our observations, the downward transport of luminophores within this network resulted from small-scale transfer toward depth during each retraction of the siphon. A network of galleries with a similar inverse conical shape was described earlier for the network of siphonal channels in *A. nitida* (Wikander 1980). However, we did not observe any such structures during our experiments with *A. nitida*. Grémare et al. (2004) found that *A. nitida*

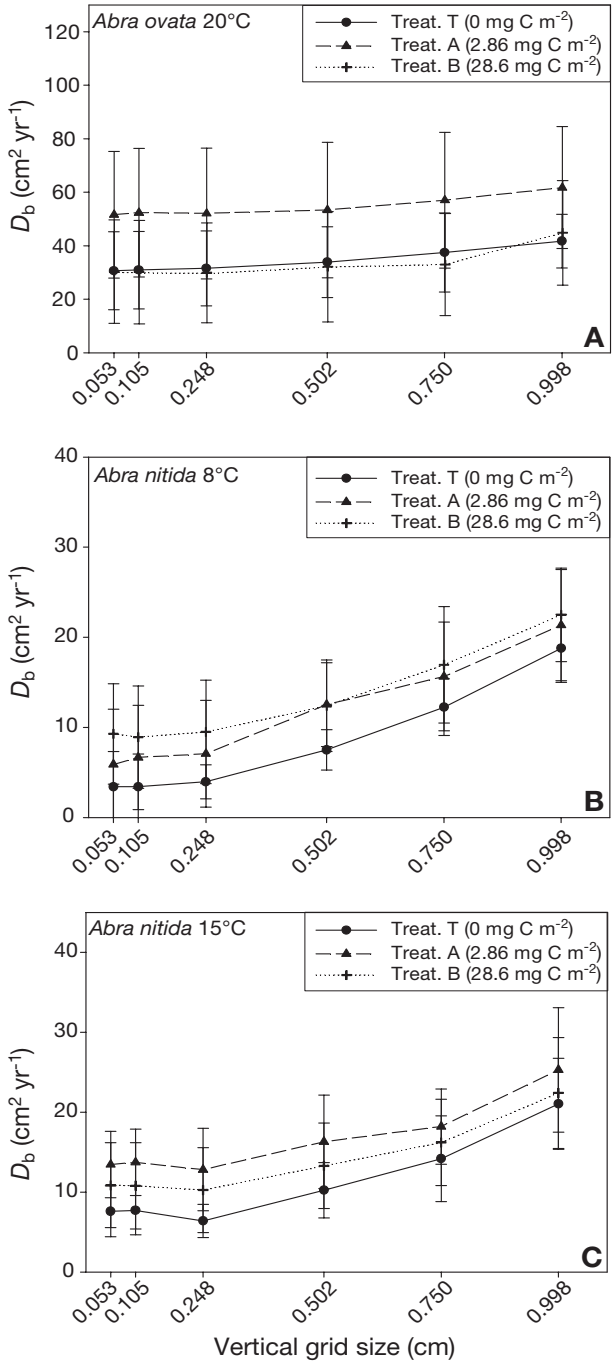

Fig. 11. *Abra* spp. Effect of vertical grid size on the computation of D_b: (A) *A. ovata* (20°C), (B) *A. nitida* (8°C) and (C) *A. nitida* (15°C). Vertical bars are standard deviations

could move quickly in the sediment in response to food addition. This was probably causing the sediment reworking patterns of *A. nitida.* The similarity of those patterns between the 3 food treatments constituted indirect evidence that *A. nitida* was moving in the sediment even when food availability was low.

During the first 24 h of the experiments, luminophore concentration decreased exponentially (Type 1) with depth in most cases for both *Abra ovata* and *A. nitida*. Later, most of the luminophore profiles exhibited a subsurface secondary peak in luminophore concentration (Type 2). In *A. ovata* only, subsurface primary peaks (Type 3) were often observed after 48 h. Vertical profiles are often used as indicators of sediment reworking types. They classically allow for the distinction between biodiffusion and non-local transport. Exponential profiles are associated with biodiffusion (Guinasso & Schink 1975, Cochran 1985, François et al. 1997, 2001), whereas profiles featuring a subsurface peak are often considered as indicative of non-local transport (Boudreau 1986, Meysman et al. 2003, Mugnai et al. 2003). Observations carried out during the beginning of our experiments strongly suggested that both *A. ovata* and *A. nitida* were biodiffusers (François et al. 1997). However, vertical luminophore profiles showing subsurface peaks were dominant in both species after 48 h. This was probably not indicative of non-local transport, since we did not observe either luminophore transport over long distances or luminophore patches indicative of non-local transport. Instead, our observations showed that subsurface peaks resulted from both: (1) the initial penetration of luminophores in the galleries formed by bivalves when burying and (2) the depletion of luminophores at the sediment surface due to feeding by the bivalves. Subsurface peaks, therefore, did not result from the transfer of luminophores to depth, but rather from differential intake at the sediment surface and at the depth of the subsurface peaks, where luminophores were initially introduced but later slowly reworked. A similar process has already been described for a natural Mediterranean species assemblage (Gérino et al. 1994). Based on our results, *A. ovata* and *A. nitida* clearly appeared as biodiffusers.

There were also considerable differences between *Abra ovata* and *A. nitida* in PRS and MPD. After 48 h with no food addition, *A. nitida* reworked on average 40% of the total surface of the aquarium versus 93% for *A. ovata*. As observed by Grémare et al. (2004), the extension of the inhalant siphon in *A. nitida* is limited, whereas *A. ovata* can extend its inhalant siphon over larger distances (typically a few centimetres) and is thus capable of exploring larger areas of the sediment without moving its shell. In the absence of food addition, this behaviour resulted in larger reworked areas than in *A. nitida*, despite *A. ovata* being stationary. It should, however, be pointed out that the surface affected by sediment reworking in *A. ovata* quickly decreased with depth, whereas it was constant over the whole reworked sediment layer for *A. nitida*. *A. ovata* also tended to rework a thicker sediment layer (3 cm on average) than *A. nitida* (1.5 cm on average). Differ-

ences in sediment reworking between the 2 species may be a consequence of specific adaptations to avoid and reduce predation and to handle hydrodynamic conditions prevailing in their respective habitats. Grémare et al. (2004) suggested that the low extension of the inhalant siphon in *A. nitida* could be an adaptation to avoid siphon nipping by juvenile benthic fishes. Along the same line, deeper burying in *A. ovata* may constitute an adaptation to avoid resuspension by strong currents and waves (Millet & Guelorget 1994), which may occur in shallow Mediterranean lagoons during winter storms.

Quantification of sediment reworking: effects of food availability

Quantification of sediment reworking based on optic measurements of luminophores mixing along the thin planes adjacent to the glass of an experimental aquarium or a sediment profiler has already been used both during *in situ* (Solan et al. 2004b) and laboratory experiments (Gilbert et al. 2003). One of the key assumptions of this approach is that the sediment mixing observed on the photographs is representative of that taking place in the entire sediment column. To our knowledge, this hypothesis has never been tested. Our results showed that there was a strong correlation between the concentrations of luminophores derived from: (1) the analysis of the photographs of the walls of 5 experimental aquaria and (2) the direct counting of sediment slices of the whole corresponding aquarium. As a consequence, the vertical profiles obtained by these 2 approaches were almost identical, which clearly supports the experimental approach used during the present study.

Bivalve densities used during our experiments were compatible with field abundances reported for the 2 tested species (Guelorget & Mayere 1981, Josefson 1982). Our results clearly showed that sediment reworking by the 2 tested bivalves quickly led to a major reduction of luminophore concentrations at the water–sediment interface, which may have biased the computation of D_b should experiments have been prolonged. More generally, all experiments carried out under non-steady conditions are limited by the tracer availability at the water–sediment interface (Gérino et al. 1994). Moreover, it should be stressed that such a depletion of luminophores may well occur faster in preferentially reworked area and may thus not be apparent if vertical luminophore profiles are computed for whole experimental aquaria. To overcome this bias, our experiments were restricted to 48 h, which is very short term compared to radioisotope measurements (Sharma et al. 1987, Soetaert et al. 1996, Wheatcroft & Martin 1996, Alperin et al. 2002, Green et al. 2002) or even to most experiments involving luminophores (Table 2).

It is essential that such short-term experiments are carried out on well-acclimated animals. During the present study, we introduced the bivalves into the experimental aquaria 24 h before the beginning of the experiments. Grémare et al. (2004) used a similar procedure to study the feeding activity of *Abra ovata* and *A. nitida* and concluded that this acclimation period resulted in a constancy of feeding behaviours during short-term experiments. Due to the strong correlation between biological activity and sediment reworking, we are thus confident in stating that the acclimation period used during the present study was sufficient to insure adequate quantification of D_b.

The validity of our experimental approach is further supported by a comparison between the D_b values recorded during the present study and literature data. Without food addition, our D_b values averaged 30.7 and 3.7 $cm^2\ yr^{-1}$, based on 48 h studies for *Abra ovata*

Table 2. Compilation of literature values of D_b, computed based on luminophore experiments and the biodiffusive model

Geographical area	Species	D_b ($cm^2\ yr^{-1}$)	Experiment duration (h)	Temperature (°C)	Density (ind. m^{-2})	Source
Gulf of Fos	Field community	219.80	528			Gérino (1990)
Gullmarsfjord	Field community	14.00	216			Gilbert et al. (2003)
Gullmarsfjord	Field community	126.00	16			Solan et al. (2004b)
Laboratory	*Ruditapes decussatus*	5.11	168	15	724	François et al. (1998)
Laboratory	*Venus aurea*	2.78	168	15	724	François et al. (1998)
Laboratory	*Nereis diversicolor*	3.05	360	16	800	François et al. (2002)
Laboratory	*Nereis diversicolor*	5.50	720	16	800	François et al. (2002)
Laboratory	*Cardium edule*	0.91	480	14	250	Mermillod-Blondin et al. (2005)
Laboratory	*Corophium volutator*	1.82	480	14	5100	Mermillod-Blondin et al. (2005)
Laboratory	*Abra ovata*	14.56–69.73	48	20	1470	Present study
Laboratory	*Abra nitida*	0.17–15.70	48	8	1470	Present study
Laboratory	*Abra nitida*	2.47–16.14	48	15	1470	Present study

and *A. nitida*, respectively. These values can be compared with literature data based on luminophore experiments and biodiffusive models (Table 2), even though this comparison is complicated by strong heterogeneity in experimental conditions (e.g. individual species vs. species assemblages or whole communities, field vs. laboratory measurements, abundance of macrofauna per unit surface area). Nevertheless, the values recorded during the present study are of the same order of magnitude as literature data. Our D_b values are slightly lower than community estimates carried out in the Gulf of Fos (Gérino 1990) and in the Gullmarsford (Solan et al. 2004b). Conversely our estimates of D_b in *A. ovata* are higher than those reported for other individual species, such as *Cardium edule*, *Corophium volutator*, *Venus aurea*, *Ruditapes decussatus* and *Nereis diversicolor* (François et al. 1998, 2002, Mermillod-Blondin et al. 2005). Even if caution should be taken in comparing D_b derived from the measurements of different tracers, it should be pointed out that the range of D_b recorded during the present study (i.e. between 0.2 and 252.3 cm^2 yr^{-1}) is in good agreement with the overall range of 1 to 400 cm^2 yr^{-1} reported for coastal sediments, mostly based on radioisotope measurements (Tromp et al. 1995). Moreover, the average values recorded during the present study are also close to those recorded by Lecroart et al. (2005) and Schmidt et al. (in press) in the Thau Lagoon (NW Mediterranean), based on both ^{234}Th and ^{7}Be measurements (i.e. up to 10 and 30 cm^2 yr^{-1} depending on the vertical flux of particulate organic matter caused by cultivated bivalves). Mermillod-Blondin et al. (2005) recently underlined the difficulty in comparing the results of sediment reworking experiments dealing with single species and those dealing with assemblages of species. One difficulty is clearly associated with the occurrence of interactions between species, and another one with the way of controlling biomass during comparative experiments. One possibility consists of comparing observed and expected (i.e. computed based on the sum of all the individual sediment reworking rates of the species present in the assemblage) reworking rates. The difference between observed and expected reworking rates is usually negative, pinpointing the occurrence of negative interactions between species (Mermillod-Blondin et al. 2005). Even if caution should clearly be taken in applying the same approach to D_b values recorded in *A. ovata* and those measured by Lecroart et al. (2005) and Schmidt et al. (in press) in the Thau Lagoon, our results nevertheless suggest that *A. ovata* is a major contributor to sediment reworking in Mediterranean lagoons.

D_b values were significantly affected by food treatment, both in *Abra ovata* and *A. nitida*. However, there were clear differences in the functional responses of the 2 tested species. For *A. ovata*, D_b values were highest at intermediate food concentrations and greatly reduced at high food concentrations. Conversely, for *A. nitida*, D_b increased with food concentration. This pattern is fully coherent with the functional responses exhibited by the same 2 species in terms of feeding activity (Grémare et al. 2004). This further confirms that sediment reworking is mostly caused by feeding activity in the 2 tested bivalves. Moreover, difference in functional response underlines the necessity of running comparative experiments at different food levels for the study of sediment reworking (Grémare et al. 2004). Schmidt et al. (in press) carried out a seasonal survey in the Thau Lagoon based on field measurements of ^{7}Be and ^{234}Th profiles. These authors compared 2 sites, the first one located in the middle of the lagoon and the second one close to aquaculture tables (and thus subject to much higher inputs of organic matter). They consistently reported higher D_b under the aquaculture tables. Unfortunately, no data on the composition of macrofauna are provided, and it is thus impossible to assess the effect of differences in macrofauna on sediment reworking rates. Nevertheless, our data suggest that such differences in D_b may partly result from the functional response of individuals belonging to the same species, but subjected to different levels of organic inputs. More generally, Tromp et al. (1995) reported a positive correlation between D_b and sedimentation rate. This relationship was interpreted to result from the positive correlation between sedimentation rates and vertical fluxes of organic matter to the water–sediment interface, which themselves correlate positively with benthic standing crops. This relationship is clearly associated with large spatio-temporal scales, and our results show that D_b values can also vary over a short temporal scale in response to sedimentation rates.

Sediment reworking can be assessed based on a large variety of tracers, including luminophores, chlorophyll *a* and radioisotopes such as ^{234}Th and ^{7}Be. These tracers integrate sediment reworking on different time scales (Goldberg & Koide 1962, Gérino et al. 1998, Fuller et al. 1999, Thomson et al. 2000, Lecroart et al. 2005). Due to technical constraints, field measurements of sediment reworking are most often based on the assessment of radioisotope profiles (Rice 1986, Sharma et al. 1987, Wheatcroft & Martin 1996, Soetaert et al. 1996, Alperin et al. 2002, Green et al. 2002, Kniskern & Kuehl 2003, Widdows et al. 2004, but Solan et al. 2004b). One of the important assumptions of the models used to derive D_b values from those profiles is the constancy of sediment reworking rates during the period of time integrated by isotope profiles. Our results show that sediment reworking is a process that

occurs over a short-time scale and can be affected by short-term biological events such as the sedimentation of a phytoplanktonic bloom. In the Skagerrak, for example, pelagic primary production and associated sedimentation show a sharp peak during spring (Lindahl 1988, Belgrano et al. 1999). Such events are typically restricted in time and correspond to a temporal scale shorter than those associated with the measurements of radioisotope profiles. In this sense, our results suggest that field measurements of D_b based on radioisotopes may underestimate maximal sediment reworking rates associated with the sedimentation of phytoplanktonic blooms. Moreover, radioisotopes used to assess sediment reworking generally show a strong affinity for particles. Their flux to the water–sediment interface is thus likely to correlate positively with the vertical flux of organic matter sedimenting to the water–sediment interface. As recently pointed out by Lecroart et al. (in press) this violates the assumption of steady inputs of radioisotopes to the water–sediment interface and may result in an even higher underestimation of computed D_b values during strong sedimentation events. In this context, the use of luminophores as tracers of sediment reworking appears especially suitable to measure and to detect changes in D_b over short time scales since: (1) their input to the water–sediment interface is fully controlled and (2) their measured vertical profiles are only dependent on these controlled inputs.

Effects of spatial and temporal scales

One of the main advantages of the experimental approach used during the present study relative to classical luminophore experiments is that it allows assessment of sediment reworking at spatial scales smaller than the whole studied aquarium. Both in *Abra ovata* and *A. nitida*, D_b tended to decrease with spatial scales up to 3.75 cm. Part of the relationship between spatial scale and D_b in *A. ovata* clearly resulted from the existence of biogenic structures, which were typically between 3 and 4 cm wide. D_b values computed for these structures were indeed about twice as high as those computed for the whole aquarium. In *A. ovata*, the magnitude of the changes in D_b in relation to spatial scale was, however, much larger than a factor of 2. Moreover, the relationship between D_b and horizontal scale was very similar in *A. nitida*, where biogenic conical structures were absent. One of the main differences between the 2 species was the average PRS after 48 h. In *A. ovata*, the average PRS was close to 100% after 48 h for all treatments, whereas it was between 30 and 80% in *A. nitida*. The relationship between horizontal scale and D_b thus probably partly resulted from the heterogeneity between reworked and non-reworked areas in *A. nitida* and from the heterogeneity associated with biogenic structure within a reworked area in *A. ovata*. In any case, our results clearly underline the necessity to take into account the appropriate spatial scale to precisely assess individual sediment reworking rates. The relationship between spatial scale and sediment reworking is clearly dependent on the studied animal (or community). It is thus impossible to extrapolate the results of the present study to other experimental models. As far as *A. ovata* and *A. nitida* are concerned, our results nevertheless suggest that the sizes of cores (i.e. typically between 50 and 70 cm^2) used during classical luminophore experiments are suitable to infer sound values of D_b.

Our results clearly show the significant effect of vertical grid size on the computation of D_b in *Abra nitida*, but not in *A. ovata*. These results are fully consistent with the fact that, in order to allow for sound assessment of sediment reworking, vertical grid size has to be: (1) larger than microscopic sediment variations and (2) smaller than the scale of gradient concentrations (Boudreau 1986). The luminophores used during the present study were between 100 and 160 µm in size, and we tested grid sizes between 520 and 9970 µm. All tested grid sizes were thus clearly larger than microscopic sediment variations. Our results showed that MPDs were higher in *A. ovata* than in *A. nitida*. Since the scales associated with gradient concentrations correlate positively with MPD (Boudreau 1986), differences in the effect of vertical grid size on D_b in *A. ovata* and in *A. nitida* thus probably corresponded to differences in the scales associated with gradient concentrations in these 2 species. D_b in *A. nitida* started to increase at a vertical grid size as small as 0.5 cm, which is smaller than the minimal thickness of sediment layers sliced during classical luminophore experiments (Gérino et al. 1994, François et al. 1998, 2002, Mugnai et al. 2004, Ouellette et al. 2004, Mermillod-Blondin et al. 2005). Thus, such classical experiments would not be adequate to quantify sediment reworking in *A. nitida* unless a technical procedure is found to cut thinner sediment layers. The shape of vertical concentration profiles also constitutes an indicator of sediment reworking types, and the occurrence of a secondary subsurface peak, for example, is generally considered indicative of non-local transport (Boudreau 1986, Meysman et al. 2003, Mugnai et al. 2003). Our results showed that the perception of true profiles is strongly affected by vertical grid size (Fig. 12). More specifically, the subsurface peaks, which were apparent for small grid sizes, tended to disappear for larger grid sizes (Fig.12), and the 'observed' decrease in luminophore concentrations with depth became better fitted using a biodiffusive model. Meysman et al.

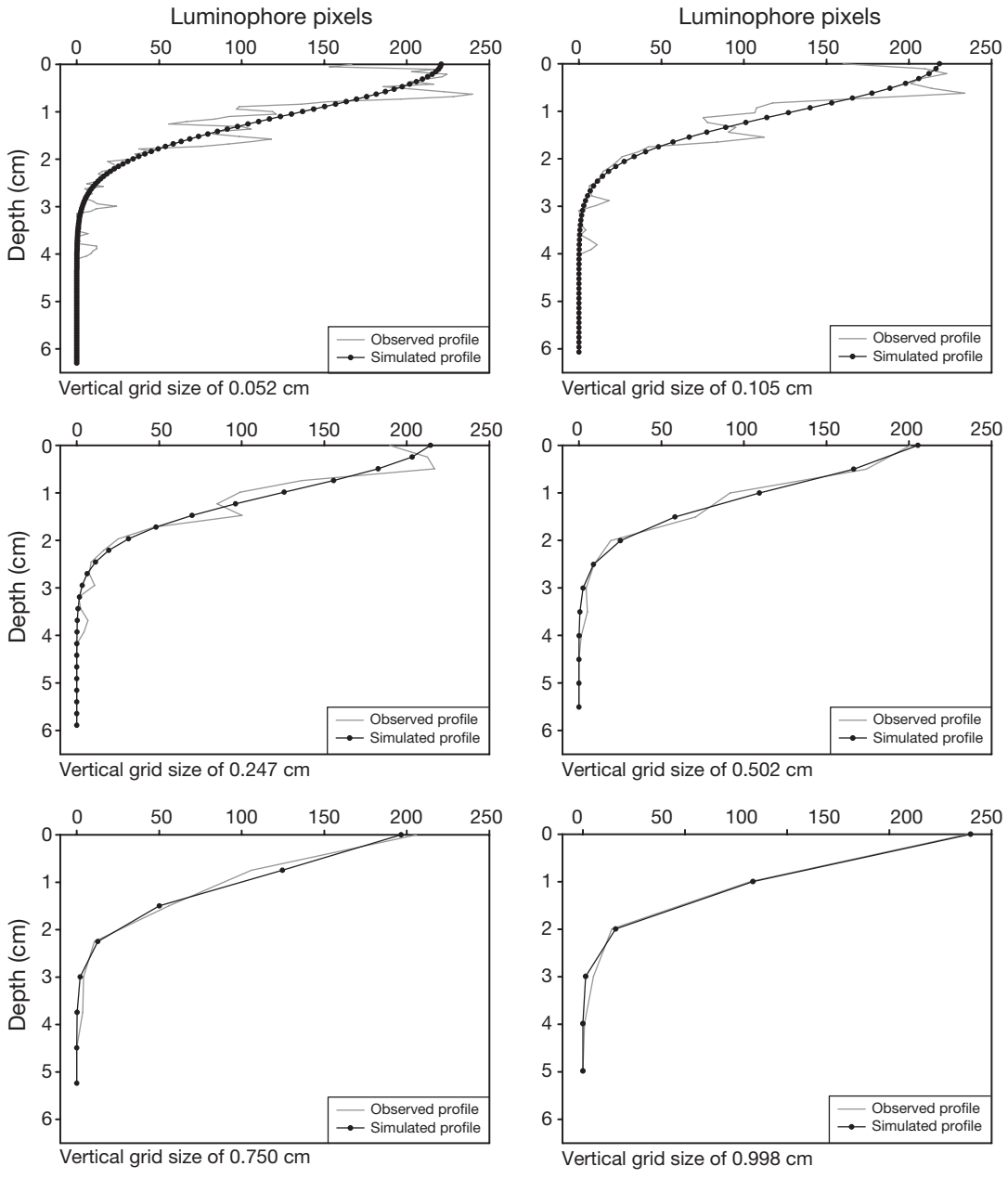

Fig. 12. Example of the effects of vertical grid size on the fitting of the biodiffusive model to vertical luminophore profiles. Data correspond to a Treatment T (0 mg C m^{-2}) aquarium with *Abra ovata* (20°C, 48 h of experiment)

(2003) recently pointed out the so-called 'biodiffusion paradox', since biodiffusion has proven to constitute an empirical model of reference for sediment reworking even though some of its theoretical requirements (especially the length criterion) are rarely met. The most often invoked explanation for this paradox is that the occurrence of several sediment reworking types within the same community, in the long run (i.e. the integration period of radioisotopes), results in exponential vertical profiles. At the single species level, and over a much shorter time scale, our results suggest that part of the paradox could also result from the low resolution of vertical profiles, which are used to fit sediment reworking models and to infer sediment reworking rates. Such low resolution indeed results in underestimation of subsurface peaks and thus enhances the fitting of biodiffusion models. This hypothesis can be tested by comparing the fittings of both biodiffusive and non-local transport models over a large range of vertical grid sizes. It is suspected that there will be no major differences between models over the whole range of vertical grid sizes in *A. ovata*

and *A. nitida*, because these 2 species are both biodiffusers (see above). However, it would be interesting to further test this hypothesis on a data set acquired with the experimental approach used during the present study, but on an animal belonging to another functional group. Overall, our results clearly underline the necessity of adapting vertical grid size to the studied animals or community when assessing sediment reworking rates. Here again, our experimental approach allows *a posteriori* and highly flexible determination of this grid size.

Another advantage of the experimental approach used during the present study relative to classical luminophore experiments is that it allows assessment of the kinetics of sediment reworking. As mentioned above, our experiments were clearly short term, and care should be taken in interpreting temporal changes. The temporal trends recorded during the present study were in good agreement with previous observations regarding the interaction between feeding activity, feeding intensity and time devoted to feeding in the 2 studied species (Grémare et al. 2004). We thus believe that the temporal trends were real and are worth being discussed. Temporal changes in D_b differed between the 2 species. D_b tended to decrease with experiment duration in *Abra nitida* and to increase with experiment duration in *A. ovata*. This discrepancy probably resulted from the different feeding strategies of these 2 species. In *A. nitida*, the increase in feeding activity mostly results from an increase in feeding intensity (Grémare et al. 2004). During our experiments sediment reworking rates were maximal immediately after food addition, which confirmed the ability of this species to rapidly exploit food pulses resulting from the sedimentation of phytoplanktonic blooms (Grémare et al. 2004). Conversely, increase in feeding activity in *A. ovata* mostly resulted from an increase in the amount of time devoted to feeding. This may explain why during our experiments sediment reworking rates increased progressively with time. In any case, such differences in temporal changes in sediment reworking rates underline the importance of carefully defining the duration of sediment reworking experiments, which are often fixed *a priori* in classical luminophore experiments (Gérino et al. 1994, François et al. 1998, 2002, Mugnai et al. 2003).

CONCLUSIONS

The combined utilisation of thin aquaria, luminophores and image analysis techniques proved efficient in visualising, characterising and quantifying sediment reworking processes at different time and spatial scales, information that is required to precisely study the behavioural ecology of infaunal benthic organisms. Our results showed that, despite being closely related, *Abra ovata* and *A. nitida* featured 2 distinct types of sediment reworking. These 2 species were efficient bioturbators, which resulted in a quick reduction of luminophore at the water–sediment interface and thus limited experimental duration. *A. ovata* and *A. nitida* featured 2 different responses to organic matter availability. These responses were coherent with recently described types of feeding activity in these 2 bivalves (Grémare et al. 2004). The experimental approach used during the present study allowed the assessment of sediment reworking at different spatial scales and for different vertical grid sizes. D_b values were strongly affected by these 2 parameters due to: (1) spatial heterogeneity associated with sediment reworking and (2) the magnitude of vertical grid sizes relative to concentration gradients. It was concluded that the sizes of cores (i.e. up to 50 cm^2) most often used during classical luminophore experiments would be suitable to assess sediment reworking in the 2 tested species. Conversely, a vertical grid size of 0.5 cm, which is commonly used during such experiments, would not be appropriate to assess sediment reworking in *A. nitida*, due to the thinness of the reworked sediment layer.

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