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# Activities by *Hediste diversicolor* under different light regimes: Experimental quantification of particle reworking using time-resolved imaging

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## abstract

Particle reworking by the ragworm *Hediste diversicolor* was assessed by quantifying the transport of fluorescent particles (luminophores) added to the surface sediment during a 10 day experiment. Plexiglass cores and thin glass aquaria with fauna and controls were exposed to either 12 hour light/dark cycles or constant darkness. Luminophore distributions were evaluated by side-view imaging of the aquaria together with destructive sectioning and quantification of tracer distributions in both types of microcosms at the end of experiments. Tracer distributions were evaluated by the gallery-diffusor model, from which the biodiffusion ( $D_b$ ) and the non-local transport ( $r$ ) coefficients were determined. In addition, the maximum penetration depth (MPD) of luminophores, the 2-D redistribution coefficient, and the transport rate were used as a suite of proxies to quantify particle reworking by fauna.

All measured reworking proxies demonstrated a general decrease in transport of luminophores in darkness compared to light/dark cycles. The difference was significant for proxies determined from sectioning. Imaging of particle transport demonstrated that rates were ~30% higher during light/dark cycles, with ~constant amount of particles transported on a daily basis. The effect of light was consistent in the two microcosm types. However, there was a significant difference in  $D_b$ , while  $r$  and MPD were not significantly different between the cores and aquaria. Overall, these results suggest a light-triggered surface feeding by *H. diversicolor*. Our study highlights the importance of experimental settings for quantification of particle transport by fauna, and that light conditions and types of experimental microcosms need to be carefully considered during investigations of bioturbation in illuminated environments.

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### Keywords:

Bioturbation  
Gallery-diffusor  
*Hediste diversicolor*  
Luminophores  
Sediment reworking  
Time-resolved imaging

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## 1. Introduction

Benthic macrofauna significantly influences early diagenesis and the fate of organic material deposited on the sediment surface. Faunal activities not only alter the physical geometry and biogeochemical structure of the sediment, but also affect the transport of particles, solutes and gases across the sediment water interface and across redox boundaries within the sediment (Aller, 1994; Rhoads, 1974). This is done mainly by redistributing material during e.g. feeding, burrowing, ventilation, tube construction, and fecal pellet formation (Aller, 1982; Kristensen et al., 2012). It is therefore essential to quantify particle and solute mixing induced from bioturbating fauna and to include faunal reworking activities in qualitative and quantitative models describing biogeochemistry and element cycling in surface sediments. Reworking parameters for particle transport by fauna are

normally extracted from experimental quantification of the vertical distribution of tracers and subsequent reaction-transport modeling (Aller, 1982; Berner, 1980; Boudreau, 1997). The most frequently applied model for particle transport relies on the biodiffusive approach in which the conservation of mass and Fick's first law of diffusion are combined into the biodiffusion coefficient,  $D_b$  (Boudreau, 1986; Goldberg and Koide, 1962; Guinasso and Schink, 1975). The underlying theoretical assumptions in the biodiffusion model have recently been reevaluated and partly revised (Meysman et al., 2010). Despite that model constraints are often violated (Lecroart et al., 2010; Wheatcroft et al., 1990), tracer distributions obtained from the biodiffusion model are frequently in close accordance with those obtained from direct measurements (Meysman et al., 2003). Models for particle reworking have occasionally been modified to also include biological transport of particles in a non-diffusive manner. Such modifications are referred to as the non-local transport or anomalous reworking (Fisher et al., 1980; Meysman et al., 2010; Smith et al., 1986). Francois et al. (1997, 2002) have developed a suite of

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models that couple to the reworking modes of individual species. Of these, above all the gallery-diffusor model (François et al., 2002) has successfully been applied to evaluate reworking activities by *Hediste diversicolor* (Duport et al., 2006; Mermillod-Blondin et al., 2004). The rapid transport of particles from the sediment surface to deeper sediment layers is estimated by the non-local transport coefficient,  $r$ , while the diffusive-like transport in surface sediments is estimated by the biodiffusion coefficient,  $D_b$ .

A vast majority of previous experiments designed to assess particle reworking activities by *H. diversicolor* have integrated particle reworking over the experimental period (often 10 to 30 days). In these studies, the vertical distributions of tracers were mainly quantified by manual efforts following sectioning of sediment cores in discrete sediment layers with inherent destructive sampling protocols (Duport et al., 2006; François et al., 2002; Hedman et al., 2011). Therefore, the temporal evolution of reworking parameters (or proxies) is normally excluded and not quantified. Gilbert et al. (2003) developed a technique for 2-dimensional experimental quantification of particle displacement by fauna using side-view imaging of fluorescent particles (luminophores). With the non-destructive side-view imaging of luminophores emerging as a complementary technique to estimate particle reworking, there is also a potential for high-resolution (temporal and spatial) studies of bioturbation activities by benthic macrofauna (Maire et al., 2008; Schiffers et al., 2011). A number of bioturbation experiments have since been conducted using concepts originating from the fluorescence imaging approach (Maire et al., 2006; Piot et al., 2008; Schiffers et al., 2011), including imaging of faunal activities in situ (Solan et al., 2004). However, the thin aquaria used for imaging experiments may bias quantification of reworking proxies. Potential artifacts associated with experimental settings include e.g. alterations in reworking behavior due to wall effects and the physical geometry of the microcosm. To our knowledge, there is no previous study that quantifies and compares reworking proxies in cores and aquaria.

The common ragworm *H. diversicolor* (Polychaeta, Nereididae), normally classified as a gallery-diffusor in terms of mode of particle reworking (François et al., 2002), is a frequently used model species for experimental studies of bioturbation by benthic macrofauna. The polychaete is commonly found in marine and brackish waters of the littoral zone across the Atlantic coasts of North America and Europe (Scaps, 2002). *H. diversicolor* has during several studies been demonstrated as a major bioturbator with significant influence on the biogeochemical structure, function, and dynamics of surface sediments. For example, bioturbating activities by *H. diversicolor* may have considerable, but often not easily predicted, effects on organic matter cycling and nutrient fluxes in shallow-water sediments (Kristensen et al., 1992; Nizzoli et al., 2007; Papaspyrou et al., 2010). In addition, activities by *H. diversicolor* may have significant effects on phytoplankton production (Vedel et al., 1994), as well as on the initiation, growth and sustenance of macroalgal mats and seagrass beds in shallow-water embayments (Hughes et al., 2000; Nordström et al., 2006).

There are several environmental factors that affect the behavior of *H. diversicolor* and thereby also the magnitude and extent of bioturbation. Availability of food supply (Nogaro et al., 2008; Papaspyrou et al., 2010), tidal cycles (Esselink and Zwartz, 1989) and availability of light (Lambert et al., 1992; Tang and Kristensen, 2007; Wenzhöfer and Glud, 2004) provide examples that have been included in studies focused on the behavior of *H. diversicolor*. Investigations that specifically describe how the availability of light affects bioturbation and sediment reworking by *H. diversicolor* have up to now concentrated on the short-term (24–36 h) behavior during feeding (Lambert et al., 1992), the oxygen uptake (Wenzhöfer and Glud, 2004) and the benthic metabolism (Tang and Kristensen, 2007).

The overall aim of the study was to quantify particle displacement by *H. diversicolor* using the non-destructive fluorescence imaging approach and the destructive manual sectioning technique. Imaging was primarily used for high resolution quantification of reworking

proxies in both time and space. Specific objectives were to investigate how experimental proxies for particle reworking were affected by 1) the exposure of *H. diversicolor* to constant darkness as compared to 12 h light/dark cycles, and 2) sediment water incubations in thin glass aquaria as compared to incubations in Plexiglass cores.

## 2. Materials and methods

### 2.1. Experimental design and general overview

In this tracer-pulse experiment, the effects of i) light/dark cycles compared with constant darkness (statistical factor Light) and ii) Plexiglass cores compared with glass aquaria (statistical factor Microcosm) on particle reworking activities by *H. diversicolor* were investigated in a factorial design. Individuals of *H. diversicolor* were added to microcosms (Plexiglass cores (C) or thin glass aquaria (A)) and were either exposed to 12 h light/dark cycles (Li/Da), or to constant darkness (Dark) ( $n = 6$ ). There were also corresponding control treatments without fauna added ( $n = 6$ ).

Particle displacement was evaluated from vertical profiles obtained by sectioning of sediment and modeling (François et al., 2002) ( $n = 6$ ) and from time-resolved imaging of the fluorescent particles in thin aquaria (Gilbert et al., 2003) (I;  $n = 3$ ). In addition to  $D_b$ ,  $r$  and maximum penetration depth of luminophores (MPD) (Maire et al., 2006), the fluorescence imaging approach provided bioturbation proxies associated with 2-D redistribution, and transport rates.

### 2.2. Sampling and set-up

Surface sediment (0–5 cm) and *H. diversicolor* were sampled by hand from a shallow bay on the Swedish west coast (Rågårdsvik, N 58°12'32"; E 11°26'47") in May 2007 (Table 1). The sampling area is microtidal with an average tidal amplitude of ~20 cm and a salinity between 20 and 25 (Pihl and Rosenberg, 1982). The sampling area has been described as silty and semi-enclosed with high organic matter content and high porosity in the surface sediment. The benthic macrofaunal community is dominated by detritivores (Engelsen et al., 2008).

Porosity was measured at the sampling occasion by weight loss after drying the sediment at 70 °C until constant weight. Solid phase organic carbon (TOC) and organic nitrogen (TON) were determined by an elemental analyzer NA 1500 NC, Fison instruments. Grain size was determined by sieving and re-weighing a known amount of dry sediment through a stack of sieves with progressively decreasing mesh size. The analysis on dry sediment may have underestimated the silt and clay fraction due to particle aggregation during the drying process.

Sediment and fauna were transported to temperature-controlled (15 °C) facilities at the Lovén Centre Kristineberg. The sediment was dry-sieved (1 mm) to remove macrofauna and larger debris, homogenized, and placed in large (75 × 55 × 45 cm) plastic containers. Animals were placed in sediment from the sampling location. Sediment and animal aquaria were continuously supplied with unfiltered seawater from the Gullmarsfjord ( $S = 31$ ,  $T = 15$  °C) for a period of 11 days to stabilize biogeochemical characteristics and chemical

Table 1  
Overview of experimental activities.

Day	Activity
23/24 14	Sampling of animals and sediment Plexiglass cores inserted into the sediment and thin aquaria filled with sediment
7	Additions of <i>Hediste diversicolor</i> to the experimental microcosms
0 1	Additions of luminophores and start of sediment water incubations
1 11	Capture of images
10 11	Termination of incubation

gradients in the sediment pore water system before Plexiglass cores ( $\emptyset = 10$  cm, height = 30 cm) were carefully inserted into the sieved sediment ( $\sim 14$  cm deep). In parallel, sieved sediment (height  $\approx 14$  cm) and bottom water from the sampling location were added to thin glass aquaria ( $16 \times 33 \times 1.4$  cm). The overlying water was replaced regularly and each microcosm was continuously aerated by aquaria pumps.

*H. diversicolor* was introduced seven days after sediment was added to cores and aquaria. Individuals were wet weighed (ww) and the volume of each animal was measured using a graded measuring cylinder before the addition. Faunal density was 530 ind.  $m^{-2}$  in the cores (4 individuals) and 446 ind.  $m^{-2}$  in the aquaria (1 individual). This is within the lower range typically observed on the Swedish west coast (35–3700 ind.  $m^{-2}$ ; Scaps, 2002). The biomass varied between 58 and 71 g ww  $m^{-2}$  in the cores and between 28 and 41 g ww  $m^{-2}$  in the aquaria, and the biovolume ranged between 66 and 200 mL  $m^{-2}$  in the cores and between 110 and 170 mL  $m^{-2}$  in the aquaria. All animals burrowed within 25 min, a majority (N70%) within 10 min. The Li/Da and Dark treatments started at the addition of fauna. Microcosms were either exposed to 12 h light/dark cycles ( $\sim 8 \mu\text{mol photons } m^{-2} s^{-1}$ ), or to constant darkness (covered with opaque black plastic).

*H. diversicolor* was allowed to acclimate for another week in the experimental set-up before luminophores were added and image acquisition started. At the start of tracer incubations, fluorescent particles (Partrac; 63–125  $\mu\text{m}$ ,  $\lambda_{\text{ex}} = 500$  nm,  $\lambda_{\text{em}} = 602$  nm) were dispersed into the overlying water of the aquaria to a final density of  $\sim 265$  g luminophores  $m^{-2}$ . Image acquisition started when the luminophores had settled on the sediment surface. In total, 32 images were captured of each aquarium, of which 17 were used for evaluation of reworking proxies. Images for quantification of particle transport were evaluated on a daily basis for all proxies, while more frequently at the beginning of the experiment for the 2-D redistribution (4, 8, 12, 24 and 36 h). The experimental period of the sediment water incubations with luminophores was 10 days.

### 2.3. Optical instrumentation and image analysis

In brief, the optical set-up included a 300 W xenon lamp used as light source for luminophore excitation. The excitation wavelength was obtained by a bandpass filter (Thorlabs; 510 nm  $\pm$  10 nm) mounted on-line with the light source. Fluorescence emission was captured by a Canon EOS 350D digital (CMOS image sensor) camera with a bandpass filter (Thorlabs; 610 nm  $\pm$  10 nm) mounted on-line with the CMOS chip. The aquaria were manually positioned in front of the camera during experiments and image acquisition was controlled from an adjacent room.

All images were captured in the RAW (.CR2) format with a  $3456 \times 2304$  pixel resolution (corresponds to  $\sim 8$  M pixels), saved in Exif-TIFF (8 bit, Digital Photo Professional 2.2.0.1) and processed in Matlab7.5 (R2007B). The effective pixel size was  $\sim 80 \mu\text{m pixel}^{-1}$ . There was a need to re-align the images (pixel by pixel) prior to processing due to the manual positioning of the aquaria. This was done using the plugin TurboReg (Thevenaz et al., 1998) in the image processing program ImageJ (Version 1.45).

Fluorescence was quantified using the red layer of the CMOS chip. Image threshold was obtained by maximizing visualization of luminophore fluorescence while simultaneously minimizing artifacts caused e.g. by reflections from the glass wall of the aquaria. The total fluorescence from luminophores varied with time for individual sets of aquaria, indicating non-conservative parameterization. The threshold value was therefore adjusted throughout the experimental period to maintain a stable total fluorescence and thereby correct for this apparent instability in mass conservation over time. By this procedure, variations in the total fluorescence narrowed from a range of 79 to 165% to a range of 91 to 102% compared to the initial fluorescence at the start of the experiment.

The position of the sediment water interface was determined in the resulting binary matrix. Before the analysis of  $D_b$ ,  $r$  and MPD, the sediment surface was flattened. During this process, each column was individually adjusted so that pixels corresponding to the sediment surface were in the first row (Solan et al., 2004). Prior to the quantification of 2-D redistribution and daily transport rates, pixels above the sediment water interface were set to 0 in order to remove artifactual fluorescence from luminophores attached to the glass wall of the aquaria. Compaction was compensated for by correcting the image matrix upwards at the same rate as compaction affected the sediment during the time between the images. This gravity-driven transport process of particles would otherwise artifactually contribute to these proxies, especially close to the sediment surface. Total compaction was estimated to  $\sim 2$  mm during the 10 day experiment.

### 2.4. Quantification of reworking proxies

The Optical Reworking Coefficient (ORC) was initially described by Gilbert et al. (2003). A slightly modified approach was used in this work and three other reworking proxies were derived from images. The basic idea was similar, i.e. the binary image obtained at time  $t$  ( $M_t$ ) was subtracted from the binary image at the start of experiments ( $M_0$ ). The sum of the resulting matrix  $M$  therefore described the total relocation of luminophores in the sediment. In our study, no further modeling was performed and results were directly presented as a function of incubation time. In contrast to the ORC, absolute values of pixels ( $|X|$ ) were summed and results were not normalized for time.

The 2-D redistribution was calculated from the binary image obtained at time  $t$  ( $M_t$ ), subtracted from the binary image at the start of experiments ( $M_0$ ). A daily particle transport was calculated from the binary image obtained at time  $t$  ( $M_t$ ), subtracted from the binary image captured the previous day ( $M_{t-24\text{h}}$ ). The integrated transport ( $\sum M_i$ ) was obtained by adding the daily transport to the total sum of particle displacements from previous days of incubation. The derivative of the integrated transport over time ( $\delta \sum M_i / \delta t$ ) described the rate of luminophore transport ( $\text{cm}^2 \text{d}^{-1}$ ). For the maximum penetration depth of luminophores (MPD), seven pixels with a positive fluorescence response were set as the detection limit to exclude background noise.

In addition to quantification of luminophore tracers by means of side-view imaging, luminophore distributions were assessed by sectioning and counting of the tracer. At the termination of the experiment, cores and aquaria were sectioned in 0.5 cm layers down to 2 cm, and in 1 cm layers down to the bottom of the microcosm (12–13 cm). The sediment from each section was homogenized, a subsample collected and freeze-dried. Quantification of luminophores was made under UV-light using a Synergy Mx microplate reader (Lagauzere et al., 2011).

The obtained distributions of luminophores were evaluated by the gallery-diffusor model (François et al., 2002). Two main mechanisms for particle transport are included in the model 1) diffusive mixing in the shallow sediment with dense burrows, and 2) non-local transport within burrows. The model is time- and space dependent and employs ordinary differential equations. The biodiffusion coefficient ( $D_b$ ) and the non-local transport coefficient ( $r$ ) were calculated by minimizing a weighted sum of squared differences between the observed and modeled tracer concentrations with depth in the sediment. The MPD was also determined from the vertical distributions of luminophores.

### 2.5. Statistical analysis

$D_b$ ,  $r$ , and the MPD determined from the sectioning method were evaluated by a two-way analysis of variance (ANOVA) for significant ( $\alpha = 0.05$ ) differences between faunal treatments (Light and Microcosm). As the main focus in this work was to explore effects from light

availability and the type of microcosm (i.e. not the technique for fluorescence evaluation per se) on particle reworking by *H. diversicolor*, comparisons between the imaging technique and the slicing technique were not made for  $D_b$ ,  $r$  and MPD. Differences in the 2-D redistribution and the MPD at the termination of the incubation and the transport rate between the treatments Li/Da-I and Dark-I were tested with t-tests. Prior to analysis, assumptions of normality and homogeneity of variances were assessed by graphical exploration of box plots (Quinn and Keough, 2002). Homogeneity of variance was also evaluated with Levene's test. In order to homogenize variances  $r$  was  $\log(x + 1)$  transformed and the MPD was  $\log(x)$  transformed. Statistical analyses were performed with IBM SPSS Statistics for Windows (version 20.0).

### 3. Results

#### 3.1. General observations, recovery of fauna and sediment properties

All faunal microcosms were affected by reworking at the time of luminophore addition. New burrows and sediment constructions were created progressively throughout the incubation. In addition to the perforation of the surface sediment with shallow (~1–2 cm) burrows, there were burrows visible down to ~5 cm depth. Burrows were preferentially observed along the side-wall of the aquaria in the thin aquaria. Control microcosms were devoid of larger burrow constructions. There were thin traces of burrows in the top cm of the sediment and occasional visual observations of a removal of luminophores from the sediment surface that indicated the presence of small fauna in control microcosms.

Upon termination of experiments, 41 of 48 (~85%) individuals were found in the cores and 11 of 12 (~92%) individuals were recovered in the aquaria. *H. diversicolor* was mainly retrieved down to ~5 cm depth, and a majority within the 1.5–4 cm sediment layer (Fig. 1). During sectioning of the aquaria, several individuals were observed burrowing downwards and were subsequently found at the bottom of the sediment column (10–12 cm depth). However, as they were all initially recognized in the top 5 cm, this observation was considered an escape reaction associated with the sectioning procedure and therefore an artifact related to the experimental protocol. Further, as the number of luminophores retrieved below 7 cm was negligible (see below), the artifactual downward movement of fauna upon sectioning seemed not to have affected the qualitative or quantitative interpretation of bioturbation activities by *H. diversicolor*.

The sediment was defined as silty sand with a grain size distribution of 44% silt and 52% fine sand. The TOC of the sediment used for

incubations was 1.8% and TON 0.16% at the start of the experiment. Sediment porosity in the cores at the termination of the experiment was  $0.79 \pm 0.11$  ( $n = 6$ ) in the 0–0.5 cm layer and  $0.58 \pm 0.03$  ( $n = 6$ ) in the 11–12 cm layer.

#### 3.2. Distribution of luminophores

In the control microcosms, deposited luminophores were exclusively found in the top cm of the sediment. 98–99% were observed in the 0–0.5 cm sediment layer (including the lower fraction of the bottom water just above the sediment surface), and 1–2% were recovered in the 0.5–1 cm layer by the sectioning technique. Close to 100% of the luminophores were found in the 0–0.5 cm layer by the imaging technique.

In contrast, luminophores were removed from the top sediment layer as a consequence of reworking activities by *H. diversicolor*. In the light/dark cycles, 72–89% of deposited luminophores were recovered in the first 0.5 cm of the sediment in the cores (Li/Da-C) and 72–88% in the aquaria (Li/Da-A). Under the dark conditions, 86–93% were retrieved in the cores (Dark-C) and 94–99% in the aquaria (Dark-A). Imaging of luminophore distributions demonstrated similar patterns of luminophore removal from the sediment surface: 62–81% and 73–95% of luminophores were found in the 0–0.5 cm sediment layer during light/dark (Li/Da-I) and dark (Dark-I) conditions, respectively. Further, observations of subsurface peaks of luminophores were less evident in the dark compared to the light/dark treatment (Fig. 2).

Similar patterns emerged when the MPD was examined at the termination of the experiment (Fig. 3). Luminophores were observed significantly deeper in the light/dark cycles (Li/Da-C,  $6.3 \pm 1.3$  cm, and Li/Da-A,  $6.8 \pm 2.9$  cm) compared to cores and aquaria exposed to constant darkness (Dark-C,  $3.5 \pm 1.3$  cm, and Dark-A,  $5.2 \pm 2.7$  cm). There was no significant difference in MPD between the microcosm treatments (2-way ANOVA, Table 2).

With the results from imaging, the evolution of MPD could be resolved in time. In the *H. diversicolor* treatments luminophores were quickly (within a day) transported downwards to a depth in close accordance with the final MPD (Figs. 4 and 5). The MPD from image analysis at the termination of the experiment (10 days) supported the observed deeper penetration of luminophores under the light/dark (Li/Da-I,  $2.8 \pm 1.6$  cm) compared to dark (Dark-I,  $1.1 \pm 0.4$  cm) conditions, although the difference was not significant (t-test,  $p = 0.147$ ). The MPD in the controls was shallower ( $0.4 \pm 0.1$  cm for both light/dark and dark conditions) compared to that in the faunal aquaria.

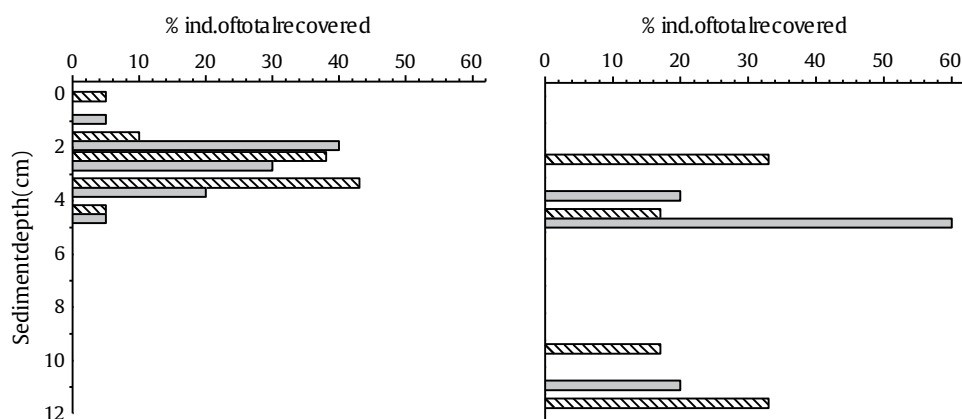


Fig. 1. Distribution of *Hediste diversicolor* recovered at the end of experiment in Plexiglass cores (left,  $n = 6$ ) and thin aquaria (right,  $n = 6$ ) incubated under light/dark (striped) and dark (filled) conditions.

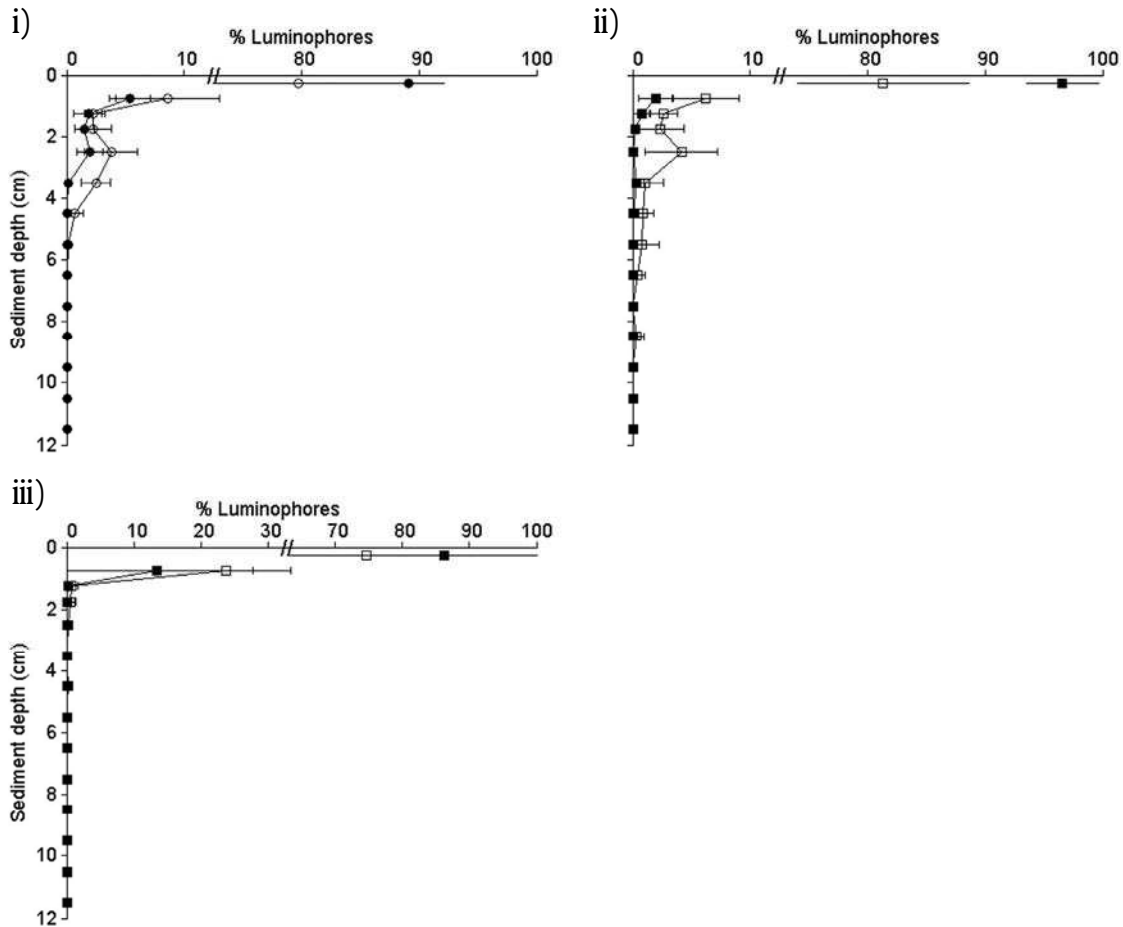


Fig. 2. Distribution of luminophores (mean  $\pm$  SD) following incubations of sediment with *Hediste diversicolor* in Plexiglass cores (i, n = 6) and thin aquaria (ii, n = 6). Distributions from using side-view imaging are also illustrated (iii, n = 3). Experiments were performed under light/dark (open symbols) and dark (filled symbols) conditions. Note the different scales.

### 3.3. Quantification of particle reworking by imaging

The 2-D redistribution quantitatively described and visualized the temporal and spatial change in distribution of luminophores following bioturbation by *H. diversicolor* (Fig. 6). Over the whole experimental period, there was a logarithmic increase in 2-D redistribution with time of incubations. The logarithmic trend was more pronounced for

the faunal treatments compared to the controls. During the initial ~two days of experiment, the 2-D change in luminophore distribution increased in a seemingly linear fashion for all aquaria (Li/Da-I,  $0.83 \pm 0.21 \text{ cm}^2 \text{ d}^{-1}$ , and Dark-I,  $0.64 \pm 0.29 \text{ cm}^2 \text{ d}^{-1}$ , linear fit). After this period, the increase in 2-D redistribution with time was less pronounced (Li/Da-I,  $0.11 \pm 0.03 \text{ cm}^2 \text{ d}^{-1}$ , and Dark-I,  $0.10 \pm 0.05 \text{ cm}^2 \text{ d}^{-1}$ , linear fit) compared to that observed within the first

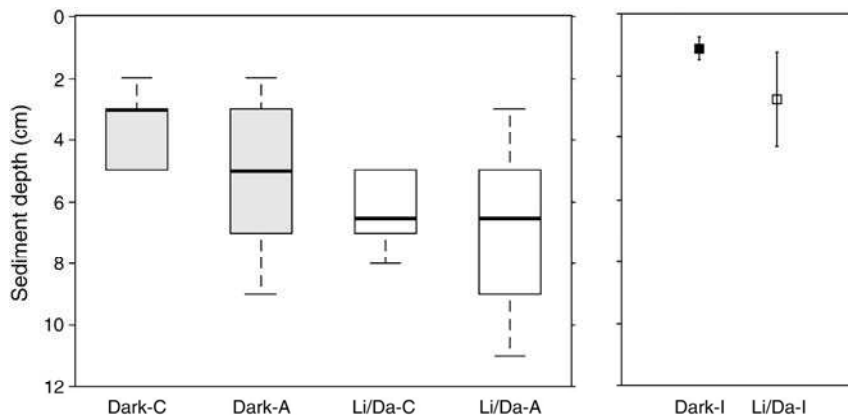


Fig. 3. Maximum penetration depth of luminophores (MPD) as a consequence from particle displacement by *Hediste diversicolor* during sediment water incubations in cores (C) and aquaria (A). Reworking activities were quantified by sectioning and semi-automatic counting of luminophore distributions (left) or from side-view imaging (I) of luminophore fluorescence in the aquaria (right). Incubations were performed under light/dark (Li/Da-C, Li/Da-A and Li/Da-I) and dark (Dark-C, Dark-A and Dark-I) conditions. Boxplots (n = 6) demonstrate the median and the 25th and 75th percentiles (edges of the box) of the data set. The whiskers extend to the most extreme data points. MPD quantified by the imaging technique is represented as mean  $\pm$  SD (n = 3).

Table 2

Statistic analysis (two-way analysis of variance, ANOVA table) of the biodiffusion coefficient ( $D_b$ ), the non-local transport coefficient ( $r$ ) and the maximum penetration depth of luminophores (MPD). \* denotes differences significant at  $\alpha = 0.05$ .

Source of variation	d.f.	$D_b$			$r$			MPD		
		m.s	F	p	m.s.	F	p	m.s.	F	p
Light	1	1.571	14.208	0.001*	1.242	34.834	0.000*	0.536	18.223	0.000*
Microcosm	1	1.042	9.422	0.006*	0.038	1.070	0.313	0.006	0.206	0.655
Light $\times$ Microcosm	1	0.141	1.276	0.272	0.127	3.566	0.074	0.064	2.194	0.154
Residual	20	0.111			0.036			0.029		
Total	23									

2 days (Fig. 6). The highest values for 2-D redistribution were observed at the termination of the tracer incubation (Li/Da-I,  $3.54 \pm 0.69 \text{ cm}^2$ , Dark-I,  $2.85 \pm 0.83 \text{ cm}^2$ ). Control aquaria were similar at the termination of the experiment (light/dark cycles:  $1.06 \pm 0.12 \text{ cm}^2$  and darkness:  $1.08 \pm 0.11 \text{ cm}^2$ ) with lower values compared to aquaria with fauna. The difference between 2-D redistribution under light/dark compared to dark conditions was not significantly different for the faunal aquaria (t-test,  $p = 0.328$ ).

Averaged over the experimental period, the daily transport was  $1.7 \pm 0.4 \text{ cm}^2 \text{ d}^{-1}$  during light/dark (Li/Da-I) and  $1.3 \pm 0.5 \text{ cm}^2 \text{ d}^{-1}$  during dark (Dark-I) conditions. After 10 days of incubation, the total area of luminophore relocation (i.e. the integrated transport) in aquaria with fauna corresponded to  $16.9 \pm 2.3 \text{ cm}^2$  during light/dark and  $12.9 \pm 3.0 \text{ cm}^2$  during dark conditions (Fig. 7). For the controls, the integrated transport was lower and values were similar in light/dark ( $5.5 \pm 0.8 \text{ cm}^2$ ) and dark ( $6.5 \pm 1.8 \text{ cm}^2$ ) conditions at the termination of the tracer incubation. The rate of luminophore transport was calculated from a linear fit of the integrated transport with time of incubation ( $\delta(\sum M_i)/\delta t$ ;  $R^2 = 0.9899$  to  $0.9996$ ). Under light/dark conditions, rates were slightly higher compared to dark conditions ( $1.66 \pm 0.21 \text{ cm}^2 \text{ d}^{-1}$  and  $1.25 \pm 0.28 \text{ cm}^2 \text{ d}^{-1}$ , respectively), although the difference was not significant (t-test,  $p = 0.117$ ).

### 3.4. The biodiffusion ( $D_b$ ) and non-local transport ( $r$ ) coefficients

The transport coefficients were generally higher in the light/dark treatment as compared to the dark treatment (Fig. 8).  $D_b$  in microcosms exposed to light/dark cycles (Li/Da-C,  $1.37 \pm 0.41 \text{ cm}^2 \text{ y}^{-1}$ , and Li/Da-A,  $1.08 \pm 0.56 \text{ cm}^2 \text{ y}^{-1}$ ) was significantly (2-way ANOVA, Table 2) higher compared to the dark microcosms (Dark-C,  $0.96 \pm$

$0.34 \text{ cm}^2 \text{ y}^{-1}$ , and Dark-A,  $0.46 \pm 0.40 \text{ cm}^2 \text{ y}^{-1}$ ). There was also a significant difference in  $D_b$  between types of microcosms, with higher values in the cores. The imaging technique supported a higher  $D_b$  under light/dark (Li/Da-I;  $1.81 \pm 0.01 \text{ cm}^2 \text{ y}^{-1}$ ) compared to dark (Dark-I;  $1.31 \pm 0.44 \text{ cm}^2 \text{ y}^{-1}$ ) conditions.

The non-local transport coefficient,  $r$ , was also significantly higher under light/dark conditions (Li/Da-C,  $4.39 \pm 2.40 \text{ y}^{-1}$  and Li/Da-A,  $4.56 \pm 3.12 \text{ y}^{-1}$ , 2-way ANOVA, Table 2) compared to dark conditions (Dark-C,  $1.75 \pm 1.62 \text{ y}^{-1}$ , and Dark-A,  $0.26 \pm 0.26 \text{ y}^{-1}$ ). There was no statistical difference between types of microcosms. Model predictions to estimate  $D_b$  and  $r$  were in good agreement with experimental data and the distribution patterns of luminophores (error ranged between 1.06 and 5.89). In Li/Da-I,  $r$  was also slightly higher than in Dark-I ( $4.06 \pm 4.32 \text{ y}^{-1}$  compared to  $2.41 \pm 4.01 \text{ y}^{-1}$ ), but the variability was high (Fig. 8).

## 4. Discussion

### 4.1. Distribution of luminophores

*H. diversicolor* has been observed to construct semi-permanent U, Y- or J-shaped mucous-lined burrows that can extend about 20 cm deep into the sediment (Davey, 1994; Esselink and Zwarts, 1989). The particle transport induced by *H. diversicolor* is characterized as mainly diffusive in the surface layer while non-local in deeper sediment layers (François et al., 2002). The general distribution of luminophores obtained in the present study confirmed an exponential decrease of luminophores with depth over the first ~cm and subsurface peaks of the tracer at depth (Figs. 2 and 4). Similar distribution patterns were observed in aquaria and cores exposed to 12 h light/dark cycles. The

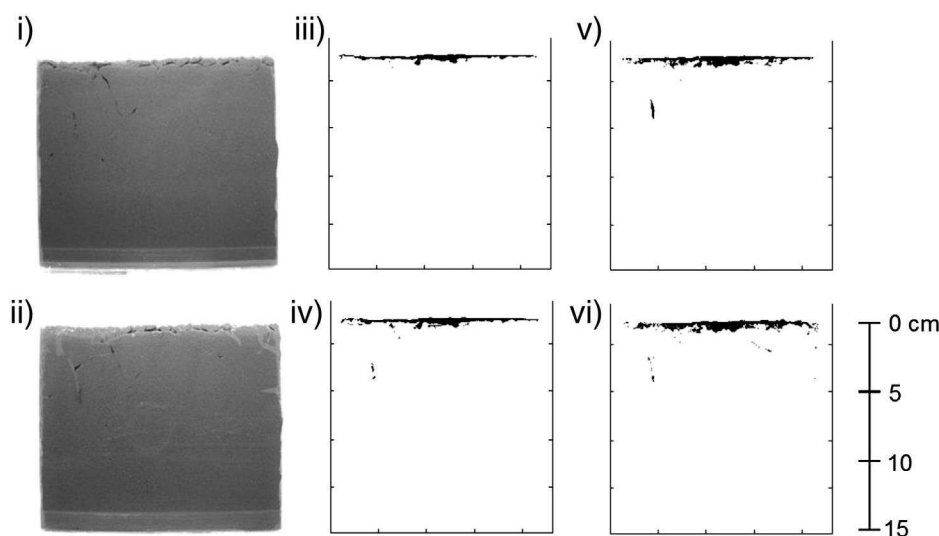


Fig. 4. Examples of sediment reworking patterns by *Hediste diversicolor* (i and ii) and time-resolved distributions of fluorescent particles (luminophores) following particle displacements during sediment-water incubations in glass aquaria (iii-vi). Images were captured 2 days before (i) and 10 days after (ii) the addition of luminophores under normal light conditions and without bandpass-filters to visualize the burrows. Images of fluorescence were thresholded before quantification of reworking patterns 1 h (iii), 8 h (iv), 24 h (v) and 10 days (vi) after addition of luminophores. The circular shape in image ii is a camera reflection.



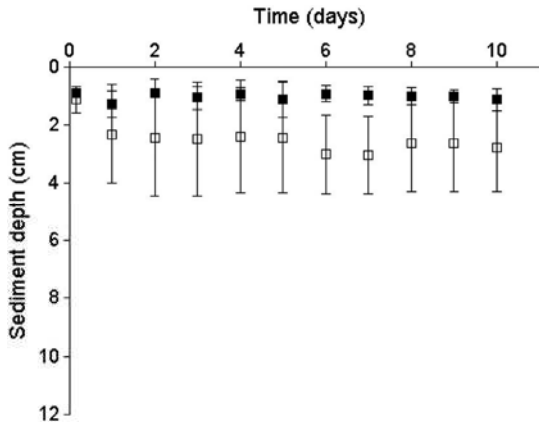


Fig. 5. Maximum penetration depth of luminophores (MPD) with time during sediment water incubations. Luminophore distributions were quantified by side-view imaging of fluorescence in aquaria with *Hediste diversicolor* exposed to light/dark (open symbols) and dark conditions (filled symbols). Values are mean  $\pm$  SD (n = 3).

relative amount of luminophores found in the subsurface peaks was, however, lower under darkness compared to light/dark cycles. Fewer luminophores were also recovered in the subsurface peaks in the aquaria compared to the cores. It therefore seemed that the final distribution of luminophores depended on the light conditions as well as the type of microcosm.

Visual inspections and calculated proxies quantitatively and qualitatively used to describe particle reworking (luminophore distributions,  $D_b$ , r, MPD, 2-D redistribution and transport rates), confirmed no significant displacement of particles in the control cores or the control aquaria. Indications of small-scale transport of luminophores to a maximum depth of  $\sim 1$  cm were most probably due to activities by small fauna.

It appeared that temporal patterns in the redistribution of luminophores by *H. diversicolor* could be described in at least two phases (Figs. 5 and 6). During the initial phase of 1–2 days there was a pronounced change in the 2-D redistribution and the MPD. The transport and removal of particles from the thin layer of luminophores initially deposited on the sediment surface were associated with a logarithmic increase in the 2-D redistribution and a rapid transport of surface luminophores deeper into the sediment. After 2 days, the redistribution of particles reached 64–70% of its final value and the progressive increase leveled off. In parallel, following this initial phase the MPD was fairly stable until the end of the tracer incubation. The integrated transport revealed a rather constant daily relocation of luminophores by *H. diversicolor* during the

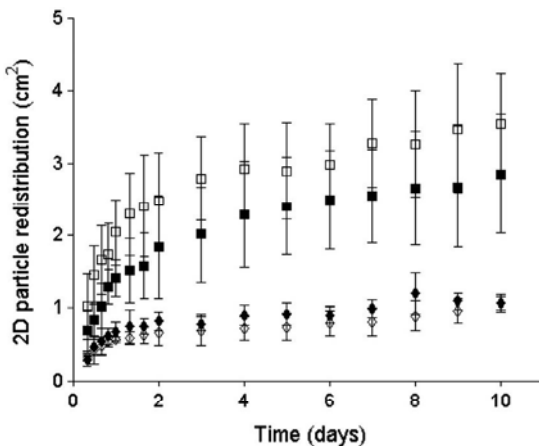


Fig. 6. 2-D redistribution of particles with time in the presence of *Hediste diversicolor* (squares) or controls (diamonds) in aquaria exposed to light/dark (open symbols) and dark (filled symbols) conditions. Values are mean  $\pm$  SD (n = 3).

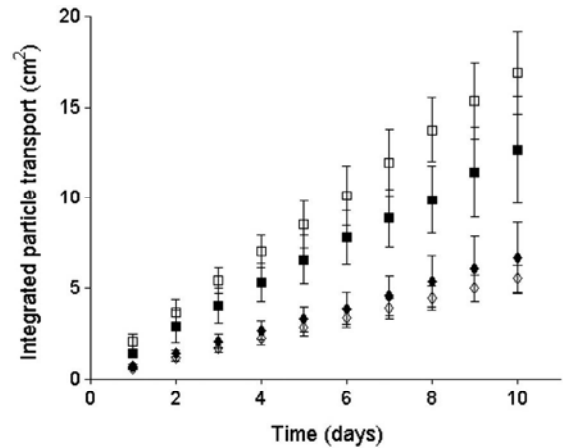


Fig. 7. Integrated particle transport in the presence of *Hediste diversicolor* (squares) or controls (diamonds) as identified by side-view imaging of fluorescence under light/dark (open symbols) and dark (filled symbols) conditions. Values are mean  $\pm$  SD (n = 3).

tracer incubation in all aquaria (Fig. 7). This was interpreted as a constant particle reworking behavior by the polychaete throughout the experimental period. This is in agreement with the study by Davey (1994), where imaging by X-radiography progressively demonstrated complex burrow constructions by *H. diversicolor* over a three week long experimental period.

#### 4.2. Methodological considerations

Thin aquaria have frequently been used as experimental systems to study particle reworking by single-species (Maire et al., 2006; Piot et al., 2008; Schiffers et al., 2011) or natural communities (Gilbert et al., 2003; Solan et al., 2004), likely as a result of the development of the fluorescence imaging technique. Introducing bioturbating macrofauna to thin aquaria is a technique associated with potential artifacts mainly related to changes in behavior due to wall effects and alterations in the physical geometry of the sediment matrix (Dorgan et al., 2006). In addition, the number of individuals added to experimental microcosms often differs within the experiment due to normalization of e.g. biomass, biovolume or density between treatments (Gilbert et al., 2007; Michaud et al., 2006; Norling et al., 2007).

In this study, additions of *H. diversicolor* varied between cores (4 ind.) and aquaria (1 ind.). This was a direct consequence of the desire to maintain a similar density ( $\text{ind. m}^{-2}$ ) despite an  $\sim 4$  times smaller surface area in the aquaria than in the cores. Thus, both the type of microcosm and potential interactions between individuals should be considered when comparing the obtained reworking proxies for cores and aquaria. A possible consequence of the difference in actual number of *H. diversicolor* in cores and aquaria relates to an increased complexity of the burrow system in aquaria with single individuals (Davey, 1994). For example, a territorial behavior of *H. diversicolor* including aggressive intraspecific interactions (Esnault et al., 1990) may lower the variation among replicates in Plexiglass cores with several individuals than thin aquaria with a single individual. Further, such behavior may also have influenced reworking proxies, e.g.  $D_b$ . The overall importance for the complex interactive effects that may have occurred between individuals was, however, not within the scope of the present study. Due to the comparably low density of fauna ( $\sim 500 \text{ ind. m}^{-2}$ ), these effects were considered not to have influenced the main conclusions of the study.

*H. diversicolor* has been documented to preferentially bury along walls under experimental conditions (Davey, 1994). Individuals were therefore expected to preferentially create burrows along the side of the walls in both types of microcosms. The wall area normalized to sediment volume was larger in the aquaria compared to the cores ( $1.6 \text{ cm}^{-1}$  compared to  $0.4 \text{ cm}^{-1}$ ). A larger ratio between wall area

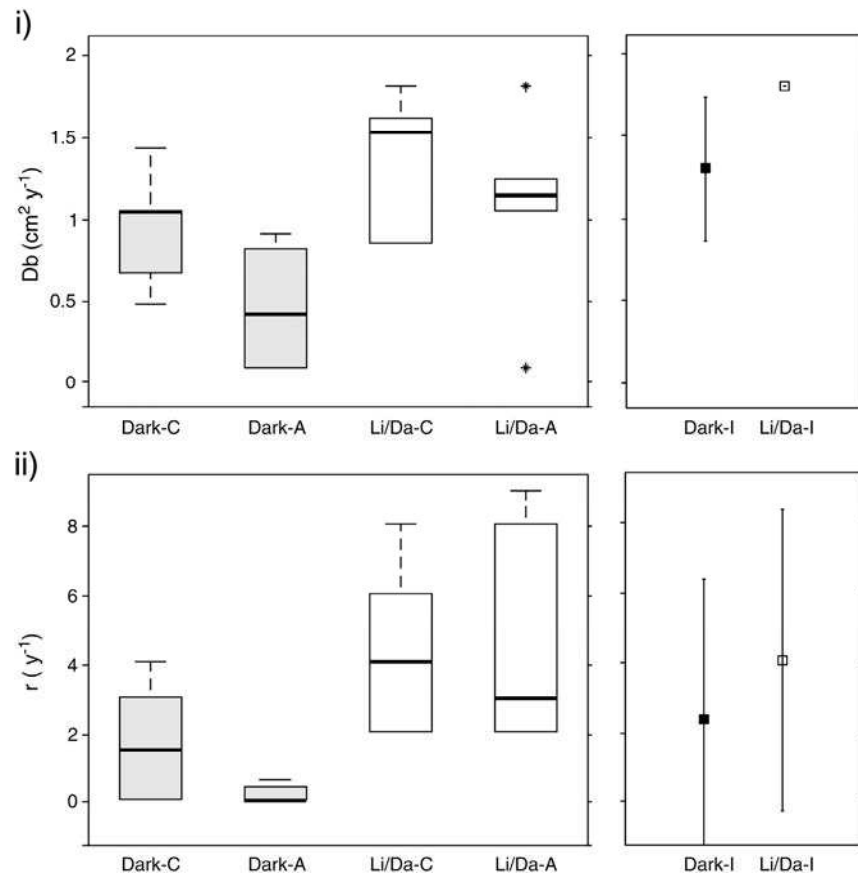


Fig. 8. The biodiffusion coefficient ( $D_b$ ) (i), and the non-local transport coefficient ( $r$ ) (ii) for *Hediste diversicolor* during sediment water incubations in Plexiglass cores (C) and thin aquaria (A). Reworking variables were quantified from sectioning (left), or from side-view imaging (right). Incubations were performed under light/dark (Li/Da-C, Li/Da-A and Li/Da-I) and dark (Dark-C, Dark-A and Dark-I) conditions. Boxplots ( $n = 6$ ) demonstrate the median and the 25th and 75th percentiles (edges of the box) of the data set. The whiskers extend to the most extreme data points not considered as outliers. Outliers ( $D_b$ , Li/Da-A) are indicated by an asterisk. Reworking variables quantified by the imaging technique are represented as mean  $\pm$  SD ( $n = 3$ ).

and sediment volume in the aquaria than in the cores could have stimulated animal activities and particle reworking close to the wall of the aquaria. Of the three measured particle reworking proxies, there were significant differences between the two types of microcosms only for  $D_b$ . However,  $D_b$  was lower in the aquaria compared to the cores. In contrast to a stimulated activity in aquaria, it therefore seemed that the observed difference in particle reworking was mainly caused by a restriction in lateral movements in the thin aquaria rather than by general wall effects.

#### 4.3. Effects from light exposure on reworking activities of *H. diversicolor*

*H. diversicolor* inhabits the littoral zone and is naturally exposed to pronounced variations of environmental conditions (e.g. light, temperature, salinity, availability of oxygen and organic material (Möller, 1985; Rasmussen and Jørgensen, 1992)). In temperate regions such as the west coast of Sweden, the diurnal availability and variations in light intensity over the year can be expected to be important factors for the reworking behavior of *H. diversicolor*. In the present study, all measured proxies for particle reworking demonstrated a general decrease in transport of luminophores in darkness compared to light/dark cycles. The difference was more pronounced using the sectioning compared to the imaging technique. This discrepancy was probably caused by the low number of replicates for the latter technique, rather than an indication of similar reworking proxies between the two light treatments using imaging.

From imaging it was discerned that rates of particle transport were  $\sim 30\%$  higher during the light/dark cycles compared to darkness (Fig. 7). There appeared to be no difference in the long-term behavior

of the *H. diversicolor* in the light/dark cycles compared to darkness as the daily particle transport was constant over the duration of the experiment, only the magnitude was affected. This reduced reworking activity under dark conditions could be explained by direct light-triggered behavior. Indeed, a pulsed and temporarily enhanced transport of surface deposited particles into the sediment and burrow systems occurs normally during prospecting/foraging for food on the sediment surface. Assuming that luminophores were transported into the sediment mainly during surface feeding, the general lower values of particle reworking proxies in the dark treatment would be associated with a decreased surface feeding by *H. diversicolor* in the darkness compared to light/dark cycles. It has been suggested that *H. diversicolor* has a diurnal feeding rhythm and preferentially feed on the surface during the day (Lambert et al., 1992). It was further shown that the worm kept the feeding rhythm even if the light was turned off for 24 h (Lambert et al., 1992). Food prospecting would be governed by an endogenous diurnal rhythm. In contrast to this observed diurnal surface feeding, Wenzhöfer and Glud (2004) related a dramatic increase in oxygen consumption to increased surface activities by *H. diversicolor* at sunset. In support of this conclusion, Tang and Kristensen (2007) found that ventilation activity by *H. diversicolor* decreased significantly during the night and inferred this behavior to nocturnal surface feeding similar to that of *Alitta (Nereis) virens* (Caron et al., 2004; Last, 2003; Miron et al., 1992). Although somewhat conflicting observations on the response, a bioturbation behavior directly coupled to and possibly triggered by the availability of light has therefore been demonstrated in several investigations. In this study, the lower rates of both biodiffusive and non-local transport observed over 10 days indicated a lower activity by *H. diversicolor* in the upper surface layer (0–2 cm)

under dark conditions. It also suggested a decreased activity between the deeper burrows (2–6 cm) and the surface sediment. In addition to the decreased non-local transport, the shallower MPD supported a decreased activity at depth under dark conditions (Fig. 3). Depth of burrow constructions has been correlated to body conditions where worms in good condition are able to maintain deeper burrows (Esselink and Zwarts, 1989). Shallower burrows could therefore be a sign of stress and worm activity under non-optimal conditions in the dark treatment.

Besides the direct effect of light on the feeding behavior of *H. diversicolor*, feeding characteristics are also indirectly affected by light through the diurnal activities by benthic microalgae. *H. diversicolor* is known to feed on benthic diatoms (Tang and Kristensen, 2007) and a possible decrease in the availability and amount of food in the dark treatment would also decrease particle reworking activities by *H. diversicolor* (Nogaro et al., 2008).

These observations of a light dependent particle reworking behavior by *H. diversicolor* may have direct implications for the biogeochemistry and element cycling in surface sediments. For example, during the day, surface sediments in shallow-water environments are oxidized by benthic microalgae during photosynthesis (Revsbech and Jørgensen, 1983). Under oxidizing sediment conditions, Fe and Mn predominately occur as particulate Fe(III)- and Mn(IV)-oxides rather than  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  dissolved in the pore water (Froelich et al., 1979). In addition to the particle redistribution and enhanced transport of e.g. organic material to deeper sediment layers, the transport of particulate Fe- and Mn-oxides across redox boundaries may therefore under diurnal variations in light conditions be enhanced in sediments inhabited by *H. diversicolor*. Such transport of reactants across redox zones and the exposure of organic material to a suite of oxidants have been found to stimulate rates and extent of organic matter mineralization and thus reduce the fraction of organic material that is buried in the sedimentary record (Aller, 1994; Hulthe et al., 1998; Sun et al., 2002).

## 5. Conclusions

Imaging of luminophores was proven to be a successful technique to quantify time-resolved particle reworking by *H. diversicolor*. Overall, patterns of particle translocation from imaging agreed with results from the destructive sampling protocols. The main advantage using the imaging technique was the ability to quantify particle reworking and estimate reworking proxies throughout the incubation. This feature allowed time-resolved quantification of 2-D particle redistribution (small variation after ~2 days), MPD (reached an asymptotic value after ~1–2 days), and daily transport rates (constant throughout the incubation) with spatial resolution better than  $80 \mu\text{m pixel}^{-1}$ .

The influence of light/dark cycles and constant darkness on reworking activities by *H. diversicolor* was consistent in the two microcosm types (Plexiglass cores and thin glass aquaria). However, while the non-local transport and MPD were statistically similar between microcosms, the biodiffusion coefficient was significantly lower in the aquaria compared to the cores. The reworking behavior of *H. diversicolor* was therefore likely affected by the type of microcosm.

The sediment reworking behavior by *H. diversicolor* was significantly affected by the light treatments with generally lower values of  $D_b$ ,  $r$ , and MPD during the darkness compared to the light/dark cycles. The rate of particle transport was constant during the 10 day incubation period, and rates were ~30% lower in the darkness compared to the light/dark cycles. Observations were suggested to be associated with a decreased surface feeding by *H. diversicolor*.

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