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Diurnal bioturbating activities of *Monoporeia affinis*: effects on benthic oxygen and nutrient fluxes

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ABSTRACT: A laboratory experiment was carried out to investigate the overall effects from the diurnal bioturbating activities of *Monoporeia affinis* on benthic solute fluxes. Investigations were performed on Baltic Sea sediments during a 12:12 h light:dark cycle, where light conditions (1 µmol photons m^{-2} s^{-1}) corresponded to those at a simulated water depth of 25 m in July. Oxygen consumption, nutrient [NO_2^- + NO_3^-, NH_4^+, $Si(OH)_4$ and HPO_4^{2-}] fluxes and denitrification rates were analysed after organic material enrichment (5 g C m^{-2}) of the sediment. Significant effects from the diurnal activity of *M. affinis* could be observed, as enhanced solute fluxes of O_2, NO_2^- + NO_3^-, NH_4^+ and HPO_4^{2-} were found during day (light) compared to night (dark) treatments. The diurnal activity of *M. affinis* showed no effect on denitrification rates. Irrigation of *M. affinis*, however, stimulated denitrification of the nitrate supplied from the overlying water (Dw) by about 50% compared to the control (no macrofauna). No effect on coupled nitrification/denitrification (Dn) was found. Diurnal activity of meiofauna seemed to have effects on solute fluxes as control cores (no macrofauna) also showed enhanced fluxes during the day compared to at night. In addition, effects from the endogenous diurnal activity of *M. affinis* were observed in cores kept in total darkness. This study highlights the importance of diurnal activity patterns of benthic fauna during studies of nutrient dynamics and sediment mineralisation processes.

KEY WORDS: *Monoporeia affinis* · Diurnal activity · Nutrient fluxes · Oxygen consumption · Denitrification · Baltic Sea

INTRODUCTION

Monoporeia affinis, a deposit-feeding amphipod, is one of the most common and widespread species of benthic macrofauna in the Baltic Sea. It may occur in very high abundances, up to 10000 ind. m^{-2}, but the numbers can fluctuate greatly, both spatially and temporally (e.g. Andersin et al. 1984, Laine et al. 1997, Lehtonen & Andersin 1998). As the brackish Baltic Sea is charaterised by relatively low biodiversity and functional diversity (Rumohr et al. 1996, Bonsdorff & Pearson 1999), *M. affinis* is of high ecological significance, e.g. as a food source for different species of fish (e.g. the Baltic herring *Clupea harengus* and the fourhorn sculpin *Myoxocephalus quadricornis*) (Aneer 1975).

The amphipod is an important bioturbator and bioirrigator affecting various biogeochemical processes in the sediment. For example, field and laboratory studies have shown that the activity of *Monoporeia affinis* can extend the oxic zone of the sediment, enhance the mineralisation rates of benthic organic matter, affect the rates and pathways of nutrient cycling and stimulate the denitrification rate (Lehtonen & Andersin 1998, Gran & Pitkänen 1999, Tuominen et al. 1999, Modig & Ólafsson 2001, van der Bund et al. 2001, Karlson et al. 2005).

Through burrow constructions, benthic macrofauna may increase the overall area of sediment available for diffusive exchange. Generally, irrigating activities release pore water nutrients and reduce compounds from the sediment, while oxygen and other electron acceptors are transported into the sediment (Aller & Yingst 1985, Aller 2001). Accordingly, macrofaunal activities enhance the oxygen concentration of the

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sediment and improve the general conditions for the fauna by removing toxic metabolites. Furthermore, sharp gradients of O_2, NH_4^+ and NO_3^- often occur across linings of burrow constructions, which have a stimulatory effect on coupled nitrification and denitrification (Brune et al. 2000, Hulth et al. 2002, Gilbert et al. 2003).

Faunal activities in the surface sediment redistribute particulate material within the sediment, alter the surface to volume ratio of particles, and expose new surfaces for microbial colonisation (Aller & Aller 1998). Benthic macrofauna may also affect the general characteristics of the microenvironment by promoting temporally oscillating sediment redox conditions, by grazing microbes and by excreting important N-nutrients such as ammonium and urea (Mayer et al. 1995, Hulthe et al. 1997, Aller & Aller 1998).

Monoporeia affinis exhibits a diurnal rhythm (Donner & Lindström 1980, Lindström & Fortelius 2001). During the day it normally stays burrowed in the sediment, while at night it actively migrates into the overlying water. The main factor regulating swimming activity is visible light, but the amphipod also possesses an endogenous diurnal activity rhythm (Donner & Lindström 1980). According to Donner & Lindström (1980), weak light reduces swimming activity, while higher light intensities completely abolish it.

Seasonal variations in the vertical migration of *Monoporeia affinis* have also been documented, which may primarily be attributed to the reproduction cycle of the amphipod and the changed light intensity during the year (Lindström & Lindström 1980, Donner et al. 1987). Swimming behaviour has also been found to depend on (1) quality of the bottom substrate and the availability of food; (2) hydrographical factors such as water temperature, salinity, currents and oxygen concentration; and (3) population density (Donner et al. 1987, Lindström 1991, Lindström & Fortelius 1992, 2001).

The overall objective of the present study was to assess the effect of the diurnal activity of *Monoporeia affinis* on benthic oxygen and nutrient fluxes in the sediment.

MATERIALS AND METHODS

Field sampling. Surface sediment and *Monoporeia affinis* were collected in May 2004 by an epibenthic sled from 25 m depth (salinity 8‰) near the Askö field station, the north-western Baltic Proper (58°49′N, 17°31′E). A 1 mm sieve was used to remove ambient macrofauna from the sediment and to sort out *M. affinis*. Amphipods and sediment were kept cold in separate containers immediately after sampling and during immediate transport to a temperature-controlled (5°C) laboratory where the experiments were later performed. Seawater was collected outside Kristineberg Marine Research Station, Göteborg University, and appropriate salinity (8‰) was obtained by diluting the seawater with distilled water.

Experiment setup and sampling. The amphipods were stored in a dark environment in aquaria with sieved sediment and aerated water (8‰) before the start of the experiment. The sediment was homogenised, and thereafter Plexiglas core tubes (inner diameter of 10 cm) were carefully inserted into the sediment. The sediment cores were connected to a closed system with aerated recirculating water (8‰, 5°C) and left for acclimatisation for 14 d before *Monoporeia affinis* were added. The overlying water volume was 1.2 l.

Adult *Monoporeia affinis* (7 to 9 mm) were sorted out from the aquaria and added to the sediment cores (30 ind. core^{-1}, equivalent to 3800 m^{-2}) according to Table 1. All treatments were replicated 3 times.

Half of the treatments were exposed to a 12 h light/12 h dark cycle (light:dark cores) (Table 1). Individual 5 W halogen bulbs over each core were used as the light source. Bulbs were adjusted to give an approximate irradiance of 1 µmol photons m^{-2} s^{-1} at the sediment surface. The chosen light intensity was estimated to be equivalent to a water depth of 25 m in the Askö area in July (place of sampling and time of the experiment) (Ærtebjerg Nielsen & Bresta 1984, Wallentinus 1991). Separate pre-incubations (30 h with no recirculating water) of core tubes with sediment and water revealed that no elevation of the water temperature occurred due to exposure to the halogen bulbs.

The remaining cores were treated in the dark (24 h) (dark cores). Dark cores were used to control potential stimulation of microphytobenthos from the light used

Table 1. Treatments (1 to 8). Sediment cores with *Monoporeia affinis*, 30 ind. core^{-1} (Treatments 1, 3, 5 and 7) and controls, no fauna (Treatments 2, 4, 6 and 8). Light:dark cores (Treatments 1, 2, 3 and 4) were exposed to weak light (1 µmol photons m^{-2} s^{-1}) during the day (07:00 to 19:00 h) and were kept dark during the night (19:00 to 07:00 h). Dark cores (Treatments 5, 6, 7 and 8) were kept in darkness over 24 h. In day incubations (Treatments 1, 2, 5 and 6), flux measurements were performed during the day (07:00 to 19:00 h) and, in night incubations (Treatments 3, 4, 7 and 8), flux measurements were performed during the night (19:00 to 07:00 h)

Incubation (12 h)	Light:dark cores (12:12 h)	Dark cores (24 h)
Day	(1) *Monoporeia*	(5) *Monoporeia*
	(2) Control	(6) Control
Night	(3) *Monoporeia*	(7) *Monoporeia*
	(4) Control	(8) Control

in light:dark treatments, and to control eventual effects from the endogenous diurnal rhythm of *Monoporeia affinis*.

After addition of *Monoporeia affinis,* cores with sediment and recirculating water were left to adjust for another 17 d. Solid phase and pore water solute concentrations during similar incubations using coastal marine sediment from the Gullmar Fjord (western Sweden) confirmed that the sediment/pore water system had reached pseudo-steady-state conditions after this period of acclimatisation (Skoog et al. 1996, S. Hulth pers. comm.). Following frequent visual inspections of cores, dead *M. affinis* at the sediment surface were continuously replaced to keep the number of bioturbators constant. However, dead amphipods within the sediment were not known or replaced, as biogeochemical structure of the surface sediment would then be destroyed. The amphipods added were considered homologues to the ones initially added to the sediment cores. In addition, macrofaunal recovery and survival rate were performed at the end of the experiment after sampling for denitrification analyses (see below).

One day before flux incubations, the sediment surface of the cores was enriched with organic material. A microalgal concentrate of intact, non-living cells of the chlorophyte *Tetraselmis* sp. (Reed Mariculture) was added in wet form to the sediment surface. The degree of enrichment (5 g C m^{-2}) was estimated to be equivalent to approximately half a spring bloom event in the Baltic Sea (Graf et al. 1982, Lehtonen & Andersin 1998).

After the period of acclimatisation (17 d), samples for measuring oxygen consumption and benthic nutrient fluxes [NH_4^+, $\sum NO_3^- + NO_2^-$, HPO_4^{2-} and $Si(OH)_4$] were taken from the overlying water. Before sampling, the recirculating system was turned off and the cores were sealed with air-tight Plexiglas lids. Lids included 2 polypropylene valves, 1 for sample removal and 1 for diluted seawater that simultaneously replaced water removed during sampling (Hulth 1995). A Teflon-coated magnetic stirring bar was attached to the top of the lid, for mixing of the overlying water. Water samples (60 ml) were taken at starts and stops during the 2-point flux incubations (12 h). Incubations were performed during the day (07:00 to 19:00 h, day incubations) and during the night (19:00 to 07:00 h, night incubations) (Table 1).

The exact time of sampling was adjusted so that oxygen concentrations did not decrease by >20% from initial values (oxygen saturated). Benthic flux rates were calculated from the change in solute concentration of the overlying water with time of incubation.

Water samples for determination of oxygen concentration were immediately analysed after sampling, while water samples for nutrient determination were first filtered through 0.45 µm cellulose acetate filters and then frozen for later analyses.

Sampling for denitrification was performed at the end of the experiment (6 d after organic material enrichment) due to a destructive sampling protocol (Nielsen 1992). The recirculating system was turned off, and incubations were performed during the day and during the night as described above for the nutrient and oxygen incubations. ^{15}N-labelled $K^{15}NO_3$ (Isotec) was added to the overlying water in all cores to a final concentration of about 50 µM. Initial concentrations of nitrate in the overlying water were 0.82 ± 0.16 µM. At the end of incubations (12 h), saturated zinc chloride (7.5 ml l^{-1}) was added to each core to stop microbial activity. Sediments and water were then mixed. Before water samples were taken for denitrification analyses, the cores were left for several minutes for the sediment to settle. The water samples were transferred to gas-tight vials (10 ml); an additional 250 µl of saturated zinc chloride was added to each gas-tight vial. The gas-tight vials were kept cold (8°C) until the time of analyses.

Analyses and calculations. Oxygen concentrations were determined using Winkler titrations. Nutrients (NH_4^+, $\sum NO_2^- + NO_3^-$ and HPO_4^{2-}) in the overlying water were analysed using an auto-analyser (TRAACS 800) according to standard colorimetric methods (Strickland & Parsons 1972). The detection limit was estimated from variations in the baseline drift during nutrient analysis. Samples taken to determine denitrification rates were sent to the National Environmental Research Institute in Silkeborg, Denmark, and analysed according to the nitrogen isotope pairing method (Nielsen 1992).

Steady-state saturation concentrations of $^{29}N_2$ and $^{30}N_2$ at the start (t_0) of incubations, calculated from salinity and temperature, were <10 nM, i.e. <4% of $^{29}N_2$ and $^{30}N_2$ produced between start (t_0) and stop (t_1). Organic carbon and nitrogen in *Tetraselmis* sp. and in the solid phase of the sediment were measured by the EA1198 CHNS-O analyser (Fison instruments) and evaluated with the EAGER 200 software (Fison instruments). The light level was measured by a planar detector (LI-1400 data logger). All measurements are reported as means (±SE). Flux measurements are given in µmol or mmol per 12 h, to indicate activity during the day (07:00 to 19:00 h) and during the night (19:00 to 07:00 h). Data were tested with factorial analysis of variance (ANOVA, 1-, 2- and 3-way) with treatment (levels: *Monoporeia* and control), diurnal period (levels: day and night) and light condition (levels: light:dark and dark) as fixed factors. Homogeneity of variances were checked using Cochran's test, and, when found to be heterogeneous, the data were log transformed (Underwood 1997). Pairway testing of the

means were made using Student-Newman-Keul's (SNK) test. Differences were accepted as significant at $p < 0.05$.

RESULTS

Overall sediment characteristics and macrofaunal observations

Organic C and N content in the solid face of the surface sediment (before enrichment) were 4.4 and 0.60% of the dry weight, respectively, and the organic material (*Tetraselmis* sp.) contained 38% C and 2.1% N of the dry weight. The C:N molar ratios for the sediment and *Tetraselmis* sp. were calculated at 7.3 and 18, respectively.

Recovery and survival rates of *Monoporeia affinis* were found to be from 73 to 96% and from 59 to 87%, respectively (Table 2). The highest recovery (96%) and survival (87%) rates were found in light:dark-core night incubations, while the lowest recovery (73%) and survival (59%) rates were found in dark-core day incubations (Table 2). Also, light:dark-core night incubations had the lowest replacement rate (∑3 *M. affinis* of 3 replicate cores) and dark-core day incubations had the highest (∑12 *M. affinis* of 3 replicate cores) (Table 2). Thus, light:dark-core night incubations were least affected by mortality of *M. affinis* and dark-core day incubations were most affected. The differences found in recovery, survival and replacement rates between treatments may have had some effect on measured solute fluxes.

No direct documentation of swimming activity was carried out. However, swimming activity by *Monoporeia affinis* was confirmed by individuals found stuck at the water surface film, in those cases when air bubbles had been formed between the water surface and the lid of the cores, during the acclimatisation period before the flux incubation. During periods of darkness (light:dark core during night and dark cores during the 24 h) a higher number of *M. affinis* was normally found stuck (up to about 5–6 ind. core^{-1}) compared to when cores were exposed to light (light:dark cores during day). Under these latter circumstances, only 1 to 2 ind. core^{-1} were occasionally found stuck.

Oxygen consumption and nutrient fluxes

Overall, there was a stimulatory effect from the activity of *Monoporeia affinis* on mineralisation processes in the sediment. Solute fluxes were, in general, enhanced in cores with amphipods compared to control cores. Cores treated with *M. affinis* had a significantly (ANOVA: $p < 0.001$) higher oxygen consumption compared to control cores (Fig. 1). There were also significantly (ANOVA: $p < 0.001$) higher fluxes for nitrate, ammonium and silicate in cores treated with *M. affinis* (Figs. 2 to 4). For phosphate, on the other hand, a significantly (ANOVA: $p < 0.001$) higher flux was found in control cores compared to *M. affinis* cores (Fig. 5).

In Table 3 statistical tests (2-way ANOVA and SNK) of solute fluxes in light:dark cores (A) and dark cores

Table 2. Replacement of *Monoporeia affinis* (number of individuals in each of the 3 cores) during time between addition and end of experiment (22 d), and recovery and survival rates of *M. affinis* in experimental cores at the end of the experiment. Standard error given in parentheses. Originally added: 30 ind. core^{-1}

Core	Incubations	Replacement (no. core^{-1})	Recovery (%)	Survival (%)
Light:dark	Day	2, 3, 6	79 (6.8)	71 (7.8)
	Night	0, 1, 2	96 (2.9)	87 (1.9)
Dark	Day	2, 4, 6	73 (0)	59 (2.9)
	Night	1, 2, 3	81 (2.9)	74 (6.2)

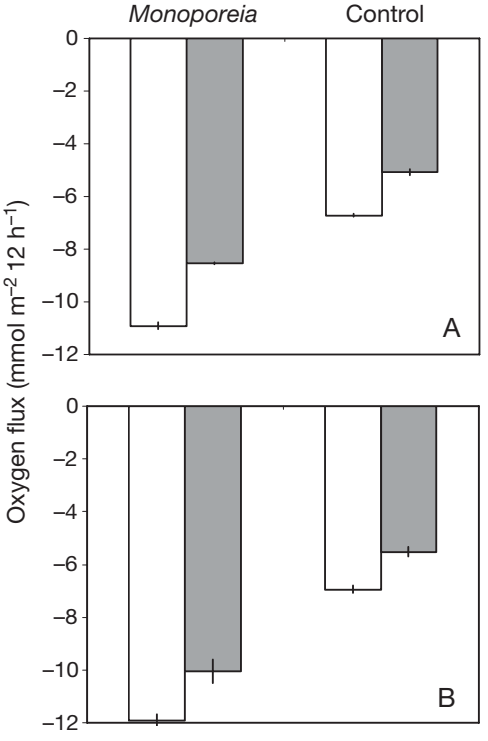

Fig. 1. Oxygen flux in control cores and in sediment cores treated with *Monoporeia affinis* during the day (white bars) and night (grey bars). Sediment cores exposed to (A) a 12:12 h light:dark cycle and (B) total darkness (24 h). Error bars = ±SE (n = 3)

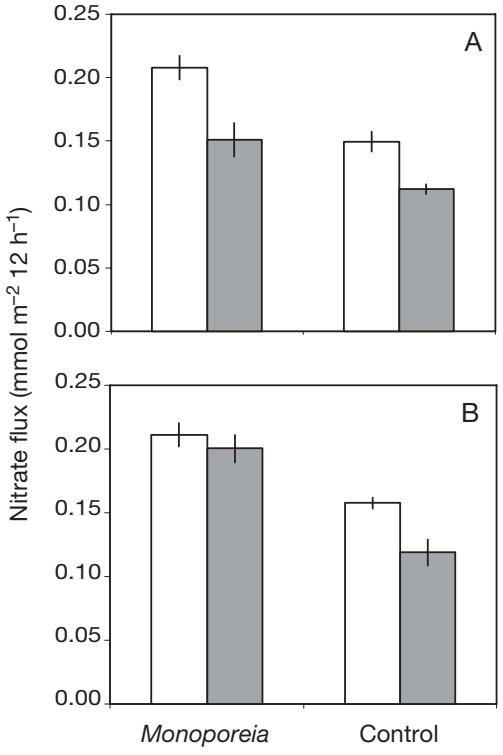

Fig. 2. Nitrate flux in *Monoporeia affinis* cores and control cores. Flux incubations were performed during the day (white bars) and night (grey bars). Sediment cores exposed to (A) a 12:12 h light:dark cycle and (B) total darkness (24 h). Error bars = ±SE (n = 3)

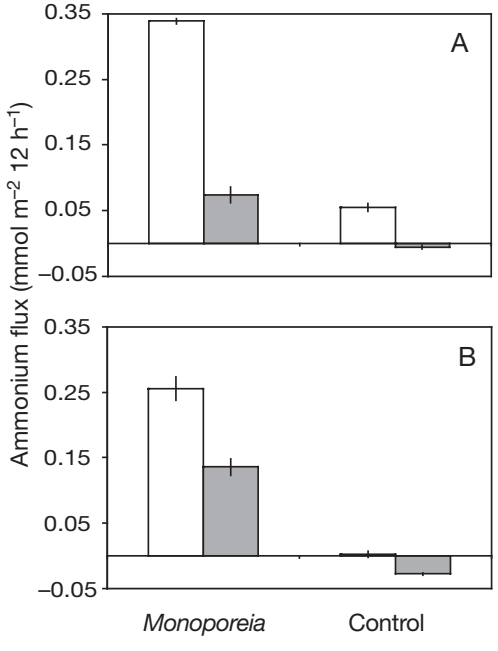

Fig. 3. Ammonium flux in *Monoporeia affinis* cores and control cores. Flux incubations were performed during the day (white bars) and night (grey bars). Sediment cores exposed to (A) a 12:12 h light:dark cycle and (B) total dark-ness (24 h). Error bars = ±SE (n = 3)

(B) are presented. For light:dark cores significant interaction between treatment and diurnal period (T × D) was found for oxygen, ammonium and phosphate, while for nitrate and silicate no interaction was found. For dark cores significant interaction between treatment and diurnal period was only found for ammonium.

A pronounced difference comparing day and night in *Monoporeia affinis* light:dark cores was recorded. light:dark cores treated with *M. affinis* had a significantly (SNK) higher oxygen consumption during the day compared to at night (Table 3A, Fig. 1A). Significantly higher ammonium and phosphate (SNK) fluxes were also found during the day (Table 3A, Figs. 3A & 5A). The higher solute fluxes during the day compared to the night indicate that diurnal activity of *M. affinis* was important for overall mineralisation and solute transport in the sediment. However, significantly higher oxygen consumption (SNK) and ammonium flux (SNK) were also found in the control light:dark cores during the day compared to at night (Table 3A, Figs. 1A & 3A). For nitrate and silicate there was a significantly higher (SNK) consumption of *M. affinis* cores and control cores combined during the day compared to at night (Table 3A, Figs. 2A & 4A).

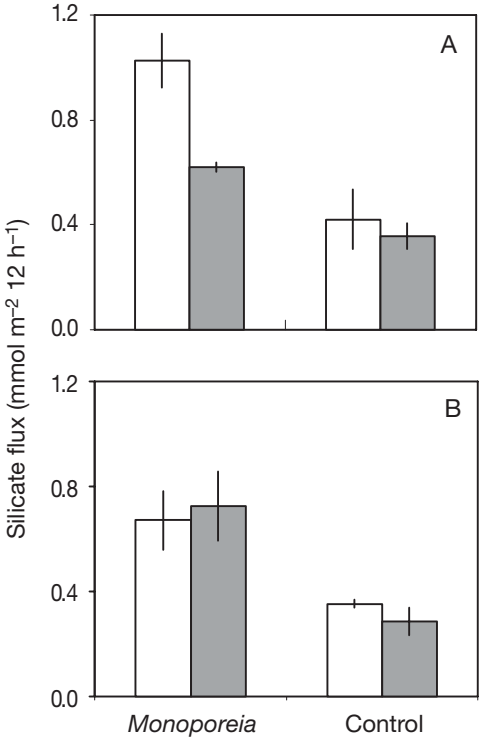

Fig. 4. Silicate flux in *Monoporeia affinis* cores and control cores. Flux incubations were performed during the day (white bars) and night (grey bars). Sediment cores exposed to (A) a 12:12 h light:dark cycle and (B) total darkness (24 h). Error bars = ±SE (n = 3)

Table 3. A 2-way ANOVA model testing effects from diurnal activity of *Monoporeia affinis* on solute fluxes in (A) light:dark cores and (B) dark cores. Mon: *M. affinis*; Con: control; Day and Night: incubation periods from 07:00 to 19:00 h and 19:00 to 07:00 h, respectively. Pairway testing of the means using Student-Newman-Keul's (SNK) test is also indicated. ***p < 0.001, **p < 0.01, *p < 0.05, ns: p > 0.05

Source of variation	df	Oxygen MS	Nitrate MS	Ammonium MS	Phosphate MS	Silicate MS
(A) Light:dark cores						
Treatment (T)	1	43.8***	7.12×10^{-3}**	0.0990***	4.29×10^{-3}**	0.502**
Diurnal period (D)	1	12.1***	6.66×10^{-3}**	0.0795***	1.35×10^{-3}*	0.146*
T × D	1	0.429**	2.86×10^{-4} ns	0.0312***	1.85×10^{-3}*	0.0774 ns
Residual	8	0.0272	2.50×10^{-4}	1.59×10^{-4}	2.01×10^{-4}	0.0223
SNK		Mon: Day > Night Con: Day > Night Day: Mon > Con Night: Mon > Con	Mon > Con Day > Night	Mon: Day > Night Con: Day > Night Day: Night:	Mon: Day > Night Con: Day = Night Mon > Con Mon > Con	Mon > Con Day > Night Day: Mon = Con Night: Mon < Con
(B) Dark cores						
Treatment (T)	1	67.6***	0.0137***	0.130***	2.65×10^{-3}**	0.497***
Diurnal period (D)	1	8.02***	1.84×10^{-3}*	0.0169***	4.84×10^{-5} ns	9.64×10^{-3} ns
T × D	1	0.146 ns	5.83×10^{-4} ns	6.05×10^{-3}**	6.04×10^{-4} ns	1.72×10^{-4} ns
Residual	8	0.227	2.38×10^{-4}	3.55×10^{-4}	1.67×10^{-4}	0.0188
SNK		Mon > Con Day > Night	Mon > Con Day > Night	Mon: Day > Night Con: Day = Night Day: Mon > Con Night: Mon > Con	Mon < Con	Mon > Con

Generally, larger differences in average solute fluxes were found comparing day and night in *Monoporeia affinis* light:dark cores as opposed to *M. affinis* dark cores, which may reflect stimulated diurnal activity during a light:dark cycle compared to constant light conditions (darkness) over 24 h. In dark cores an endogenous diurnal rhythm of *M. affinis* was indicated by ammonium. Significantly (SNK) higher fluxes were found for this solute during the day compared to at night (Table 3B, Fig. 3B).

Enhanced fluxes for some solutes during total darkness (dark cores), compared to those during a light/dark cycle (light:dark cores), seemed to have occurred. For example, during the night increased fluxes of ammonium and phosphate were found in *Monoporeia affinis* dark cores compared to light:dark cores (3-way ANOVA treatment × diurnal period × light condition: $p < 0.001$ and $p = 0.03$, respectively) (SNK) (Figs. 3 & 5). For oxygen, the interaction 'treatment × diurnal period × light condition' was found to be non-significant (3-way ANOVA). However, during day and night combined, higher rates were indicated in *M. affinis* cores during total darkness compared to the 12:12 h light:dark cycle (treatment × light condition: $p = 0.006$) (SNK) (Fig. 1).

There was no indication of stimulated microphytobenthos photosynthesis from the light intensity used at the simulated depth (25 m), since there was no significant (2-way ANOVA) difference in oxygen consumption comparing light:dark and dark control cores during the day (Fig. 1).

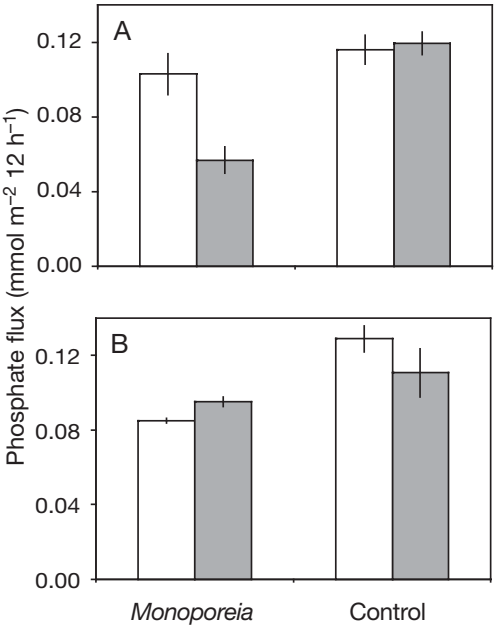

Fig. 5. Phosphate flux in *Monoporeia affinis* cores and control cores. Flux incubations were performed during the day (white bars) and night (grey bars). Sediment cores exposed to (A) a 12:12 h light:dark cycle and (B) total darkness (24 h). Error bars = ±SE (n = 3)

Denitrification

Diurnal activity of *Monoporeia affinis* showed no effect on denitrification comparing day and night in light:dark cores (Fig. 6A,C). Similarly the endogenous diurnal activity of *M. affinis* showed no difference in denitrification comparing day and night in dark cores (Fig. 6B,D). Coupled nitrification/denitrification (Dn) rates were about 3 to 4 times higher in cores with fauna and about 7 times higher in control cores compared to the denitrification rate caused by a stimulated supply of nitrate from the overlying water (Dw) (Fig. 6). *M. affinis* cores showed a significantly (ANOVA: $p < 0.001$) higher Dw compared to the control (Fig. 6A,B). Dn in *M. affinis* cores, on the other hand, was similar to that in the control cores (Fig. 6C,D).

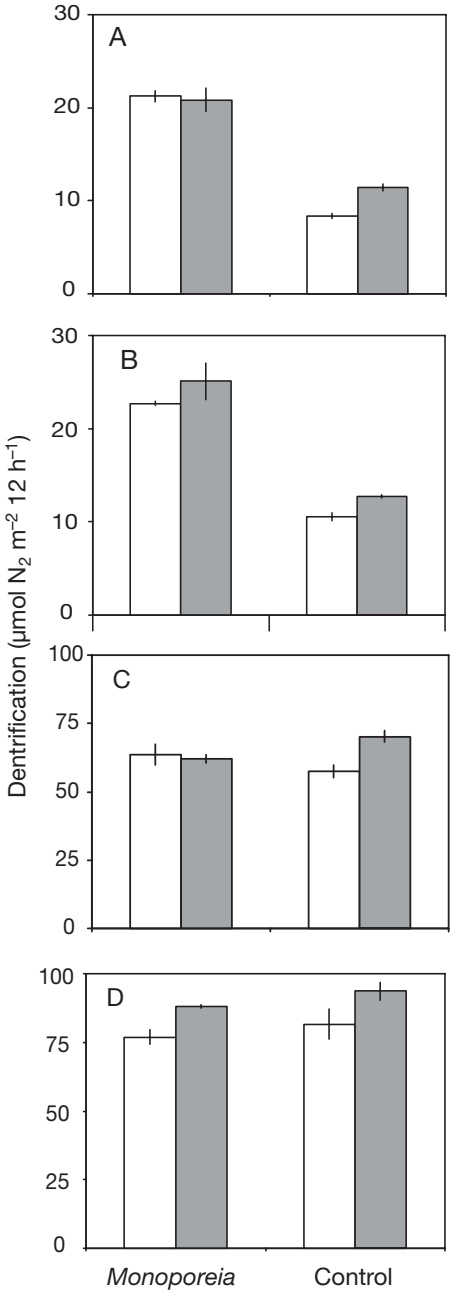

Fig. 6. Denitrification from nitrate supplied from (A,B) the overlying water and (C,D) coupled nitrification/denitrification. Rates are indicated for the control cores and cores with *Monoporeia affinis* during the day (white bars) and night (grey bars) incubations. Sediment cores exposed to (A,C) a 12:12 h light:dark cycle and (B,D) total darkness (24 h). Error bars = ±SE (n = 3)

DISCUSSION

Activities of *Monoporeia affinis* and effects on solute fluxes

Higher oxygen consumption during the day compared to at night in the *Monoporeia affinis* light:dark cores can most likely be explained by irrigation and bioturbation of *M. affinis*, mainly occurring during the day. As oxic water is drawn into the sediment during irrigation, it may increase sediment oxygen demand by stimulating bacterially and chemically mediated oxidation reactions (for example, nitrification and sulphide oxidation). Oxygen may also be consumed by macrofauna and meiofauna. The diurnal activity of *M. affinis* also had an effect on nutrient fluxes (ammonium and phosphate) across the sediment–water interface. The generally higher fluxes found during the day most likely reflected *M. affinis* bioturbating and irrigating activities. Ammonium, in particular, showed an enhanced flux in *M. affinis* cores during the day. Increased solute fluxes were an indication of enhanced mineralisation. Macrofaunal activity has been shown to stimulate mineralisation rates and, thus, pathways and transport of solutes (Diaz & Schaffner 1990, Aller 2001, Heilskov & Holmer 2001, Gilbert et al. 2003, Mermillod-Blondin et al. 2004). The role of *M. affinis* in benthic mineralisation processes has been found to be of significant importance in the Baltic Sea, either by direct metabolism or by enhancing bacterial activity through the process of bioturbation. For example, in a study by Lehtonen & Andersin (1998), it was estimated that *M. affinis* (about 5000 to 7000 ind. m^{-2}) may assimilate about 50% of the sedimented carbon during the period mid-March to mid-July. Furthermore, it has been shown that sediment reworking by *M. affinis* may result in improved oxygen conditions in the sediment, followed by stimulated nitrification and denitrification (Tuominen et al. 1999, Karlson et al. 2005).

Enhanced silicate fluxes cannot be explained by additions of organic material as such, since *Tetraselmis* does not contain significant quantities of silicate. Sili-

cate is generally considered a conservative nutrient, and does not normally participate in biogeochemical reactions other than the formation and dissolution of biogenic opal (SiO_2) (Libes 1992). Benthic fluxes of silicate are therefore sometimes used as an indication of solute transport enhancement caused by macrofaunal irrigation in sediments (Boudreau 1997, Aller 2001). In the present study the enhanced silicate flux is a clear indicator that bioirrigation by *Monoporeia affinis* stimulates solute transport.

In *Monoporeia affinis* cores the fluxes of silicate were, on average, about 2 times higher compared to control cores. There was also an indication of higher silicate fluxes during the day (on average 1.7 times) compared to at night in *M. affinis* light:dark cores; however, this was not statistically confirmed. In dark cores, on the other hand, day and night silicate fluxes were similar. The above observation supports the idea of enhanced irrigation activities of *M. affinis* and solute transports during the day.

Formation of anoxic microzones in connection with added organic material may explain the increased phosphate fluxes found in control cores. Mobilisation of phosphate to the pore water and subsequent transport across the sediment–water interface is often a consequence of a redox transition towards more reducing conditions in the overlying water, or an enhanced supply of reductants to the surface sediment (Sundby et al. 1986). In oxic sediments, on the other hand, phosphorus is readily adsorbed onto iron oxy-hydroxides (Krom & Berner 1980, Thamdrup 2000). In cores with amphipods present, formation of anoxic zones was most likely reduced by the activity of *Monoporeia affinis* through redistribution of added organic material and oxygenation of the sediment (Tuominen et al. 1999). Consequently, lower phosphate fluxes found in cores with *M. affinis* compared to control cores can be explained with the more positive redox condition found in the bioturbated cores and, thus, with the greater amount of phosphate bound in the sediment.

A decreased phosphate concentration in the pore water during oxygenation of the sediment and subsequent formation of Fe and Mn oxides may be directly associated with bioturbation and macrofaunal reworking activities (Mortimer et al. 1999, Tuominen et al. 1999).

Endogenous diurnal activity in *Monoporeia affinis* was indicated by ammonium fluxes (Fig. 3), as higher fluxes were found during the day compared to at night in the dark cores. However, the effect of diurnal activity in *M. affinis* was found to be more pronounced when exposed to light:dark stimuli.

Furthermore, it was possible to observe higher fluxes for some solutes during total darkness. Ammonium and phosphorous fluxes were higher in the *Monoporeia affinis* dark cores during the night compared to in the *M. affinis* light:dark cores, and oxygen consumption during day and night combined was higher in the *M. affinis* dark cores than in the *M. affinis* light:dark cores. During 24 h total darkness, swimming behaviour likely increased. Accordingly, higher numbers of *M. affinis* at the water surface film under dark conditions were generally observed. Stimulated swimming behaviour during the 24 h treatment may reflect the stress amphipods experienced when kept in the dark, as opposed to that of the amphipods in the light:dark cores, at almost natural light intensity (25 m). The changed condition may result in unnatural swimming behaviour of amphipods in the dark cores. It is, for example, possible that *M. affinis* repeatedly swim up and down, alternating between digging and swimming during continuous darkness. This behaviour of the amphipods may have caused enhanced particle displacement and an associated increased pore water flow, which may have had a stimulating effect on solute fluxes. Thus, enhanced solute fluxes found in the dark cores may be explained by an 'overactive' behaviour of *M. affinis* following 24 h darkness.

Oxygen consumption and ammonium fluxes were enhanced in control cores during the day compared to at night in the light:dark cores. This may indicate a diurnal activity of, for example, meiofauna (Boaden & Platt 1971, Steyaert et al. 2001). It is also possible that juvenile *Monoporeia affinis* and/or *Pontoporeia femorata* went through the 1 mm sieve and contributed to the achieved fluxes. Consequently, measured solute fluxes in *M. affinis* cores may not be attributed to the activity of *M. affinis* alone, but also to, for example, meiofauna.

Denitrification

Field studies have shown a significant correlation between benthic fauna and denitrification (both Dn and Dw) (Tuominen et al. 1998) and between benthic fauna dominated by *Monoporeia affinis* and *Pontoporeia femorata* and total denitrification (Gran & Pitkänen 1999) in the Baltic Sea.

In this study and previous studies (Karlson 2005, Karlson et al. 2005), denitrification using nitrate supplied from the overlying water (Dw) was accordingly found to be stimulated by the activity of *Monoporeia affinis*. However, no effect on coupled nitrification/denitrification (Dn) by *M. affinis* bioturbating and irrigating activity was observed.

Since Dw was between 3 and 7 times lower compared to Dn in this study, no marked effect from the activity of *Monoporeia affinis* could be seen on total denitrification (Dw + Dn). Only total denitrification in

M. affinis light:dark cores during day was significantly (SNK) higher compared to the control.

In contrast to this study, as well as to the studies of Karlson (2005) and Karlson et al. (2005), Tuominen et al. (1999) reported a stimulation of Dn and decreased ammonium flux in *Monoporeia affinis* cores. The reduced ammonium flux was explained by oxidation of the sediment caused by the activity of the amphipods and a concomitant stimulated nitrification in surface sediments. A different composition of the sediment, and the quality/quantity of the organic enrichment may explain the different observations. In this study the degree of enrichment was about 10 times greater, which likely had a larger reducing effect on the oxygen concentration of the surface sediment compared to the smaller size used by Tuominen et al. (1999). There was also a clear elevation of ammonium fluxes in *M. affinis* cores in this study, which indicates, relatively, more reduced sediment.

Under more reduced sediment conditions the relative contribution of Dw likely increases (Tuominen et al. 1998, Karlson et al. 2005). A stimulated Dw rate during recolonisation of highly anoxic Baltic Sea sediments by *Monoporeia affinis* was reported by Karlson et al. (2005), which was associated with a direct supply of nitrate from the overlying water to the sediment interior by faunal activities (Pelegrí & Blackburn 1995). Thus, the presumably more reduced oxygen conditions may be one explanation for the stimulating effect by *M. affinis* on Dw in this study compared to the Dn stimulation found by Tuominen et al. (1999).

Survival and recovery rates

Different survival and recovery rates in the cores may have affected measured flux rates. However, assuming no mortality and that a higher number of active *Monoporeia affinis* would have led to higher flux rates, the levels of significance should have been stronger rather than weaker. Therefore, animal mortality, in this sense, is not likely to have affected the main findings of this study.

On the other hand, the dead amphipods increased the amount of labile organic material in the sediment. The highest mortality (41%) of *Monoporeia affinis* in the experiment corresponded to 12 dead individuals, which corresponded to about 0.021 g C core^{-1} (Karlson et al. 2005) over a time period of 22 d (time between addition of *M. affinis* and the end of the experiment). The amount of added phytodetritus (*Tetraselmis* sp.) was 0.039 g C core^{-1}. Thus, addition of carbon by dead individuals (found or not recovered) may have influenced the overall microbial activity and the availability of oxidants/reductants in the sediment. However, it is not likely that all *M. affinis* died at the same time, but rather progressively throughout experiments. Each *M. affinis* added about 0.002 g C core^{-1}, and, considering the time of experiments (22 d) and the total amount of organic material (about 5 g C) in the surface layer where *M. affinis* was active (i.e. recovered after experiments), the effect of additional C from dead individuals was <1%. In addition, assuming a depth of residence for *M. affinis* of at least 5 cm and that added phytodetritus mainly occurred at the sediment surface (place of addition), the extra C added by dead *M. affinis* was diluted in a larger volume of sediment compared to C added by phytodetritus. Consequently, overall effects from dead individuals on mineralisation rates and additional eventual effects on the activity of *M. affinis* were considered minor.

CONCLUSIONS

The diurnal activity of *Monoporeia affinis* affected solute fluxes across the sediment–water interface. Significantly higher fluxes were found during the day in the light:dark cycle compared to measurements made during the night. Solute fluxes of oxygen, nitrate, ammonium, phosphate and silicate were enhanced on average by 22, 30, 78, 45 and 39%, respectively.

The study indicated that the endogenous diurnal activity of *Monoporeia affinis* also influenced solute fluxes. However, the effect from the diurnal activity of *M. affinis* was much more pronounced when exposed to light:dark stimuli.

Denitrification of nitrate supplied from the overlying water (Dw) was stimulated by the activity of *Monoporeia affinis*. However, no effect from the diurnal activity of *M. affinis* was observed on total denitrification (Dn + Dw).

Diurnal activity of meiofauna may have affected solute fluxes, as higher fluxes (oxygen and ammonium) were found in control cores during the day compared to at night.

This study clearly demonstrates that diurnal activity patterns among benthic fauna can have a profound effect on nutrient dynamics and mineralisation processes of the sediment and needs to be taken into consideration during, for example, experimental studies and nutrient modelling.

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