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ORIGINAL ARTICLE

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A method for rapid abundance estimation of semiplanktonic meiofauna

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Abstract Many meiofaunal copepods and plathelminths enter the tidal waters at night thus exhibiting a life-style intermediate between benthic and planktonic. At the same time, ostracods may leave their interstitial dwelling and move across the sediment surface. In laboratory experiments, the percentage of plathelminth populations emerging from the sediment varied with the species, temperature, light conditions, and the dimensions of the sediment cores studied, but not with tidal level, season, ambient density of conspecifics, or the sediment composition. Therefore, the swimming activity may be utilised for extraction of semiplanktonic meiofauna provided that the extraction procedure is standardised with respect to temperature, light and core size. For free-living plathelminths from the Wadden Sea intertidal a robust standard procedure is as follows: sediment cores 1.6 cm in diameter (2 cm^2 surface area) and 3 cm deep are fitted into cylindrical containers and submerged into aquaria containing filtered seawater (ambient salinity, room temperature, darkness) for 24 h. The sediment containers are then removed and the aquarian water filtered through appropriate meshes; the residue contains the emergent faunal component. For plathelminths, this procedure reduces sorting time by some 90% compared with the standard shaking–decantation method and thus makes it possible to process a high number of samples in a short time. Similar procedures may be developed for copepods and epibenthic ostracods.

Keywords Meiofauna · Emergence · Abundance · Extraction method · Spatial pattern

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Introduction

Meiofauna constitute a highly diverse component of the benthic fauna in coastal waters. In terms of species richness they exceed the macrozoobenthos by about an order of magnitude (e.g. Reise et al. 1998; Armonies and Reise 2000). Although the individuals are small, their high densities and high P:B ratios result in a productive share similar to that of the macrofauna (Giere 1993). Nevertheless there is a general paucity of studies on meiofauna and plathelminths in particular. One of the reasons is that meiofaunal studies depend on tedious extraction and sorting methods while a high number of replicates is needed for reliable estimates of population parameters because of strong small-scale patchiness. In addition, many meiofaunal taxa perform a life-style intermediate between benthic and planktonic. In shallow coastal areas, many harpacticoids resting in the sediment during low tide may enter the water column during high tide, preferentially in the dark (Walters and Bell 1986; Arlt 1988; Armonies 1988a, b, c; Bell et al. 1988; Hicks 1988; Palmer 1988; Walters 1988). Epibenthic ostracods moving across the sediment surface may be lifted off the sediment by the incoming tide to float at the water surface (Armonies 1988a, b, c). Finally, in free-living plathelminths life-styles seem to range continuously between exclusively benthic and planktonic (Armonies 1989). Due to transportation with the tidal currents, the benthic distributional patterns of these swimming or floating meiofauna change continuously (e.g. Palmer and Brandt 1981; Kern and Bell 1984; Coull and Feller 1988; Fegley 1988; Armonies 1990, 1994; Hicks 1992). Hence any estimate of abundance is merely a snapshot in a movie which may, or may not, be typical for a larger area or longer time, depending on the frequency of swimming and the net horizontal transportation of individuals. In order to arrive at a reliable estimate of abundance, large spatial scales need to be sampled, e.g. by using a mapping approach. However, considering the usual limitations of manpower, repeated mapping of a larger area becomes impossible in practice when using the traditional

methods for extraction. This is particularly true for the soft-bodied species such as plathelminths, which are best studied alive immediately after extraction from the sediment samples.

Therefore, a less time-consuming method is proposed here to estimate the abundance of the semiplanktonic meiofauna. It utilises the swimming behaviour itself as a convenient method for separating the specimens from the sediment. During experimental submergence of containers with sediment samples into aquaria, many specimens actively left the sediment (Armonies 1988a, b, c). They were separated by filtering the aquarian water through fitting meshes after removing the sediment containers. However, the percentage of specimens leaving the sediment samples varied with the species, the period of submergence, and physical conditions such as temperature, current velocity and salinity. In addition, the size of the sediment cores and the dimensions of the aquaria might affect the effectiveness of extraction. Therefore, some standardisation is needed. Based on two sets of experiments, a standard procedure for plathelminths in the Wadden Sea was developed.

The first set of experiments aimed to find optimum conditions with respect to (1) maximising the percentage of emergence, and (2) minimising its variability; that is, finding the most robust procedure. The second set of experiments checked for the effects of sediment composition and tidal level to give an overall estimate of the efficiency. Since the results of a single experiment often affected the experimental set-up of the succeeding ones, this paper deviates from the traditional organisation by describing the experiments one-by-one, including experimental set-up, results, and a short discussion. This is followed by a general discussion.

Although this study concentrated on plathelminths, copepods and ostracods were also enumerated in some experiments, indicating that the method may be useful for these taxa as well. Only a few experiments are needed to check whether the "Wadden Sea plathelminth" standard is also adequate for other geographical areas, respectively, to develop taxon- or habitat-specific other standards.

Methods

Study area

The study was performed using sediment and associated fauna from the Königshafen Wadden area, a semi-enclosed bight comprising about 5 km^2 of tidal flats, near the island of Sylt in the northern Wadden Sea (German Bight). Tides are semidiurnal with an average range of 1.8 m. Salinity remains close to 30 psu and the annual mean of sea water temperature is about 10°C. Compared with other parts of the Wadden Sea, the sediment is rather coarse-grained, which is due to aerial input of dune sand. A detailed description of the area is given by Reise (1985).

Experimental set-up

The following experiments all included collection of sediment cores in the field, transfer of the cores into fitting vessels, and subsequent submergence into aquaria under the specific experimental conditions. In each of the experiments, the sediment cores were simultaneously collected in the field and randomly distributed over the treatments as appropriate. The aquaria were filled with filtered seawater one day before the experiments started and allowed to adapt the experimental temperature. Prior to submersion the outer surface of the sediment containers was cleaned of adhering particles by dipping into fresh water. Then the containers were carefully filled with filtered seawater from a squeezing-bottle to the rim. This was done to prevent superficial sediment disturbance as the containers are submerged into the aquarian water. After the period of experimental submersion the containers were immediately removed and the aquarian water filtered through 0.063 mm square meshes. Specimens adhering to the aquarian walls were removed by a jet of filtered seawater from a squeezing-bottle. The residue from sieving was transferred into Petri dishes, the fauna counted, and plathelminths determined to species level using a stereomicroscope or compound microscope if necessary.

Two environmental factors, light and salinity, were not manipulated during this study. In the northern Wadden Sea, all species hitherto tested either showed a higher swimming activity in the dark than in daylight, or showed no difference between light levels (Armonies 1988a, c). Therefore, and because darkness is easier to standardise than a specific light level, all experiments were performed in the dark. However, specimens from other geographical regions or other taxa may show a different reaction to light (e.g. Bell et al. 1988). Therefore, the effect of light should be tested when dealing with specimens from outside the Wadden Sea.

While a reduced salinity increased activity of some plathelminth species (Armonies 1988c), other species might become inactive, at the same time. This is expected because in intertidal sediments short-term salinity fluctuations may either be due to precipitation, or evaporation, during low tide or in rather shallow waters. Both sources of salinity fluctuations usually affect a larger area and specimens have little chance to escape from adverse salinity by swimming. On the other hand, a sit-and-wait tactic will result in a return to the usual conditions as soon as the tidal flat is flooded again. Therefore, seawater of ambient salinity (30 psu) was used throughout. However, salinity may be a problem in estuarine habitats with strong salinity fluctuations. In supratidal salt marshes of the North Sea, activity of single species varied with salinity, with different sets of species becoming active as the salinity changed (Armonies 1986). Thus, salinity should be adapted to ambient salinity if the set of actually active rather than the potentially active species is concerned. Otherwise, several sets of samples may be submerged into water with different salinity to revive the passive (e.g. in tarpar or encysted) species as well. Note that there may be salinity–temperature interactions on the different species, as has been observed in some salt marsh plathelminths (Armonies 1986).

Results

Experiment I: core diameter

During experimental submersion of sediment cores into aquaria, semiplanktonic plathelminths and copepods were observed to swim straight upward into the water column, stay there for a while, and then sink to the sediment again (Armonies 1988b). In the absence of currents, the probability of returning to the sediment core increases with core diameter. Therefore, experiment I compared emersion from large versus small sediment cores to test whether core size would affect the number of specimens found in the aquarian water after submersion of the sediment cores (H_0: net emersion does not depend on core diameter).

Table 1 Set-up of the "aquarium size" experiment (2 July 1997; samples collected on an intertidal sandflat at mid-tide level)

Treatment	Aquarium volume (cm^3)	Water volume (cm^3)	Aquarium dimensions L×W or diameter (cm)	Water height (cm]	Bottom area (cm^2)
A	300	250	6.5	7.6	33
B	600	500	6.1×8.8	9.5	53
C	2,000	1,000	11.3	10	100
D	2,500	2,000	18×11.7	9.5	208
E	5,000	4,000	24.4×14.9	11	364

The sediment samples were collected on 30 June 1997, on a sandy tidal flat at mid-tide level. The "large" sediment cores were 3.5 cm in diameter and 5 cm high (equivalent to 10 cm^2 of sediment surface area, which is often used as a unit for meiofaunal abundance data). They were transferred into cylindrical vessels of 3.9 cm diameter and 4.4 cm height. The "small" sediment cores were 1.6 cm in diameter and 5 cm high (equivalent to 2 cm^2 surface area which is commonly used in plathelminth studies in the Wadden Sea) and were transferred into vessels of 2.0 cm diameter and 4.4 cm height. There were six replicates per treatment. The cores were individually placed into aquaria of 2,500 cm^3 volume filled with 2,000 cm^3 of filtered seawater. Submersion (for 15 h, at 18°C) started half an hour after sample collection.

Results

In absolute numbers, more specimens emerged from the larger sediment cores and the total number of recorded species was higher. However, based on volume units of the sediment, extraction efficiency from the small cores was higher for all of the abundant plathelminth species as well as for copepods and ostracods (Fig. 1). Therefore, the hypothesis claiming no effect of core size on extraction is rejected. Small sample units yielded about 50% more copepods, twice as many plathelminths (*U*-test: $P<0.01$), and nearly five times more ostracods (*U*-test: $P<0.01$).

Discussion

The superiority of smaller over larger sediment cores may generally hold true. For plathelminths in the Wadden Sea, sediment cores of 2 cm^2 surface area were a suitable size but even smaller cores may be appropriate for other taxa. However, as the statistical meaning of data becomes more doubtful as abundance decreases to <1 per sampling unit (Downing 1989), problems may arise in low-abundance habitats or with rare species. Then, submerging several small cores into a single aquarium may be better than using a few larger ones (see below). As a consequence of these results, all subsequent experiments used small cores (1.6 cm in diameter, equivalent to some 2 cm^2 of sediment surface area).

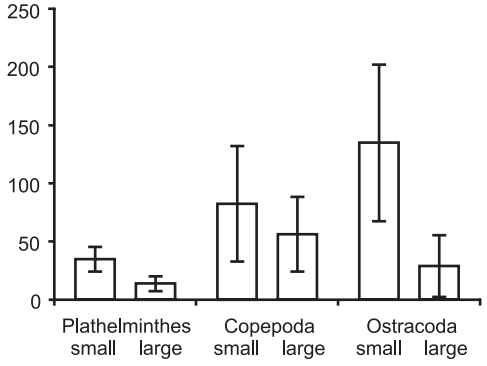

Fig. 1 Emergence from sediment cores varying in diameter. Number per 10 cm^2 of sediment surface area emerging from small (2 cm^2 surface area) and large (10 cm^2) cores, respectively

Experiment II: aquarium dimensions

Besides the diameter of the sediment cores, aquarium size might influence the probability of an accidental return to the sediment cores after swimming. Therefore, this experiment tested the hypothesis H_0: the dimensions of the aquaria do not affect net emergence.

The experiment included two runs. In the first one, a single sediment core was submerged into aquaria varying in size ("aquarian size" experiment, Table 1). In the second one, the dimensions of the aquaria were kept constant (type *D* in Table 1) but the number of cores per aquarium varied (1, 2, 4, 8 or 16 cores of 2 cm^2 surface area and 5 cm depth, collected on an intertidal sand flat at mid-tide level on 7 July 1997). Accordingly, the relation between aquarium bottom area and total area of the sediment cores varied between 104 (one core) and 6.5 (16 cores per aquarium). Both experiments were replicated six times.

Results and discussion

Within the tested range, aquarium size did not significantly affect emergence of plathelminths, copepods, and ostracods (*H*-test, $P>0.1$; Fig. 2). The smallest aquaria yielded the fewest plathelminths. Presumably, this is an artefact that does not refer to the size of the aquarium. Instead, the smallest aquaria had a wavy wall while all others had plain walls. Since many plathelminths (such as *Cheliplana remanei*, Fig. 2) may quite effectively ad-

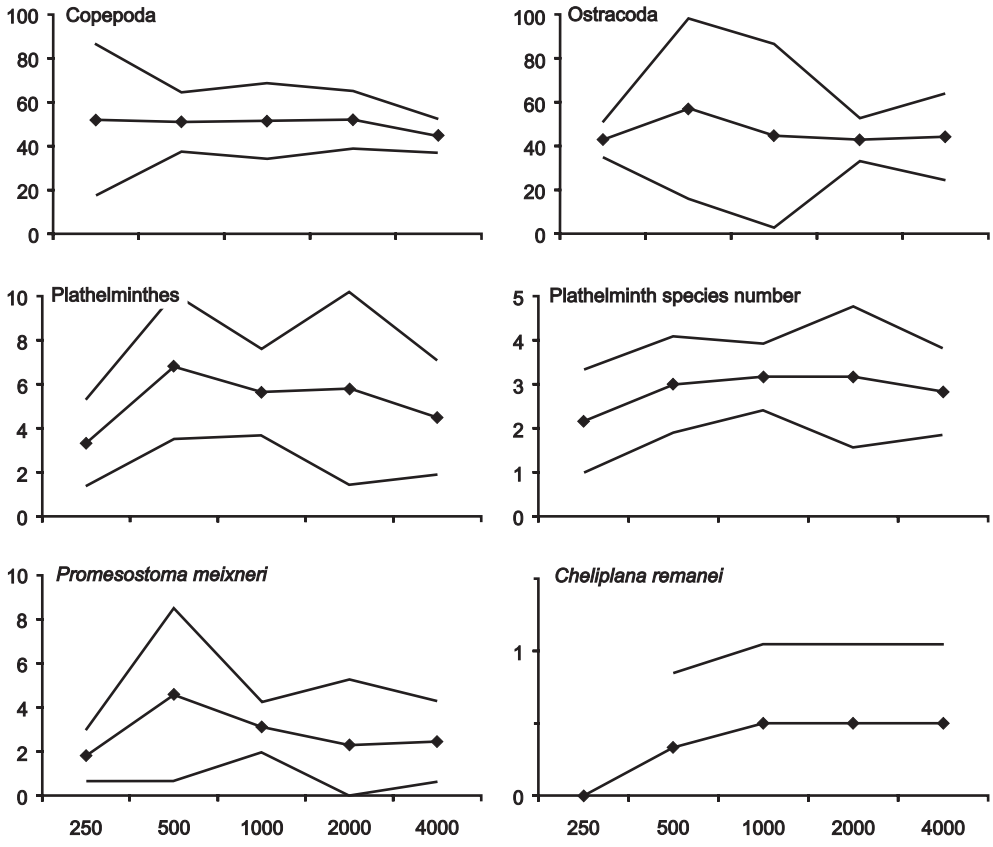

Fig. 2 Meiofaunal emergence from equally sized cores submerged into aquaria with varying dimensions (means per core and 90% confidence limits; water volume 250–4,000 cm^3)

here to solid surfaces, a jet of water from a squeezing-bottle may have been ineffective in removing them from the wavy walls of the smallest aquaria. Therefore, smooth-walled aquaria should be used when dealing with specimens able to adhere to solid surfaces.

Varying the number of cores per aquarium did not affect emergence of plathelminth taxa (*H*-test, $P>0.1$; Fig. 3). In some taxa (e.g. *Microstomum* spp. and *Promesostoma meixneri*) the numbers emerged per core show a (non-significant) tendency towards a lower efficiency in the treatments with a high number of cores. Presumably, this is a result of counting error. As an average of 70 plathelminths emerged from the 16-core treatment, small and sluggish individuals are more likely to be missed than in treatments with fewer individuals. In addition, predators such as *Pseudograffilla arenicola* are more likely to find a victim. Thus, since aquarium dimensions did not affect emergence within the tested range, an aquarium size of 500 cm^3 (e.g. a beaker) may be convenient for submergence of single cores while an aquarium capacity of 2,500 cm^3 was still handy for the simultaneous submersion of several sediment cores.

Experiment III: height of the sediment cores

The vertical distance between the sediment surface in the core containers and the upper rim of these containers might also influence extraction efficiency. This distance may either be reduced by using shorter containers or the sampled sediment depth may be varied. In the Wadden Sea, plathelminths occupy the top oxygenated sediment layer plus the uppermost zone impoverished in oxygen (Reise 1985; Scherer 1985). Depending on sediment composition and season, these zones vary in thickness between 1 mm and about 5 cm (excluding the more exposed beaches). However, emergent plathelminths are usually restricted to the uppermost few millimetres of the sediment; thus sampling the top 1 cm would suffice to collect these species. Experiment III tested shallow cores against deeper cores that harbour no additional extractable fauna but leave less distance between the core surface and the rim of the core containers ($H_{0,1}$: emersion does not depend on the core height; including the anoxic sediment layer does not affect the percentage of specimens leaving the cores). In addition, cores placed into empty glasses were compared with cores of the same size which were placed into glasses previously filled with paraplast to half their volume ($H_{0,2}$: emersion does not depend on the wall height between the sediment surface and the rim of the core containers).

Each treatment was replicated ten times (one core per aquarium, aquarium size 500 cm^3). Treatment A cores included a 5 cm sediment layer (top 1 cm of oxygenated or micro-oxic sediment and 4 cm of black anoxic sediment). Treatment B cores included the top 2 cm of sedi-

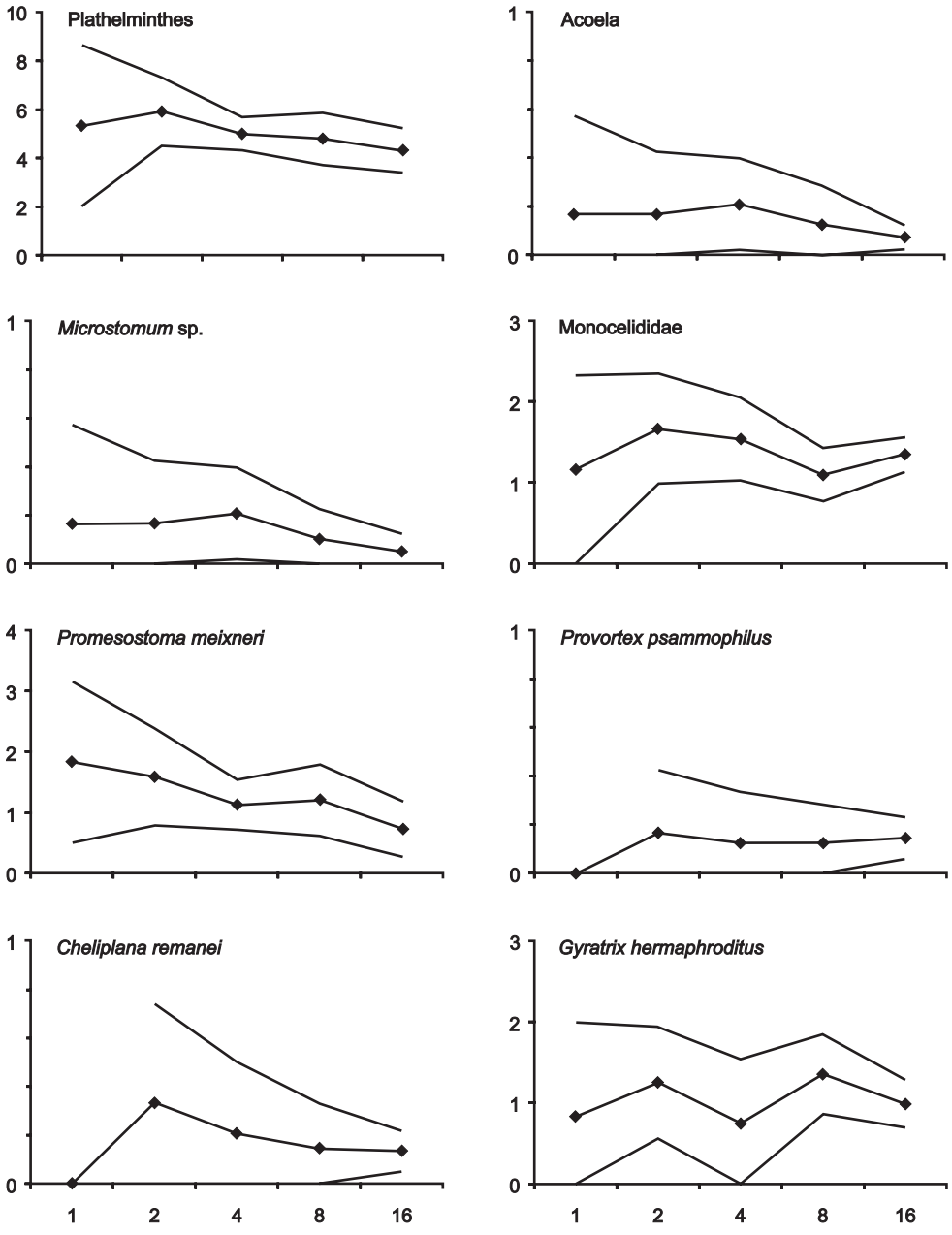

Fig. 3 Plathelminth emergence from a variable number of cores (1, 2, 4, 8, 16) submerged into aquaria with constant dimension (means per core and 90% confidence limits)

ment only. Treatment C: as B, but filled into containers that were previously filled with melted paraplast to half their volume. The core containers were rolled-rim glasses 4.5 cm high with an inner diameter of 1.8 cm. The wall height between the sediment surface and the upper rim of the containers was 1 cm in treatment A, 3.0 cm in treatment B, and 0.5 cm in treatment C. Samples were collected on 10 July 1997, from an intertidal sandflat at mid-tide level. Submersion (17 h) started immediately afterwards. Since this experiment gave significant results for copepods and ostracods but only a tendency for plathelminths, treatments A and B were repeated for the latter group using larger aquaria (2,500 cm^3) equipped with five sediment cores each (14 July 1997).

Results

Most ostracods left the containers with the smallest wall height above the sediment (*H*-test, $P<0.05$) and there was the same tendency in copepods (*H*-test, $0.1>P>0.05$). Comparing treatment A (top 5 cm layer of sediment) with treatment C (top 2 cm layer placed onto paraplast) indicates that the presence of the black sediment layer did not influence emergence (*U*-test, $P>0.05$). Finally, there was no significant difference between the treatments in plathelminths (Table 2).

Repetition of treatments A and B with five sediment cores per aquarium showed that the height of the sediment column is not important in most of the plathelminth species. Only *Gyratrix hermaphroditus* tended to emerge

Table 2 Emergence from sediment cores varying in height (mean and (SD) of ten replicates)

Treatment	A	B	C
Core height	5 cm	2 cm	2 cm/paraplast
Plathelminths	5.6 (1.8)	4.2 (1.7)	3.4 (2.5)
Copepoda	9.4 (4.6)	7.8 (3.6)	13.4 (6.1)
Ostracoda	107.0 (21.7)	82.6 (29.4)	128.0 (47.7)

Table 3 Plathelminth emergence from high (top 5 cm of ambient sediment) versus low (top 2 cm) sediment cores [means and (SD) per aquarium; only species with >ten individuals listed]

	High cores	Low cores
Promesostoma meixneri	2.1 (1.8)	2.2 (1.7)
Promesostoma caligulatum	2.8 (1.9)	3.7 (1.9)
Archilopsis spp.	16.4 (4.7)	16.8 (5.2)
Acoela	0.9 (0.9)	0.6 (0.8)
Gyratrix hermaphroditus	4.7 (4.9)	1.4 (1.5)
Cheliplana remanei	2.6 (2.0)	2.4 (1.5)
Provortex psammophilus	0.5 (0.5)	0.9 (0.9)
All species	30.7 (9.9)	28.9 (5.8)

in higher numbers from the higher cores (*U*-test, $0.1>P>0.05$; Table 3).

Discussion

The sediment cores should fit tightly into the sediment containers leaving a small (and, if possible, constant) distance between the sediment surface and the rim. The sediment depth that needs to be sampled to include all of the (potentially) emerging specimens may vary with the habitat and the season. While this may not be a problem in muddy sediments, meiofauna may, at times, retreat deep into coarse sand such as beaches exposed to wave action. In this case the sediment column may be split into horizontal layers and the same may be done if the vertical distribution in the sediment of the studied species is unknown.

Experiment IV: shape of the sediment containers

This experiment tested whether sediment containers with a straight vertical wall were superior to rolled-rim glasses (H_0: the shape of the sediment containers does not affect emersion). The rolled rim glasses were 4.5 cm high and 1.8 cm in inner diameter while aluminium caps (3.0 cm high and 1.8 cm wide) were used as straight-walled vessels. Sediment samples were collected on 15 October 1997, from ten intertidal sites with medium, fine, or muddy sand. Each aquarium (ten replicates per treatment) was equipped with five sediment cores from one of the ten sites.

Results and discussion

Fitting the samples into aluminium caps instead of glass tubes significantly increased the numbers of ostracods and plathelminths (ostracods, total=532 from aluminium caps versus 496 from glass vessels, sign test $P<0.05$; plathelminths 29 versus 20, sign test $P<0.05$). In part, this may be an effect of the smaller vertical distance between the sediment surface inside the containers and their upper rim. However, the rolled rim of the glasses might also be a barrier for these organisms as they try to climb the vertical walls. Therefore, straight-walled vessels are recommended as containers for the sediment.

Table 4 Plathelminth emersion after variable periods of storage and submersion. Totals of six replicates; only taxa represented by ≥10 individuals listed

Treatment	A	B	C
Storage	0 h	24 h	0 h
Submersion	24 h	24 h	48 h
Acoela	4	4	7
Bresslauilla relicta	5	5	0
Monocelididae	17	35	27
Promesostoma marmoratum	10	1	0
Promesostoma meixneri	10	10	9
Provortex psammophilus	5	7	4
All species	64	78	59
Number of species	15	16	11

Experiment V: sample storage and the period of submersion

In a previous experiment, storage of the sediment samples prior to submersion significantly increased swimming activity in some species (Armonies 1988c), possibly because of oxygen depletion during storage. On the other hand, species differ in the period needed to leave the sediment, which may be due to differential swimming frequency (Armonies 1988a, b, c). Experiment V tested the combined effects of storage ($H_{0,1}$: storage of the sediment samples prior to submersion does not affect emersion) and the period of submersion ($H_{0,2}$: prolonged submersion does not affect emersion).

There were three treatments (each replicated six times using aquaria of 2,500 cm^3 volume equipped with five sediment cores of 2 cm^2 each). Treatment A and C samples were submerged for 24 and 48 h, respectively, without intermittent storage. Treatment B samples were stored for 24 h (20°C, darkness) prior to submersion for another 24 h. The sediment was collected along a transect of six sites between low tide level and mid-tide level (25 August 1997) and each of the replicates received sediment samples from a different site.

Results

A comparison of treatments A and C indicates that a prolonged period of submersion increased the abundance of juvenile monocelidids only (Table 4). Since some of them were very small, hatching from egg-capsules dur-

ing the experiment cannot be excluded. On the other hand, prolonged submersion tended to decrease the abundance of *Promesostoma marmoratum* but did not result in an overall increase in efficiency.

A comparison of treatments B and C indicates that storage of the samples prior to submergence did not significantly affect any of the plathelminth species (species-wise *U*-tests, all $P>0.05$). Thus, storage was not harmful, indicating that submersion does not need to be started immediately after sample collection. A 1 day delay is unlikely to affect the results.

Discussion

As the species living in the Wadden Sea need to be adapted to irregular short-term extremes of physical factors, intermittent storage of the sediment samples for up to 24 h was not a problem. This facilitates the timing of sample collection because the samples may be collected during an evening low tide and stored until the next morning, thus avoiding night-work after the 24 h of submersion. However, studying subtidal or tropical habitats, intermittent storage may no longer be possible.

A 24 h period of submersion may be equally suited for non-tidal regions and habitats with a diurnal or semi-diurnal tidal cycle. Since many species respond to either tidal cycles or dark/light-rhythms (e.g. Bell et al. 1988; Decho 1988) a shorter period of submersion is not recommended unless specifically tested. A longer period of submersion might be useful in Arctic regions. However, as we observed that some plathelminth species with a boreal–arctic distribution were equally active in the Wadden Sea in winter as are species with a distribution in temperate regions in summer (personal observations), this may not be necessary.

Experiment VI: temperature during submersion

In previous experiments (Armonies 1988c) swimming activity in copepods and plathelminths correlated positively with temperature, suggesting that submersion in a warm environment would speed up emersion. During these experiments a temperature of 20°C (±2°C) was used, which is close to the average sea water temperature in the northern Wadden Sea in summer. Also when the ambient temperature increased to a record maximum of 26°C in August 1997 (up to 32°C in the intertidal) the experimental temperature was kept at a constant level of 20°C; otherwise some species of plathelminths may have become inactive by encystment (Armonies 1987) and thus cause underestimation of abundance. However, even 20°C might be a too high temperature as specimens are adapted to a much lower one during the cold season. Therefore, experiments VI and VII tested the effect of temperature during submergence of the samples, and previous sample storage, respectively.

Table 5 Meiofaunal emergence during submergence in cold (10°C) versus warm (20°C) seawater (totals from ten replicates)

	Season	Cold	Warm	Sign test
Plathelminth abundance	October	33	45	n.s.
Plathelminth species number	October	6	8	n.s.
Ostracods	October	251	282	n.s.
Plathelminth abundance	March	15	102	$P<0.05$
Plathelminth species number	March	6	14	$P<0.05$
Ostracods	March	51	56	n.s.
Copepods	March	213	511	$P<0.05$

The experiment on temperature effects during submergence was run twice, first in October 1997, when the Wadden Sea had cooled down to 12°C, and second in March 1998, when ambient temperature reached 6°C again. The experimental temperatures tested were 10°C and 20°C in both cases (H_0: increasing temperature does not affect emersion). Each treatment was replicated ten times using sediment cores of 2 cm^2 submerged into aquaria of 500 cm^3 volume. The sediment came from ten different intertidal sites.

Results

Increasing temperature resulted in significant increases in plathelminth and copepod emergence in March (ambient seawater temperature 6°C; Table 5) while there was only a slight increase of plathelminth and ostracod emergence in October (ambient sea water temperature 12°C). Thus, submerging the sediment cores in a rather warm environment accelerated emersion. On the other hand, considering plathelminths at the species-level there was no sign for a detrimental effect to any species.

Experiment VII: temperature during storage

This experiment tested for possible effects of storage when the temperature is outside the ambient range (H_0: temperature during storage does not affect emersion). Samples were collected on 30 March 1998, when the ambient seawater temperature was 7°C. There were four treatments varying in the temperature during storage and extraction. Treatments A and B were stored at 10°C, while treatments C and D were stored at 20°C. Temperature during submersion was 10°C in treatments A and C, and 20°C in B and D. Each treatment was replicated six times using sediment from six different intertidal sites.

Results

In all taxa, emergence was highest in the samples kept in the warm throughout and lowest in the cold/cold combination (Table 6). This is similar to the results of experiment VI. In plathelminths, storage of the samples in the cold did not affect emergence if succeeding submersion

Table 6 Meiofaunal emergence from sediment samples either stored cold (10°C) or warm (20°C) with subsequent submersion in the cold or warm (totals of six replicates from different intertidal sites)

Treatment	A	B	C	D
Storage	Cold	Cold	Warm	Warm
Submersion	Cold	Warm	Cold	Warm
Plathelminths	12	46	23	46
Ostracods	50	115	100	144
Copepods	103	339	225	465

was in the warm (sign test, $P>0.05$). However, when submersion was done in the cold, significantly more plathelminths emerged from samples previously stored in the warm (sign test, $P<0.05$; Table 6). In copepods and ostracods storage of the samples in the warm significantly increased emergence (sign test, $P<0.05$) irrespective of the temperature during submergence. Detrimental effects of the warm environment were not detected in any species.

Discussion

The results fit previous findings that meiofaunal populations of the Wadden Sea intertidal generally show the highest abundance in summer. From this it is concluded that both storage and submersion should be done in quite a warm environment; the average summer temperature of the ambient water may be adequate. Possible effects of temperature increases beyond that level should be tested. However, the situation may be different in supratidal salt marshes and beaches because these habitats may harbour species with a more boreal–arctic distribution which are adapted to lower temperatures. They become active when their supratidal habitats are flooded during storm tides in winter (Armonies 1987; Hellwig 1987). In such cases a lower temperature may be more appropriate.

Experiment VIII: efficiency of the method

Swimming activity of single species might vary with the sediment composition, the density of conspecifics, season and tidal level. Therefore the overall efficiency of the method was tested by evaluating both the emergent fauna and the specimens that rested in the sediment after submergence ($H_{0,1}$: emergence does not vary with the sediment composition; $H_{0,2}$: emergence does not depend on the ambient density; $H_{0,3}$: emergence does not vary with the season).

Sediment cores (1.6 cm in diameter and 3 cm height) were fitted into aluminium containers (1.8 cm in diameter and 3 cm high) leaving a vertical distance ≤5 mm between the sediment surface and the upper rim of the containers. Submersion in filtered seawater (one core per aquarium of 500 cm^3 volume) started the day after sample collection at 8.00 a.m. Intermittently, samples were stored in the dark (20°C). After submersion for 24 h, the sediment containers were removed and the emergent fauna separated from the aquarian water by sieving. The fauna that remained in the sediment cores were extracted by a shaking–decantation procedure using seawater for the first six runs, then twice with fresh water, and finally 90% ethanol [methods combined from Noldt and Wehrenberg (1984) and Armonies and Hellwig (1986)]. In total ten sites varying in tidal elevation, exposure to wave action, and sediment composition were studied (Table 7, sites 11–20; five replicates per site). Half of the sites were studied in April 1998, when the Wadden Sea had warmed up to 6–8°C. The other five sites were studied in July, when ambient sea water temperature was 14–16°C.

A second set of ten sites was studied using a slightly different method. Sediment cores (1.6 cm diameter, 5 cm deep) were transferred to rolled-rim glasses, experimental submersion started immediately afterwards (i.e. no storage), and the period of submersion was 16 h only. In this set, the granulometric sediment composition was analysed from the pooled replicates ($n=5$) of any site (Table 7, sites 1–10) according to the procedures described by Buchanan (1984).

Results

On average over the ten sites, about half of all plathelminths left the sediment cores during experimental submergence for 24 h (equivalent to two semi-diurnal tidal cycles) and a quarter when submersion was for 16 h (including a single tidal cycle; Table 8). Most species showed the same tendency with respect to the period of submersion while the most active swimmers (*Cheliplana remanei*, *Promesostoma caligulatum* and *Promesostoma meixneri*) were notable exceptions (Table 8).

The percentage of all plathelminths leaving the sediment did not correlate significantly with tidal level (Spearman's rank correlation coefficient, $r_s=0.224$, $n=10$, $P>0.1$; Fig. 4). However, fine grained sediment (respectively, habitats with a low exposure to wave action) harboured significantly more emergent species (Spearman's rank correlation coefficient, $r_s=0.665$, $n=10$, $P<0.05$; Fig. 4).

Considering emergence from single cores, the percentage of emerging plathelminths did not correlate significantly with ambient density (Spearman's rank correlation coefficient calculated on all cores that harboured >1 individual; $r_s=-0.220$, $n=40$ cores, $P>0.05$ for the 16 h submersion series; $r_s=+0.283$, $n=45$ cores, $P>0.05$ for the 24 h submersion series). The same was true for single species; however, this may in part be due to low abundance. Therefore, density-dependent emersion cannot generally be excluded.

In the 24 h submersion series, five of the sites were studied in April and five in July. Using chi-square tests on 2×2 contingency tables (with the entities months, April or

Table 7 Sampling sites for Experiment VIII. Sediment gives the modal diameter of grain size in µm and tidal level refers to cm above mean low tide level (average tidal range is 180 cm). Exposition gives the relative influence of wave action; 1=low, 2=moderate, 3=strong

Site no.	Habitat	Sediment	Tidal level	Exposition	Date
1	Sandy beach	334	110	3	24.07.1997
2	Sand flat	196	90	2	28.07.1997
3	Sand flat	203	80	2	30.07.1997
4	Sand flat	218	50	2	07.08.1997
5	Sand flat	164	30	2	10.08.1997
6	Sand flat	163	20	2	11.08.1997
7	Muddy sand	148	90	1	13.08.1997
8	Sand flat	262	150	2	14.08.1997
9	Sand flat	173	140	2	18.08.1997
10	Muddy sand	143	120	1	25.08.1997
11	Sand flat		0	2	24.04.1998
12	Sand flat		40	2	21.04.1998
13	Sand flat		90	2	23.04.1998
14	Sand flat		40	2	27.04.1998
15	Sand flat		90	2	27.04.1998
16	Sand flat		150	1	01.07.1998
17	Muddy sand		130	1	01.07.1998
18	Sand flat		50	2	07.07.1998
19	Sand flat		80	2	07.07.1998
20	Sand flat		110	2	07.07.1998

Table 8 Plathelminth emergence during 16 h (including one tidal cycle) or 24 h (two tidal cycles) of experimental submersion (*n*, totals and %, percentages from ten intertidal sites; only species with >10 individuals in at least one of the two sets)

Submersion period	16 h		24 h	
	n	%	*n*	%
Acoela indet.	34	35%	47	74%
Acrorhynchides robustus	14	7%	21	24%
Bresslauilla relicta	3	33%	19	11%
Cheliplana remanei	11	91%	11	82%
Gyratrix hermaphroditus	50	40%	6	33%
Macrostomum pusillum	17	18%	8	63%
Microstomum cf. *jenseni*			25	60%
Monocelididae[a]	55	20%	93	54%
Neoschizorhynchus parvorostro	22	0%	29	0%
Paromalostomum dubium	4	0%	23	17%
Parotoplanidae[b]	15	7%	5	80%
Pogaina suecica			19	74%
Promesostoma caligulatum	7	86%	21	81%
Promesostoma meixneri	13	85%	148	84%
Provortex psammophilus	22	9%	40	10%
Ptyalorhynchus coecus	5	0%	19	5%
Total plathelminths (54 spp.)	361	26%	590	52%

[a] Mainly *Archilopsis arenaria*
[b] Mainly *Parotoplana papii* and *Parotoplanina geminoducta*

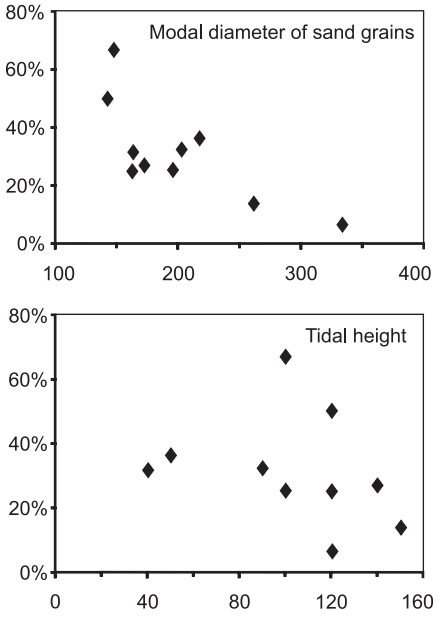

Fig. 4 Percentage plathelminth emergence from different sediment types (*top*) and different tidal levels (*bottom*). Sediment types are given as modal diameter of sand grains in µm and tidal level in cm above mean low tide level; mean tidal range is 180 cm

July, and residence after submersion, sediment or water; *df*=1) showed no seasonal differences in single species. Plathelminths as a whole, however, showed a significantly higher percentage emersion in July (Table 9). This was mainly caused by a higher abundance of *Promesostoma meixneri* which is one of the most active swimmers in this intertidal plathelminth assemblage.

Discussion

The percentage of emergent plathelminths varied with the species composition but not with tidal elevation, indicating that emergent species do not differ in swimming activity throughout these intertidal flats. A few cores from the subtidal Wadden Sea, collected in August 1998, showed that emergent plathelminths and copepods occur down to a water depth of 30 m (which was the deepest site tested) while epibenthic ostracods occurred down to 10 m at least (personal observations). However, the percentages of emergent specimens in the meiofaunal community was not evaluated in these subtidal sites. Similarly, emergence also occurred in supratidal salt marsh meiofauna when these marshes were flooded during a storm tide (personal observations). Thus, emergence is not restricted to intertidal areas.

Table 9 Seasonal differences in plathelminth emergence. *N* = total of specimens per five sites (25 sediment cores of 2 cm² surface area), E% = percentage emersion during 24 h. Only species represented by >5 individuals per season, n.s.=*P*>0.05

Taxon	April		July		
	N	E%	*N*	E%	Chi²
Acoela	13	92%	34	68%	3.008 n.s.
Acrorhynchides robustus	7	43%	14	14%	2.100 n.s.
Bresslauilla relicta	13	15%	6	0%	1.032 n.s.
Microstomum cf. *jenseni*	13	46%	12	75%	2.163 n.s.
Monocelididae	35	51%	58	55%	0.123 n.s.
Paromalostomum dubium	11	18%	12	17%	0.009 n.s.
Promesostoma meixneri	18	83%	130	84%	0.003 n.s.
Provortex psammophilus	12	17%	28	7%	0.847 n.s.
Total of all species	185	43%	405	56%	9.402 *P*<0.01

With respect to sediment composition, emergent species were most abundant in sheltered habitats (with muddy sediments) and least in sandy beaches exposed to wave action (coarse-grained sediment; Fig. 4). However, pure mud (i.e. a sediment containing >50% of dry weight of particles <0.063 mm) does not occur around the island of Sylt, and therefore was not tested. There were no seasonal differences in emergence of single species; however, as the community composition changed towards a higher percentage of active swimmers in July, total plathelminth emergence was higher in summer.

General discussion

A standard screening procedure for plathelminths in the Wadden Sea

Based on the results of experiments I–VII, the following "screening standard" is proposed for plathelminths in the Wadden Sea intertidal:

- Sediment cores of 2 cm² surface area and 3 cm depth are fitted into cylindrical containers.
- Submerge for 24 h into aquaria filled with filtered seawater of ambient salinity, at room temperature, in the dark.
- Remove the sediment containers after the submersion period, and filter the aquarian water through appropriate meshes. Study the emergent fauna immediately afterwards.
- If necessary, the sediment samples may be stored for up to 24 h before submersion; this should be done at room temperature. Use the same period of storage within a set of experiments.

Some experiments showed (statistically non-significant) trends in single species which may deviate from the average of the other species. Since only single or few factors have been tested in each of the experiments, the combination of such trends might achieve significant effects, particularly when several factors strongly deviate from the above recommendations. As an example, prolonging the period of storage and/or submersion in a warm room may result in oxygen depletion, causing emergence of specimens that would otherwise not leave the sediment, or it might kill others. In addition, species interactions may change the faunal composition during submersion. As an example, plathelminth predators of the taxon *Promesostoma* (distributed world-wide in coastal waters) on an average consume 1–2 harpacticoids (or ostracods, depending on species) per day (Menn and Armonies 1999). Assuming typical intertidal abundances of these predators and their prey, prolonging submersion may reduce harpacticoid abundance by some 5% per day.

Table 10 Time needed to separate plathelminths from the sediment by the screening standard method (min per set of six replicates, estimated from experiment II)

	Number of cores per aquarium				
	1	2	4	8	16
Placing and filling aquaria	3	3	3	3	3
Adding/removing the sediment cores	1	2	4	8	16
Filtering the aquarian water	10	10	10	12	15
Cleaning of equipment	5	5	5	5	5
Sorting of plathelminths	35	40	50	65	90
Total per six replicates	54	60	72	93	129
Total per aquarium	9	10	12	15	22
Total per sediment core	9	5	3	2	1.5

Since several samples may be submerged into a single aquarium at a time, the replicates needed to compensate for small-scale spatial heterogeneity may be evaluated simultaneously – provided that there is no need for an estimate of within-site variability. High core-number treatments are the most cost-efficient in terms of handling time (Table 10). For comparison, shaking–decantation extraction of plathelminths takes an average of 30 min per core, depending on the sediment composition. Thus, using the proposed screening standard may estimate abundance in all replicates in the same time that is usually needed to handle a single core. However, this tenfold increase in efficiency is counterbalanced by a restricted subset of species.

Since emergence did not vary with tidal elevation or season (experiment VIII), species-specific factors might be calculated to correct for an extraction efficiency <100%. However, as emergence is influenced by a multitude of other factors (e.g. Armonies 1988a, b, c), caution is advised. In fact, the proposed "screening method" was designed to compare large-scale spatial distribution pat-

terns over a longer period of time. For this purpose, the number of emergent specimens may be an equally valid indicator of change as (absolute) density.

Modifications for other geographical areas or other taxa

The procedure suggested above is based on the physical conditions in the northern Wadden Sea. Studying other geographical areas it may require some modifications. The effects of intermittent sample storage and light should always be tested. A time series of submergence periods may be run if, for some reason, a 24 h period of submersion is not deemed adequate. Temperature and salinity effects should be tested if values outside the seasonal mean are used. After these experiments, the efficiency of the adapted procedure should be checked by counting both the emergent fauna and the fauna resting in the sediment after submersion.

While sediment composition did not affect plathelminth emersion in the Wadden Sea, this may not hold true for other habitats or taxa. Particularly in copepods, swimming behaviour may vary over habitats (e.g. sand versus seagrass; Hicks 1986). Within habitats, emergence may vary with food concentration, intraspecific density, and age composition (Service and Bell 1987; Kern 1990), the latter may in part derive from differential feeding habits of adults and juveniles (Decho and Fleeger 1988). Therefore, when including markedly different habitats some extra experiments should be done to check for habitat-specific differences in emergence. Otherwise the number of emerged specimens may be a biased estimate of abundance.

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