Effect of food and sediment pre-treatment in experiments with a deposit-feeding amphipod, *Monoporeia affinis*

Albashir A. Aljetlawi, Jan Albertsson, Kjell Leonardsson

Department of Ecology and Environmental Science, Umeå University, SE-901 87 Umeå, Sweden

Received 9 December 1999; received in revised form 9 March 2000; accepted 28 March 2000

Abstract

We experimentally investigated the effects of different pre-treatments of the sediment, and the effect of daily addition of fresh phytoplankton, on the growth and survival of 1-year-old (1+) individuals of the deposit feeder *Monoporeia affinis* (Amphipoda). We used three different types of sieved sediment: pre-frozen muddy clay, non-pre-frozen muddy clay, and fine sand. The muddy clay contained phytoplankton originating from the surface sediment sampled in the field during the late spring bloom. No phytoplankton was initially present in sand. The experiment lasted for 18 days. *M. affinis* responded to the daily phytoplankton addition by increasing growth. Phytoplankton addition had no significant effects on the survival of *M. affinis*. Upon phytoplankton addition, the sandy and non-frozen muddy clay gave similar growth and survival responses. In contrast, the pre-frozen sediment resulted in significantly lower growth and survival. The growth was negative in all treatments without phytoplankton. Thus, the high initial chlorophyll content in the muddy clay was not of sufficient quality or concentration to allow a positive growth response in *M. affinis*. The growth of *M. affinis* was significantly correlated with the reduction of the chlorophyll. Our results indicated that *M. affinis* is capable of assimilating settled phytoplankton with no, or only a few days’ time delay. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Deposit feeder; Growth; *Monoporeia affinis*; Phytoplankton addition; Sediment treatment; Survival

1. Introduction

To understand how species interact, it is fundamental to have a basic knowledge about their food resources and environmental requirements. A negative influence of one
species on another could be explained either by resource competition, interference, or predation. One group of animals for which information on food sources is difficult to derive is for deposit feeding benthic animals. In addition, experiments with infauna species are difficult to perform without disturbing their environment—the sediment. To get rid of unwanted species or individuals prior to the experiment, the sediment is often sieved (Hill and Elmgren, 1987; Bianchi et al., 1988; Leonardsson, 1991; Hill, 1992; Webb and Montagna, 1993; Sparrevik and Leonardsson, 1998, 1999; Sparrevik, 1999) or frozen (Kern and Taghon, 1986; Smith and Brumsickle, 1989; Turner et al., 1997; Vrissier, 1998). Little is known about how such treatment affects (a) the amount and quality of food for deposit feeders, and (b) the behaviour of the species.

Several different approaches have been used to find out the food source of benthic animals. One method is to use stable isotopes to find out the origin of the assimilated carbon and nutrients (Miyazaki et al., 1985; Couch, 1989; Gu et al., 1994; Hobson et al., 1995; Newell et al., 1995). However, if the resources come from several trophic levels, and different geographical origins, it may be difficult to estimate the relative contribution of each food source due to the additive inclusion of the isotopes in the somatic tissues. This method can provide a rough estimate of which trophic levels the main food sources come from, but it cannot tell us if prey were dead or alive when eaten. This question is crucial when discussing species interactions.

The traditional gut content analyses do not usually provide an answer to the question concerning the essential food source of deposit feeders. Such studies often show that unspecified detritus makes up a dominant fraction of the gut content (Moore, 1977; Johnson, 1987a,b). This is the case with the swimming and burrowing amphipods of the Pontoporeididae, Monoporeia affinis (Lindström), and the closely related species Pontoporeia femorata Kröyer, and Diporeia hoyi (Smith). These species are semelparous and live in substrate types ranging from sandy to muddy sediments (Marzolf, 1965), in marine or freshwater environments. They are suggested to be non-selective in ingesting the organic fraction of the sediment (Marzolf, 1965; Ankar, 1977; Lopez and Elmgren, 1989), as many other deposit feeders (Hylleberg and Gallucci, 1975; Lopez and Kofoed, 1980; Taghon, 1982; Lopez and Levinton, 1987). Bioturbation by Pontoporeia and other deposit feeders dilute the food in the top few centimetres of the sediment (McCall and Tevesz, 1982; Robbins, 1982; Tevesz and McCall, 1983; Elmgren et al., 1986). In gut content analyses of M. affinis, detritus represented 97.6% and algae 2.3% in a freshwater lake (Johnson, 1987a). Moore (1977) found similar gut contents in D. hoyi, for which the corresponding figures were 98.5 and 1.5%, respectively. Bacteria and meiofauna seem not to be of major importance since their production cannot cover the energy requirements of M. affinis (Uitto and Sarvala, 1991; Goedkoop and Johnson, 1992, 1994). Despite the small fraction of algae in the gut, Johnson and Wiederholm (1992) found that the yearly growth rate of M. affinis in an oligotrophic lake was highly correlated with the diatom biovolume during the spring bloom. Sedimented phytoplankton is therefore the most likely food source, despite its minor fraction in the gut.

To be able to understand the intra- and interspecific competitive interactions among deposit feeders it is important to know whether the phytoplankton are available as food as soon as they reach the sediment, or if some degradation is necessary. Only field observations on growth rates and production estimates are available for interpretation of
this issue for *M. affinis*. These observations are somewhat ambiguous. Cederwall (1977) found a direct response of *M. affinis* growth to the phytoplankton spring bloom, while others have found a delayed response (Elmgren, 1978; Sarvala, 1986; Uitto and Sarvala, 1991; Lehtonen and Andersin, 1998). Such observations raise the question of whether *M. affinis* can grow on newly settled phytoplankton or if some degradation is necessary.

Does the pre-treatment of the sediment, and the way food is offered in experiments prevent us from observing a natural response in deposit feeders? How does the choice of sediment type affect the results? Here we investigate these questions using an experimental design focusing on the effects of (a) different pre-treatments of the sediment, (b) different sediment types, and (c) addition of fresh phytoplankton. We used *M. affinis*, for which we studied growth and survival to investigate these questions.

### 2. Materials and methods

#### 2.1. Experimental set-up and accomplishment

To investigate the impact of fresh phytoplankton, and sediment type and pre-treatment on the growth of *M. affinis* we used a two-way factorial experiment. The impact of phytoplankton was investigated with and without addition of fresh phytoplankton. The other factor constituted sediment type and pre-treatment of the sediment, with three levels; non-frozen and pre-frozen muddy clay, and non-frozen terrestrial fine sand.

The population of *M. affinis* consisted of three age cohorts from the northern Bothnian Sea. We only used 1-year-old (1+) individuals for the experiments. These were collected by dredging at a depth of 16–18 m from an inshore area near Norrbyn (63° 33.5’ N, 19° 49.7’ E), 40 km south of Umeå, northern Sweden. Oxidised surface sediment, the upper 2–3 cm, was collected from the same area by using a sledge-like dredge during the late spring bloom on May 20, 1998, about 2 weeks before we started the experiments. We first sieved the sediment through a 0.5-mm mesh to remove the macrofauna, then homogenised the sediment–water mixture by stirring, and left it outdoors for sedimentation, covered with a black plastic lid. After 1 week we split the sediment into two portions, of which one was kept in a freezer (−21°C) for 4 days. The remaining portion was left outdoors, covered with a black plastic lid. The aim of the freezing was to reduce potential competitors, i.e., meiofauna and bacteria, in the sediment. Since pre-freezing of sediments prior to experiments is often used in marine ecological experiments, we wanted to evaluate the general impact of freezing. In addition to non-frozen and pre-frozen muddy clay, we used wet-sieved (0.5-mm mesh size), clean, fine, terrestrial, non-frozen, sand as separate sediment without organic content.

We ran the experiments in PVC cylinders that were 50 cm in height and 18.6 cm in diameter. In total we ran six different treatments with five replicates of each. Each cylinder was filled with a 7-cm thick layer of sediment, and 40-cm (8.5–10 l) brackish seawater. We filtered (100-μm mesh) the brackish seawater used in the treatments to remove most meso-zooplankton. In addition, we filtered (borosilicate microfiber 0.8-μm mesh size filters GF75) the brackish seawater used in treatments without phytoplankton.
The initial chlorophyll $a$ (chl $a$) content of the non-phytoplankton treatments was $0.035 \pm 0.014 \mu$g l$^{-1}$ (±1 S.D., $n = 5$), while for the phytoplankton treatments it was $2.12 \pm 0.01 \mu$g l$^{-1}$. These levels are negligible when compared with the total amounts added to the treatments. The cylinders were randomly assigned to two large tanks with flowing water ($5.4 \pm 0.4^\circ C$) to keep temperature variation to a minimum. The surface level of the water was the same inside and outside the cylinders. The tanks were covered with black plastic lids to mimic deep-water light conditions and to prevent phytoplankton growth during the experiment.

One week after the addition of the sediments, we placed 50 randomly picked $M. affinis$ individuals in each cylinder (1840 ind m$^{-2}$). We excluded individuals with an empty gut (examined by eye), to avoid individuals close to the moulting stage. Before adding the animals to the cylinders, we kept them in darkness in aquaria at $7^\circ C$ for 48-h acclimation, to allow for accidentally injured animals to die before the onset of the experiment. From these animals we also preserved five replicates with 50 $M. affinis$ in each for analysis of the initial ash-free dry weight (AFDW) (Table 3). We measured the AFDW following HELCOM (1988).

From the onset of the experiment, we added 100 ml of a phytoplankton suspension corresponding to $56 \pm 24 \mu$g chl $a$ cylinder$^{-1}$ day$^{-1}$ (±1 S.D.) from a phytoplankton culture (see Section 2.4) on a daily basis to the cylinders belonging to the phytoplankton addition category. The variation given was over the time and originated from daily variation of chl $a$ in the algal culture. The amount of phytoplankton added daily to the cylinders corresponded to the daily sedimentation of phytoplankton during the spring bloom of 1991 in the southern Bothnian Sea (Kuparinen et al., 1994). To minimise handling differences between the treatments the same volume, 100 ml, of filtered seawater was added to each of the cylinders belonging to non-addition treatments. The experiment began on June 19 1998 and lasted for 18 days. The experiment was terminated by sieving the contents of the cylinders through a 0.5-mm mesh size net. The $M. affinis$ were collected from the sieve and preserved in a 4% formaldehyde solution for 7 days. Thereafter we counted the animals and measured their AFDW. The specific growth rate of $M. affinis$ was calculated from $G = \{\ln(W_f) - \ln(W_i)\}/time$, where $W_f$ is the final weight (i.e., AFDW), $W_i$ is the initial weight, and time is the length of the experimental period in days.

2.2. Estimation of time delay

Although the experiment was not originally intended to investigate the presence of a time delay between the settling of the phytoplankton and the onset of feeding, it provides useful information on this issue. While a time delay can be explained by several different mechanisms, the absence of a time delay suggests that the Monoporeia started feeding immediately, without any need for the algae to degrade. For this estimation, we included results from a pilot experiment, identical to the main experiment except that it only lasted for 10 days and the daily resource addition amounted to $15.2 \pm 14.3 \mu$g chl $a$ day$^{-1}$. If there is no time delay in feeding, the growth occurs over the entire period and should therefore be calculated from the onset of the experiments. However, if there actually is a time delay from the settling of the algae to the onset of feeding, a more

An accurate measure of the specific growth rate would be to calculate the growth rate using the number of days actually feeding. This would also require an adjustment of the initial individual mass because of starvation during the initial phase. We calculated the growth rates for a range of time delays for both experiments to evaluate the resulting growth rates from this scenario. To estimate the initial mass for different time delays we used the following differential equation:

\[ \frac{dW}{dt} = -m_1 W^{m_2} \]  

This equation accounts for loss of weight due to metabolic costs, where \( m_1 \) and \( m_2 \) are constants, and \( W \) is the individual mass. We estimated \( m_1 \) from the sandy sediment treatments without addition of algae. The exponent of the allometric scaling of these costs was \( m_2 = 0.75 \), obtained from Lehtonen (1994). Under the assumption of \( T \) days initially without feeding in the experiment, we calculated the individual mass, \( W_f \), at the onset of feeding. For each value of \( T \), between 0 and 9 days, we calculated the expected growth rate in each of the experiments. We only used data from the sandy sediment with addition of phytoplankton for these calculations, since in the other treatments some additional food should have been originally present in the sediment. Our goal with these calculations was to find the delay when the growth rates were equal in the two experiments. In that situation, the value of \( T \) gives us the time delay, given that the individuals had maximum food supply in both experiments. If the food supply were below this threshold, the time delay would be shorter than the time \( T \) where the growth rates were equal. The reason for this is that less food was added in the pilot experiment, which could make the growth rate without a time delay lower in this experiment.

2.3. Sediment and water analyses

Sub-samples for sediment analyses were collected with thin steel cylinders of 2.3-cm inner diameter. For the sediment characteristics, we measured loss on ignition from two sub-samples per treatment the day before the start and at the end of the experiment. For these measurements, the upper 3-cm of the cylinders was used. The loss on ignition was analysed following standard techniques (Dybern et al., 1976). The organic content in fine sand, and non-frozen and pre-frozen muddy clay at the start of the experiment was 0.14±0.01% (±1 S.D., \( n = 4 \)), 3.21±0.08 and 3.16±0.08%, respectively. The organic content did not change significantly from the start to the end of the experiment (ANOVA \( F_{1,18} = 1.85, P = 0.19 \)). In the addition treatment, the organic content was 0.15±0.001% in fine sand, 3.13±0.10% in non-frozen, and 3.17±0.14% in pre-frozen muddy clay, while its value was 0.18±0.04, 3.14±0.08 and 2.84±0.28%, respectively, in treatment without phytoplankton addition. For analyses of phaeopigment and chl \( a \) content, we collected one additional sub-sample from each of two cylinders per treatment. The uppermost 5-mm of the sediment was collected from each of these cylinders.

In addition, we collected samples for analysis of the water chl \( a \) content from the same two experimental cylinders (aquaria), in the middle and at the end of the experiment. One 100-ml water sample was collected from each of these cylinders. Each water sample was gently filtered (\( \leq 30 \) kPa) onto a 42.5-mm GF/C filter. The filter was
dried and wrapped in a piece of clean filter paper in air for 30 min. The chl \(a\) retained on the filter was extracted for 24 h in darkness at room temperature with 10 ml of 95% ethanol. After 10-min centrifugation at 3500 rpm, the extract was measured fluorimetrically (Perkin-Elmer fluorometer LS30) at 433 and 676 nm for excitation and emission wavelengths, respectively. Fifteen ml of 95% ethanol were added to the sediment samples, and after a 5-min ultrasonic bath, extracted for 24 h in darkness at room temperature. Ten ml of the supernatant were transferred to clean Falcon tubes and centrifuged and measured as above. Fluorescence was measured both before and 3 min after acidification with 1 M HCl (12 \(\mu\)l ml\(^{-1}\) extract). Chl \(a\) and phaeopigment concentration was calculated according to Edler (1979), using calibration factors obtained from a pure standard of chl \(a\) (Fluka) measured before and after acidification.

2.4. Phytoplankton culture

The phytoplankton culture was started from filtered (100-\(\mu\)m mesh size to exclude most meso-zooplankton) seawater during the spring bloom. The culture volume amounted to 80 l. To keep the algal population growing we added daily the basic nutrients nitrogen, as Na\(\text{NO}_3\) (50 mg l\(^{-1}\)), phosphorus, as Na\(\text{HPO}_4\) \(3.5\) mg l\(^{-1}\), and silica, as Si(OH)\(_4\) (2.5 ml l\(^{-1}\)). The culture was kept indoors in 12-h light and natural seawater temperature (\(\pm\)12\(^\circ\)C). We collected daily samples for chl \(a\) analysis and for the addition to the phytoplankton treatments in the cylinders. Dominating phytoplankton species in the culture were noted by visual inspection using a microscope. The species composition of the culture did not change markedly throughout the experimental period (Table 1). As in a typical phytoplankton spring bloom in our area, diatoms dominated in the phytoplankton culture but the species composition was somewhat different. *Entomoneis paludosa* and *Surirella* sp. were dominating in the culture, while *Thalassiosira baltica* and *Chaetoceros wighamii* commonly dominates in the field (Kuparinen et al., 1996). However, we do not find it likely that this difference has affected the results. The dominant species of the culture and the field do not differ markedly by size or carbon content per cell volume (data from Swedish National Monitoring Programme).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Average</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cell size</td>
<td>(l \times b) ((\mu)m)</td>
<td>(V) (%)</td>
</tr>
<tr>
<td><em>Entomoneis paludosa</em></td>
<td>40.5×17.7</td>
<td>45.4</td>
<td>38.2</td>
</tr>
<tr>
<td><em>Surirella</em> sp.</td>
<td>30.6×21.9</td>
<td>41.3</td>
<td>42.2</td>
</tr>
<tr>
<td>Pennate diatoms</td>
<td>98.0×8.7</td>
<td>8.5</td>
<td>10.8</td>
</tr>
<tr>
<td>Pigmented flagellate</td>
<td>6.6×6.6</td>
<td>1.7</td>
<td>3.6</td>
</tr>
<tr>
<td><em>Chaetoceros wighamii</em></td>
<td>9.5×8.1</td>
<td>1.6</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Aulacoseira</em> sp.</td>
<td>12.0×10</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td><em>Uroglena</em> sp.</td>
<td>0.0</td>
<td>0.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Before addition of cultured phytoplankton to the experimental treatments, we collected 25–30 l of the culture and filtered it through a 7-μm mesh, after which the sample was rinsed with filtered water to dilute the nutrients. A 7-μm filter was used to concentrate the larger phytoplankton and to remove the nutrient-rich water. Thereafter we stirred the phytoplankton into 1.5 l filtered (as in Section 2.1) water and distributed it equally among the replicates in the experiment. After the removal of phytoplankton from the culture, we replaced the removed volume of nutrient-enriched seawater.

2.5. Statistical treatment of the results

We used ANOVA to test for effects of sediment type and addition of phytoplankton on growth and survival of *M. affinis*. We checked the residuals for homogeneity and normality of variances. The survival probabilities were arcsine-transformed before the statistical analysis to achieve homogeneity of variances. To test for differences between groups we used Tukey’s test of multiple comparisons (Systat, 1992).

3. Results

3.1. Growth and survival

The addition of phytoplankton had a significant positive effect on the daily specific growth rate of *M. affinis* compared with treatments without phytoplankton addition (Table 2). With addition of phytoplankton, the specific growth rates were 0.017 in fine sand, 0.010 in non-frozen muddy clay, and negative, −0.001, in pre-frozen muddy clay (Table 3 and Fig. 1). In all treatments without phytoplankton addition *M. affinis* had negative growth rates (sand, −0.005; non-frozen muddy clay, −0.003; pre-frozen muddy clay, −0.008). Additionally, the sediment treatment showed a significant effect on the daily specific growth rate. The impact of the sediment treatment was not consistent, but differed depending on the addition of phytoplankton. The interpretation of this interaction showed that the growth rates were significantly higher with phytoplankton addition in sand and non-frozen muddy clay, compared with the other treatments (Tukey’s post-hoc, *P* < 0.05, df = 24, Fig. 1). The final body masses resulting from each treatment are given in Table 3. In contrast to growth, food addition had no

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>1</td>
<td>0.00147</td>
<td>61.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sediment</td>
<td>2</td>
<td>0.00028</td>
<td>11.88</td>
<td>0.0003</td>
</tr>
<tr>
<td>Phytoplankton × sediment</td>
<td>2</td>
<td>0.00015</td>
<td>6.37</td>
<td>0.0061</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.00002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The squared multiple r was 0.80.*
Table 3
Average and standard deviation (S.D.) of initial and final body masses (mg AFDW) and, specific growth rate (day$^{-1}$) of 1-year-old (1+) *Monoporeia affinis* amphipods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body mass (mg)</th>
<th>Specific growth rate (day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>S.D.</td>
</tr>
<tr>
<td>Initial</td>
<td>0.64</td>
<td>0.03</td>
</tr>
<tr>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytoplankton addition:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>0.87</td>
<td>0.09</td>
</tr>
<tr>
<td>Non-frozen muddy clay</td>
<td>0.76</td>
<td>0.07</td>
</tr>
<tr>
<td>Pre-frozen muddy clay</td>
<td>0.63</td>
<td>0.07</td>
</tr>
<tr>
<td>Non-phytoplankton addition:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>0.58</td>
<td>0.05</td>
</tr>
<tr>
<td>Non-frozen muddy clay</td>
<td>0.61</td>
<td>0.03</td>
</tr>
<tr>
<td>Pre-frozen muddy clay</td>
<td>0.55</td>
<td>0.04</td>
</tr>
</tbody>
</table>

overall effect on survival (Table 4 and Fig. 2). However, the sediment treatment, and its interaction with food addition had significant effects. The interaction originated from an increased survival in the food addition treatments in sand and non-frozen muddy clay, while in contrast the food addition treatments decreased survival in the pre-frozen muddy clay. The survival was similar in sand and non-frozen muddy clay (with food addition, 0.94±0.05 in each; without addition, 0.86±0.11 and 0.84±0.09, respectively).
Table 4
Two-factor ANOVA table for effects of sediment treatment (Sediment) and phytoplankton addition (Phytoplankton) on the survival of 1-year-old (1+) *Monoporeia affinis* amphipods\(^\dagger\)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>2</td>
<td>0.29452</td>
<td>28.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>1</td>
<td>0.00022</td>
<td>0.02</td>
<td>0.8871</td>
</tr>
<tr>
<td>Sediment × phytoplankton</td>
<td>2</td>
<td>0.05385</td>
<td>5.19</td>
<td>0.0133</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.01037</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^\dagger\) The squared multiple \(r\) was 0.74.

The survival was generally lower in the pre-frozen sediment, 0.52±0.16 with food addition, and 0.68±0.1 without. During the daily procedure of adding phytoplankton/water to the cylinders we observed high swimming activity in all pre-frozen muddy clay treatments. This high swimming activity could be related to the lower growth rates and survival rates in these treatments.

3.2. Estimation of time delay

Estimation of a time delay in the onset of feeding of *Monoporeia affinis* would provide information on whether this deposit feeder could use newly settled phytoplankton as a food source. The estimation of the presence of a time delay required calculation of the metabolic constant that converts respiration loss to loss in mass, \(m_1\). This constant was estimated to 0.027±0.012 and it did not differ significantly between the 10- and 18-day experiments. The masses after an initial starvation period due to any

![Fig. 2. Survival of 1-year-old (1+) *Monoporeia affinis* amphipods, with and without phytoplankton addition in different sediments (fine sand, non-frozen and pre-frozen muddy clay). Error bars denote ±1 S.D.](image-url)
given time delay in the onset of feeding were derived from the integration of differential equation (Eq. (1)). While increasing the time delay, the starvation reduce the body mass at the onset of feeding, and the daily growth rate during the remaining period had to increase to reach the observed final body mass. This yielded growth rates for the two experiments that increased rapidly when the time delay approached the length of each experiment (Fig. 3). The growth rate without any time delay was lower for the 10-day experiment with lower amounts of food addition. At a time delay of 5 days the calculated growth rate curves for the two experiments intersected. For longer time delays, the growth rates were higher in the low addition experiment. Since this is not realistic, the time delay for the onset of feeding should be less than 5 days.

3.3. Changes in the water and the sediment chlorophyll

The phytoplankton settled rapidly after being added to the cylinders. More than 80% of the added chl a had disappeared from the water in the experimental cylinders from 1 day to the next. The highest chl a contents at the end of the experiment with food addition was noted in non-frozen muddy clay, 9.8±2.9 μg cylinder⁻¹, and lowest in the pre-frozen muddy clay treatment, 6.1±0.6 μg cylinder⁻¹. The water chl a increased in all treatments without algal addition and reached the highest level, 8.5±1.6 μg cylinder⁻¹, in non-frozen muddy clay. The remaining treatments had lower final water chl a contents, 1.6±0.9 μg cylinder⁻¹.

No chl a could be detected in the fine sand before the start of the experiment in the upper 5-mm of the sediment. In this sediment type, chl a increased in phytoplankton addition treatments and reached 94±35 μg cylinder⁻¹ in this layer (Fig. 4). In comparison, the total addition of chl a amounted to 950 μg cylinder⁻¹. There were no differences between the initial chl a contents among non-frozen and pre-frozen muddy clay, 470±37 and 461±64 μg cylinder⁻¹, respectively. In treatments with phyto-

![Fig. 3. Estimated specific growth rates, assuming different time delays between the settling of the phytoplankton and the onset of feeding and assimilation. The two curves denote the results from the 18-day (55.7 μg chl a day⁻¹) and the 10-day (15.2 μg chl a day⁻¹) experiments, respectively.](image)
plankton addition, chl $a$ increased in both non-frozen and pre-frozen muddy clay to 677±190 and 596±67, respectively, at the end of the experiment. In the treatments without addition, chl $a$ deceased in both non-frozen and pre-frozen muddy clay, 107±35 and 315±66 μg cylinder$^{-1}$, respectively.

The reduction of total chl $a$ content in the cylinders during the experiment was somewhat less than the amount added for all treatments (Fig. 5). The specific growth rate of $M$. affinis showed a significant positive correlation ($r = 0.67, n = 12, P = 0.017$) with the reduction of the chl $a$ (total reduction in water and uppermost 0.5 cm in the sediment) (Fig. 6). The interpretation of such a correlation would be that $M$. affinis consumed most of the phytoplankton. The reduction in chl $a$ was about equal in all sediment types, about 80–90% of the added chl $a$. Despite this similarity, the growth of $M$. affinis was only similar in the sand and non-frozen muddy clay with phytoplankton addition (Fig. 1). However, a high reduction in chl $a$ also occurred in the non-frozen muddy clay when no phytoplankton was added (Fig. 5). The corresponding reduction of the initial sediment chl $a$ was much smaller in the pre-frozen sediment.

### 3.4. Estimation of the consumed amounts of added phytoplankton

One question of interest is how large a fraction of the added phytoplankton was actually consumed by $M$. affinis. We determined a conversion constant, from 30±5 μg chl $a$ l$^{-1}$ to 1 μg C l$^{-1}$ (Aljetlawi and Leonardsson, in preparation) to find the
conversion from μm chlorophyll to μm carbon. This yielded a total carbon addition of 25–30 mg C cylinder⁻¹. Since at least 10% of the chlorophyll remained in the cylinders at the end of the experiment, we estimate that the lost fraction amounted to 23–27 mg C cylinder⁻¹. By using the initial and final weights of M. affinis from the non-phyto-

Fig. 5. Initial chl a contents of sediment (columns); reduction of total chl a (chl a in water and uppermost 5-mm of the sediment combined) content in cylinders with (filled circles) and without (open circles) addition of phytoplankton. The chl a addition (squares) to cylinders exposed to phytoplankton addition amounted to 950 μg cylinder area⁻¹ irrespective of sediment (fine sand, and non-frozen and pre-frozen muddy clay). Error bars denote ±1 S.D.

Fig. 6. Relationship between reduction of total (water and uppermost 5-mm sediment combined) chl a and specific growth rate (day⁻¹) of 1-year-old (1+) Monoporeia affinis amphipods.
plankton addition treatment in Eq. (1), we calculated the energetic loss of carbon as 2.2 mg C cylinder\(^{-1}\). The absolute growth in sand with phytoplankton addition was 5–7 mg C cylinder\(^{-1}\). By using the relationship: 
\[
growth = \text{assimilation efficiency} \times \text{ingestion} - \text{energetic costs}
\]
we can estimate the total ingestion by *M. affinis* per cylinder. Assuming an assimilation efficiency in the range 0.7–0.8, we obtained a total ingestion of 9–13 mg C cylinder\(^{-1}\). Thus, about 40% of the added phytoplankton carbon was ingested. The corresponding calculations for the pilot experiment with lower daily addition of phytoplankton, less than one-third of that in the 18-day experiment, yielded 70–100% ingestion of the added phytoplankton carbon.

4. Discussion

A seemingly high quality sediment, collected from the sediment surface layer during the late spring bloom was not sufficient for growth of *M. affinis*. This was the case despite a high initial chlorophyll content in this sediment. The growth condition deteriorated in this sediment when it was frozen before the experiment. In the pre-frozen sediment, the conditions became unfavourable for *M. affinis*, and we observed drastically increased swimming activity in the phytoplankton addition treatment in this sediment. Most likely it was the increased swimming activity that led to negative growth and high mortality. In order to get positive growth of *M. affinis*, fresh phytoplankton had to be added to the experimental units. When doing so, the sandy, inorganic, and the non-frozen muddy clay gave similar positive responses for both growth and survival. Our results show that the choice of pre-treatment of the sediment and the addition of high quality food in experiments with *M. affinis* is crucial for obtaining useful results. This should be especially important when performing interaction experiments. Although our study only focused on one species of deposit feeder, there is reason to believe that other species may also be sensitive to the pre-treatment of the sediment and the quality of the food.

4.1. What is the main food source?

Uitto and Sarvala (1991), and Lehtonen and Andersin (1998) assumed that *M. affinis* consumes detritus and bacteria that respond to phytoplankton. *M. affinis* in our study grew much better in response to the fresh phytoplankton added in the absence of sediment bacteria and meiofauna (sand) than on the detritus, including meiofauna and bacteria (muddy clay). According to our calculations, between 40 and 100% of the added phytoplankton were consumed in the sandy sediment. These figures suggest that fresh phytoplankton is a high quality and sufficient food type for *M. affinis*. The added phytoplankton mainly consisted of diatoms. In that sense our results support the conclusion of Johnson and Wiederholm (1992), of a coupling between *M. affinis* growth and diatom biovolume during the phytoplankton spring bloom. They found a positive correlation between inter-annual time-series of these two variables. Siegfried (1985) suggested that bacteria-rich detritus provides a wealth of food for *Diporeia hoyi*. In contrast, Goedkoop and Johnson (1992, 1994) found that the bacteria carbon represent between 1.7 and 5.2% of overall carbon demand for *M. affinis* in Swedish freshwater
lakes. Additionally, in a Baltic archipelago, bacteria and meiofauna production do not seem to cover the energy requirements of *M. affinis* (Uitto and Sarvala, 1991). However, food sources other than fresh phytoplankton may play an important role during other periods of the year, when survival is more important than growth.

The maximum growth rates observed in the 18-day experiment were close to 0.02. Our measure is somewhat lower than that of 0.04 for *1 + M. affinis*, found by Uitto and Sarvala (1991) in the Gulf of Finland. In our study the temperature was 5.4°C, somewhat lower than the 6.5°C in Uitto and Sarvala’s (1991) study. This temperature difference could partly explain the lower growth rate in our study. The optimal temperature range for *M. affinis* growth is 8–12°C (Gordeev, 1952). However, a doubling of the growth rate should not be expected from a 1°C increase, indicating that the individuals in our experiment did not reach maximum growth rate. Consequently, the growth rates in the low addition pilot experiment should have been far from the maximum rates. Our calculations of the consumption of the phytoplankton in this experiment also suggest that the growth rate should have been below maximum since almost all phytoplankton carbon was used for growth. Hence, the possible time delay between the settling of the phytoplankton and the feeding and assimilation seems to be restricted to a few days at most. The temperature is a possible explanation for observed time delays between sedimentation and growth responses observed in the field. For example, Lehtonen and Andersin (1998) reported that individual growth responded to the sedimented spring bloom after 4 weeks. As in other poikilotherms, the physiological rates affecting growth are generally temperature dependent (Segerstråle, 1937; Dermott and Corning, 1988). A simple explanation for a time delay should be expected when the spring bloom is settling, while the temperature is still increasing. Thus, at low temperature the maximum growth rate will also be low, while maximum growth rate increases as the temperature increases. Adaptation to the long period of starvation during winter may also cause a time delay, since the physiological mechanism may need time to activate the digestion processes, as found for the copepod *Calanus hyperboreus* (Head and Conover, 1983). Lehtonen (1996) concluded that the physiological condition is one of the reasons for a time-lag in *M. affinis*. This mechanism is likely to cause a time-lag between the onset of phytoplankton spring bloom sedimentation and onset of growth. It is, however, questionable if it can cause a time-lag between peak of sedimentation and *M. affinis* maximum growth rate. Our results indicate that the temperature hypothesis is a more likely explanation for the time delay observed in the field, rather than it being due to a need for bacterial or meiofauna degradation of the phytoplankton.

4.2. Starvation despite chlorophyll in the sediment

If no phytoplankton was added, the weight of *M. affinis* decreased, with no significant differences among sediment types. *M. affinis* seemed not to be able to utilise the detritus already present in the organic sediment. One possible explanation is that the chlorophyll already in the organic sediments originated from sources too small to be utilised efficiently, such as free chlorophyll, small algal species, or fragmented larger species. Alternatively, the source chlorophyll may simply have become too diluted in the sediment, as a consequence of our handling. Concerning the dilution, the upper 1 cm of
the sediment contained as much chlorophyll as was added during the entire experimental period. Consequently, the upper 2 cm contained twice as much chlorophyll as was added. The reduction of the chlorophyll in the uppermost 0.5 cm of the sediment indicates that *M. affinis* actually consumed this fraction. However, the low growth resulting from this consumption indicates that the quality was low, or the sizes of the chlorophyll particles were small. According to Dermott and Corning (1988), the ability to select high-quality particles declines quickly with increasing size of *D. hoyi*. The size of the *M. affinis* we used was probably too large to allow efficient selection of the small food particles. Field observations commonly show that 0+ *M. affinis* continue to grow during autumn, whereas 1+ and 2+ decrease in mass (e.g., Sarvala, 1986; Uitto and Sarvala, 1991). Since sedimentation of fresh phytoplankton is generally low during the autumn, the selectivity of diluted small particles in the sediment by the smaller individuals may be one explanation for the observed pattern.

4.3. Are pre-frozen sediments harmful?

The freezing process changed the sediment characteristics, which gave rise to high swimming activity and mortality. Although *M. affinis* consumed most of the added phytoplankton in this treatment, its weight decreased. The increased metabolic cost of swimming most likely reduced the energy left for growth. Spending less time in the sediment reduces bioturbation and leads to decreased oxygen concentration in these treatments. *M. affinis* also increased its swimming activity in response to decreasing oxygen concentration in experiments by Johansson (1997a). *M affinis* and *D. hoyi* are not found in anoxic sediments (*M. affinis*, Johansson, 1997b; *D. hoyi*, Sly and Christie, 1992). There may have been an escalating response in our pre-frozen sediments, where for some other reason the individuals avoided the sediment. Leaving the sediment then made the situation even worse. We can only speculate on the reason for the avoidance. One explanation could be toxic or repellent exudates from bacterial degradation of dead micro-organisms and/or meiofauna. In fact, at the end of the experiment, cylinders belonging to this treatment smelled different from the other cylinders. Alternatively, the freezing process may have changed the physical properties of the sediment, making it more difficult for *M. affinis* to penetrate.

5. Conclusions

Our results show that experiments with the deposit feeding amphipod *M. affinis* over several weeks with addition of fresh phytoplankton do not result in the expected normal behaviour. To investigate interactions within and between species where feeding cannot be directly observed, it is of utmost importance to know that the results are due to the interactions and not caused by the experimental procedure. In the case of *M. affinis*, food addition in fine sand gave similar results to food addition in non-frozen muddy clay, even though the muddy clay already contained food. However in muddy clay without phytoplankton addition, pre-frozen or not, the *M. affinis* did not grow. Hence, detritus originating from algae in the field is not necessarily high-quality food for *M. affinis* in
experiments. One possible explanation for this is that the settling phytoplankton in the field are immediately ingested, de-fragmented, or diluted into the sediment by bioturbation. Pre-treatment of experimental sediment by freezing made the sediment highly unfavourable for the amphipods. Possibly, the amphipods would have performed better if aeration and/or water circulation had been used. The results nevertheless suggest that pre-frozen soft sediment should be used with caution, or avoided.

Acknowledgements

We thank Christian Otto and Erik Bonsdorff for comments on the manuscript, Kristina Samuelsson for help with the phytoplankton culture, and Sara Jonsson for phytoplankton identifications work. Tim Hipkiss improved the English and two anonymous reviewers provided valuable comments on the manuscript. Umeå Marine Sciences Centre (UMF), Umeå University provided the facilities needed for this work. [RW]

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