Variation in hatching success and egg production of *Eurytemora affinis* (Calanoida, Copepoda) from the Gulf of Bothnia, Baltic Sea, in relation to abundance and clonal differences of diatoms

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We monitored the hatching frequencies and clutch sizes of *Eurytemora affinis*, and environmental variables in the Gulf of Bothnia, between May and October 2003. We tested the hypothesis that the hatching frequency of the copepod would be negatively affected during time periods with high diatom concentrations. Results from two stations showed significant differences between the different sampling occasions, with the lowest observed hatching frequency during the spring diatom bloom. The reverse was true for clutch size, with the highest average egg number during the diatom bloom. These results were not correlated to ambient temperature, salinity or chlorophyll a (Chl a). In a separate laboratory experiment, nine different local clones of the diatom *Skeletonema costatum* were used as food for adult *E. affinis* females, in order to screen for possible differences in toxicity within the *Skeletonema* community. The resulting average copepod hatching frequency varied between 5 and 75% for the different clones, indicating that there can be large within-species variation in the toxic properties of diatoms. The significance of such variations in natural communities remains to be tested in future studies.

**INTRODUCTION**

The phytoplankton community in temperate waters is dynamic with blooms in spring and autumn, and occasionally in summer. Fluctuations in temperature, light and amount of available inorganic nutrients are important structuring factors of succession and growth of phytoplankton (Valiela, 1984; Granéli *et al*., 1990). In the Gulf of Bothnia, large phytoplankton (>10 μm), such as diatoms and dinoflagellates, comprise more than 60% of the total annual phytoplankton biomass (Kuparinen *et al*., 1996). There is a major bloom of large phytoplankton species in spring and a minor one in autumn, whereas smaller nano- and picoplankton are dominant during the summer (Andersson *et al*., 1996). Large phytoplankton are used as food by meso-zooplankton, typically dominated by calanoid copepods in most marine environments (Mauchline, 1998). In terms of egg production, the copepod community closely follows the phytoplankton blooms, whereas the actual copepod biomass peaks in June–July, i.e. towards the end of the spring bloom.

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This time lag in zooplankton peak biomass has been explained by the fact that calanoid copepods have a long life cycle involving 11 stages, and therefore cannot respond immediately to the rapid food increase during the early spring bloom (Valiela, 1984). As an alternative, Ianora et al. (Ianora et al., 2004) have suggested that the ability of some phytoplankton species, mainly diatoms, to impair the reproductive success of copepods may explain the slow response in copepod biomass.

Diatoms, which often account for >50% of the phytoplankton spring bloom, have commonly been recognized as the most important food source of copepods in marine pelagic environments and are considered the driving force in the classical pelagic food web (Cushing, 1989; Legendre, 1990). However, in 1993, Kleppel (Kleppel, 1993) found that many other organisms were included in copepod diets and was therefore the first to seriously question the general importance of diatoms. Furthermore, Ianora and Poulet (Ianora and Poulet, 1993) found that egg viability in the calanoid copepod Temora stylifera was low during in situ studies in March and April 1990. Further laboratory experiments showed that T. stylifera had low egg viability when given a diatom diet as opposed to a dinoflagellate diet. Because copepods are a major food source of higher trophic levels including many commercially important species of fish (Runge, 1988), it is crucial to have extensive knowledge of the factors regulating phytoplankton–copepod interactions.

Since 1993, several laboratory studies have shown that hatching frequency is inhibited when copepods are fed monocultures of certain diatom species (Ianora et al., 1995, 1996, 1999, 2004; Uye, 1996; Ban et al., 1997; Lee et al., 1999). Egg production, on the other hand, is not necessarily negatively affected (Ban et al., 1997). The inhibitory substances produced by some diatom species are mainly polyunsaturated aldehydes (PUA), which are synthesized from fatty acids when the cells are physically damaged (Miralto et al., 1999; Pohnert, 2000). Three of these active aldehydes have been identified as 2-trans-1-cis-7-cis-decatrienal, 2-trans-4-trans-1-cis-7-cis-decatrien and 2-trans-4-trans-decadienal (Miralto et al., 1999). However, Adolph et al. (Adolph et al., 2003) recently demonstrated that the biological activity is not restricted to the three metabolites mentioned above but is rather a general feature of the whole class of diatom-derived α-, β-, γ-, δ-unsaturated aldehydes.

Although several studies have found evidence of negative effects of diatoms on copepod hatching success in the laboratory (see above), there are some conflicting results concerning effects in the field. Miralto et al. (Miralto et al., 2003) compared hatching frequency during bloom and post-bloom conditions in 1997 and 1998 in the North Adriatic, Eastern Mediterranean Sea. Both copepods Acartia clausi and Calanus helgolandicus showed extremely low egg viability during a Skeletonema costatum- and Pseudo-nitzschia delicatissima-dominated bloom in February 1997 and a P. delicatissima-dominated bloom in February 1998. On the other hand, Pond et al. (Pond et al., 1996) and Irigoien et al. (Irigoien et al., 2000) found that high diatom abundance did not negatively affect the in situ hatching frequency of C. helgolandicus in the English Channel. In the same area, Laabir et al. (Laabir et al., 1996) found seasonal variations in egg viability both within and between stratified and non-stratified waters. In laboratory experiments, Ianora et al. (Ianora et al., 2003) found that the hatching frequency of C. helgolandicus was negatively affected by S. costatum but not by Thalassiosira rotula and Cylindrotheca closterium. The discrepancy of the above results might be explained with a varying ability of diatoms to produce PUA and/or with varying resistance of copepods towards these molecules, which is also suggested by the results of Ban et al. (Ban et al., 1997) and Starr et al. (Starr et al., 1999). Some diatom species are also known to be nutritionally deficient, therefore causing low production (Jones et al., 2002; Jones and Flynn, 2005) and hatching (Jónasdóttir and Kiørboe, 1996) in copepods, although alternative food sources may compensate for these effects in natural communities. Prey nutrient status can also vary depending on the growth conditions, and for instance, nitrogen depletion can result in poor reproduction (Jones et al., 2002; Jones and Flynn, 2005).

The results obtained so far indicate that the nutritional and inhibitory properties of diatoms deserve continued attention, but clearly, more field evidence is needed in order to understand the variability of reproduction and hatching success, as also indicated by Paffenhofer et al. (Paffenhofer et al., 2005). Change in nutritional status of the prey, which causes significant changes in the predator and its complex trophic interactions has been termed stoichiometric modulations (Mitra and Flynn, 2005). This process has not been well documented, and clearly experimental approaches that take prey nutritional status into account need to be developed, as well, to ultimately solve these issues.

In the present study, we focus on the in situ hatching frequencies and clutch sizes of the calanoid copepod Eurytemora affinis over the productive season in the Gulf of Bothnia, Baltic Sea. We tested the hypothesis that the hatching frequency would be lowest in spring, when diatoms dominate the food base, and that hatching frequency therefore would be negatively correlated with the biomass of dominating diatom taxa. Reductions in clutch
sizes were, however, not necessarily expected to be associated with possible reductions in hatching frequency (see Ban et al., 1997). We also focused on how different clones of the diatom S. costatum, cultivated in the laboratory, affected the hatching frequency of the copepod. A set of laboratory studies were conducted in order to test the hypothesis that there can be intraspecific variation in the toxic properties of diatoms. Our results are of relevance in an evaluation of local adaptation, intraspecific variation and the question of an ongoing ‘arms race’ between the copepod predator and its diatom prey.

METHODS

Field study

Two stations in the Gulf of Bothnia, Baltic Sea, were used in this study—B3 (southern Bothnian Bay) and C14 (Bothnian Sea) (Fig. 1). B3 is an inshore station in the Ore estuary south of Umeå, slightly affected by the freshwater outlet from Ore River, which creates a weak pycnocline at 10–15 m. C14 is a deeper, more offshore station, characterized by a strong thermocline developing in May at ~10–15 m. Data concerning temperature, salinity, chlorophyll a (Chl a) and the composition of the phytoplankton community were provided by the Swedish Environmental Monitoring Program for the Gulf of Bothnia (unpublished data, Umeå Marine Sciences Centre). Averages for 0–10 m depth were calculated for each variable. Zooplankton samples were taken every third week from station B3 and at two occasions from station C14. Zooplankton could not always be collected at the same time as the other parameters, but maximum difference was only 1 week.

Zooplankton were collected by vertical net hauls from 10 m to the surface using a 90-µm-meshed zooplankton net. To make the collection as gentle as possible, a plastic bag was attached at the end of the net and the content was poured into a bucket for immediate transportation to the laboratory. In the laboratory, 10 healthy egg-carrying females of E. affinis were sorted out by using a wide-mouthed pipette, and each copepod was put in a separate plastic bottle containing 200 mL 0.22 µm filtered seawater. Generally, 200 mL per individual should be sufficient for small copepod species to minimize encounter rates between adults and their eggs or nauplii, and hence to avoid problems with cannibalism (Runge and Roff, 2000). The females were incubated at 7°C with a 16-h light : 8-h dark cycle (~11 µmol m⁻² s⁻¹). The time for egg hatching was calculated by using equation (1) (from Andersen and Nielsen, 1997):

\[
H = \frac{36.8}{T^{1.04}}
\]

where \( H \) is the hatching time (days) and \( T \) is the temperature (°C). The incubation time was defined as 120% of \( H \) to make sure that the eggs were incubated for a sufficiently long time. After fulfilled incubation time, the experiment was terminated with 60 mL 98% ethanol (giving a final concentration of 22% ethanol). Eggs and nauplii from each sample were collected on a 50-µm plankton net and gently rinsed into a glass petri dish for counting under a dissection microscope. The egg hatching frequency (%) was calculated as the number of nauplii divided by the total number of eggs produced (nauplii and unhatched eggs). The method used did not provide information on naupliar viability.

Laboratory experiments

Monocultures of the diatom S. costatum were established by isolating single filaments from surface water samples
collected at station B3 on 11 June 2003. Nine successfully isolated filaments were grown in Guillard’s F/2 medium (Guillard and Ryther, 1962) at 14°C with a 12-h light : 12-h dark cycle under dim light conditions (~11 μmol m⁻² s⁻¹). The cultures were regularly given fresh medium in order to keep them from entering a stagnant phase. These cultures were then used in two laboratory experiments to screen for possible intraspecific differences within the diatom community by looking at the copepod hatching frequency. Before the experiments, a small amount of each culture was diluted in fresh medium, and the cells were harvested after 4–5 days, when the cultures were in exponential growth phase. Mean carbon content per cell was calculated for each clone culture and was used to obtain similar carbon concentrations in all incubations (Table I). Analysis of particulate carbon and nitrogen on glass fibre filters (Whatman GF/F precombusted for 4 h at 450°C) was carried out with a Carlo Erba model 1108 high temperature combustion elemental analyser, using standard procedures and a combustion temperature of 1030°C. Acetanilide was utilized for standardization, and results were corrected for blank filter carbon content. Residual nutrient concentrations of the medium were not measured, and therefore the nutritional status, especially with regard to phosphorous, is not known. The concentration of diatom cells in each culture was obtained by counting the number of cells in a known volume using a counting chamber and a binocular microscope.

*Eurytemora affinis* was raised from resting eggs by collecting bottom sediment from the Öre estuary and incubating it in filtered seawater with aeration at 14°C. Both adult females and males were picked out from the hatching chambers, and put into glass bottles containing 200 mL 0.22 μm filtered seawater enriched with one of the *Skeletonema* clones. For each *Skeletonema* clone, five replicates were prepared with five copepods, representing both sexes, in each bottle. The final concentration of *S. costatum* in each bottle corresponded to 400 μg carbon L⁻¹, considered sufficient for maximum reproduction (Stottrup and Jensen, 1990). The animals were incubated for 4 days at 14°C. As the egg development time at this temperature is ~2.3 days [equation (1)], this should be sufficient for an adult female to produce at least one egg clutch on the *Skeletonema* diet. Although a few eggs can also be produced on earlier lipid reserves, previous work has shown that diet shifts induce rapid response in the egg quality and quantity in *E. affinis* (Koski *et al.*, 1999). The bottles were swirled twice a day to resuspend the algae. After the incubation, one egg-carrying female from each bottle was picked out and individually reared in new glass bottles containing only filtered seawater. The bottles were checked daily, and females that had released their egg sac were removed, in order to further reduce cannibalism. After three additional days [equation (1)], 98% ethanol was added to terminate the experiment. The numbers of hatched and unhatched eggs were counted under a dissection microscope, and the egg hatching frequency was calculated.

The results from the screening of these nine clones were used to select two clones (7A3 and 14A4), which appeared to have contrasting inhibitory effects on egg hatching. For these two clones, an additional experiment was carried out, using the same experimental conditions as mentioned above. In this second experiment, at least 30 copepod females per diatom clone, divided into 10 replicate bottles, were used in the 4-day egg production incubation. A few males were also included in each bottle. The water and diatoms in the bottles were changed after 2 days. After the incubation, all females with egg sacs were transferred to filtered seawater to record the egg hatching frequencies. The bottles were checked daily, and females that had released their eggs were removed. The egg hatching was recorded after 3 days. In this second laboratory experiment, we achieved an increased statistical power, and the larger number of females also enabled us to estimate daily mean egg production rates. The egg production estimate was based on the number of eggs and nauplii found in the incubation bottles after the last 2 days of incubation and the number of eggs and nauplii found in the egg hatching

![Image](https://academic.oup.com/plankt/article-abstract/28/7/683/1482840)

**Table I: A summary of the different Skeletonema costatum clones used in the laboratory experiments**

<table>
<thead>
<tr>
<th>Clones</th>
<th>6B7</th>
<th>6C3</th>
<th>7A3</th>
<th>7B2</th>
<th>14A1</th>
<th>14A2</th>
<th>14A3</th>
<th>14A4</th>
<th>14B1</th>
<th>14B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells L⁻¹</td>
<td>6.4E+08</td>
<td>4.1E+08</td>
<td>2.5E+08</td>
<td>6.3E+08</td>
<td>1.0E+09</td>
<td>1.1E+09</td>
<td>5.3E+08</td>
<td>2.3E+08</td>
<td>6.9E+08</td>
<td>4.4E+08</td>
</tr>
<tr>
<td>μg C cell⁻¹</td>
<td>1.9E-05</td>
<td>2.4E-05</td>
<td>3.4E-05</td>
<td>1.8E-05</td>
<td>2.1E-05</td>
<td>2.4E-05</td>
<td>3.0E-05</td>
<td>2.1E-05</td>
<td>2.9E-05</td>
<td>1.3E-05</td>
</tr>
<tr>
<td>Final concentration</td>
<td>403.5</td>
<td>404.6</td>
<td>403.1</td>
<td>406.7</td>
<td>409.2</td>
<td>398.6</td>
<td>401.7</td>
<td>404.2</td>
<td>401.2</td>
<td>404.8</td>
</tr>
</tbody>
</table>

Cells L⁻¹ and μg C cell⁻¹ give information about the monocultures. The final concentrations for the incubation bottles are also indicated. All clones originate from station B3, 11 June 2003.
bottles. The egg production ($P$, eggs per female per day) was calculated as

$$P = \frac{N_e}{N_f D} \tag{2}$$

where $N_e$ is the number of eggs and nauplii produced, $N_f$ is the number of females and $D$ is the development time of eggs (2.2 days at $14^\circ C$, from Andersen and Nielsen, 1997).

Data analysis

Data analysis was performed using MINITAB Release 14, Statistical Software (Version 14.12, 1972–2004 Minitab Inc.). One-way ANOVAs were used for the field study and the laboratory screening test in order to examine differences between dates and clones, respectively. Frequency data were $'x' = \sin^{-1} \sqrt{x}$ transformed to obtain equal variances. Kruskal–Wallis was used when equal variances could not be achieved. A $t$-test was used when comparing hatching ‘during’ and ‘after’ the diatom bloom in the field and for the second laboratory experiment. In order to examine relationships between reproductive variables, phytoplankton data and the environmental variables, Pearson’s correlation was used.

RESULTS

Field study

Environmental factors

The temperature showed similar patterns in both stations B3 and C14, reaching the highest values of about $+20^\circ C$ in late July (Fig. 2). At station B3, the salinity was slightly <5 during the whole year, whereas the Chl $\alpha$ concentration started to increase in March and reached a peak in April–May. There was also a second, comparatively lower, Chl $\alpha$ peak in September–October (Fig. 2). At station C14, the salinity was slightly higher and more stable than at station B3. The Chl $\alpha$ levels reached a peak in May with a slightly higher value than that at station B3. Our data do not indicate a second Chl $\alpha$ peak in September–October at station C14, but this might be explained by insufficient sampling frequency during this period (Fig. 2).

Phytoplankton

At station B3, there were two major phytoplankton blooms: one in spring and one in autumn, and a minor bloom in summer. During the spring bloom (March–June), diatoms accounted for about 50% of the total biomass of phytoplankton in the area (Fig. 3). *Achnanthes tenuicata*, which is a common cold-water diatom species (Hajdu et al., 1997), reached the highest biomass peak but only for a short period of time. *Chaetoceros* spp., on the other hand, were present during the whole diatom spring bloom and were the most abundant genus towards the end. *Thalassioira* spp. were present in the beginning of the spring bloom and also had a second peak during the autumn bloom. *Skeletonema costatum* was not very abundant at any time but reached a minor peak towards the end of the diatom bloom in May–June. Apart from diatoms, the other dominating group during this spring bloom was Dinophyceae. Ciliates dominated during the autumn bloom.

At station C14, there were also two major algal blooms, which reached somewhat higher densities than those in the Bothnian Bay. We observed no bloom during the summer, and the autumn peak was lower than would be expected (Andersson et al., 1996), which probably was a bias due to insufficient sampling frequency. The diatoms represented ~70% of the total biomass of phytoplankton during the spring bloom (Fig. 3), the remaining 30% being mostly Dinophyceae. *Chaetoceros* spp. were the dominant diatom genus during most of the diatom bloom.
bloom, although the figure may be somewhat misleading due to lack of sampling in February–April. The autumn bloom consisted mainly of ciliates, which also was the case at station B3.

Station B3
The hatching frequency (%) of *E. affinis* at station B3 was significantly different between the different sampling occasions (one-way ANOVA; *P* = 0.001, *F* = 3.95, df = 7) (Fig. 4a). The lowest average hatching frequency (58.8%) was recorded on 11 June 2003, i.e. towards the end of the diatom bloom. At this time, the variation among females was also larger than at any other sampling occasion. The highest average hatching frequency (96.7%) was recorded on 12 August 2003, and the variation among females was low. The diatom bloom declined by the end of July (Fig. 3), and when pooling the data into ‘during’ and ‘after’ the diatom bloom (Fig. 4b), there was also a significant difference (two-tailed *t*-test, *P* = 0.001), with lower hatching frequency during the bloom. There was a significant negative correlation between hatching frequency and total biomass of diatoms and of the diatom genus of *Chaetoceros* (Table II). Correlations between hatching frequency and biomass of the other dominating diatom species as well as the other phytoplankton groups were also tested, but no significances could be established.

Fig. 3. The amount (mg m⁻³) of non-diatom phytoplankton, diatoms and the four most abundant diatom species/taxons at stations B3 and C14 during the summer of 2003. The values are an average from 0 to 10 m. Data are from the Swedish Environmental Monitoring Program for the Gulf of Bothnia. Note that the phytoplankton groups are listed in the legend in the order of their abundance, and that the legend is hence different between the two graphs.

Fig. 4. (a) The hatching frequency (%) of *Eurytemora affinis* collected at eight different occasions from station B3 in 2003. (b) The hatching frequencies from the four first sampling occasions were pooled into ‘during’ and the four last into ‘after’ the diatom bloom. Values are average ± SE.
Neither were there any significant correlations between hatching frequency and temperature, salinity, Chl a or total phytoplankton biomass (Table II).

Clutch size (eggs per female) of E. affinis at station B3 varied during the whole investigation period, ranging from an upper average of 40 eggs per female in June to a lower average of 12 eggs per female in August and October. The differences between dates were significant (Kruskal–Wallis, $P < 0.001$) (Fig. 5a) and pooling the data into ‘during’ and ‘after’ the diatom bloom also resulted in a significant difference, with an average of 28 and 16 eggs per female, respectively (two-tailed $t$-test, $P = 0.001$, not assuming equal variances) (Fig. 5b). Clutch size was positively correlated to the biomass of Chaetoceros spp. and negatively to the biomass of A. taenicata, but correlations to the environmental factors could not be established (Table II).

**Station C14**

There were two sampling occasions at station C14: the first sample was taken towards the end of the diatom bloom and

### Table II: Correlation matrix (Pearson’s correlation) for the field variables

<table>
<thead>
<tr>
<th></th>
<th>Chl a</th>
<th>Diatoms</th>
<th>Dinophyceae</th>
<th>Ciliates</th>
<th>CHA</th>
<th>THA</th>
<th>ACN</th>
<th>SKE</th>
<th>Hatching</th>
<th>Clutch size</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phytoplankton</td>
<td>0.69*</td>
<td>0.72*</td>
<td>0.87*</td>
<td>0.11</td>
<td>0.67*</td>
<td>0.17</td>
<td>0.88**</td>
<td>−0.10</td>
<td>−0.32</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Chl a</td>
<td>0.65*</td>
<td>0.66*</td>
<td>0.12</td>
<td>0.67*</td>
<td>0.48</td>
<td>0.83**</td>
<td>−0.87</td>
<td>−0.03</td>
<td>0.34</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Diatoms*</td>
<td>0.68*</td>
<td>0.025</td>
<td>0.90*</td>
<td>0.58</td>
<td>0.98*</td>
<td>−0.20</td>
<td>0.88*</td>
<td>0.54</td>
<td>−0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinophyceae*</td>
<td>−0.13</td>
<td>0.55**</td>
<td>0.36</td>
<td>0.89*</td>
<td>−0.05</td>
<td>−0.07</td>
<td>0.15</td>
<td>−0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciliates*</td>
<td>−0.12</td>
<td>−0.43</td>
<td>0.63</td>
<td>0.75</td>
<td>0.70</td>
<td>−0.59</td>
<td>0.79*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHA*</td>
<td>0.35</td>
<td>0.89*</td>
<td>0.66</td>
<td>−0.89*</td>
<td>0.76**</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THA*</td>
<td>0.38</td>
<td>−0.96**</td>
<td>0.65</td>
<td>−0.86</td>
<td>−0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACN*</td>
<td>−0.27</td>
<td>0.77</td>
<td>−0.10**</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKE*</td>
<td>0.02</td>
<td>0.68</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Hatching</td>
<td>−0.69</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clutch size</td>
<td>−0.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Salinity could not be included in the matrix since the variables were constant. CHA, Chaetoceros spp.; THA, Thalassiosira spp.; ACN, Achnanthes taenicata; SKE, Skeletonema costatum.

*Data is log transformed in order to attain normality.

**Data is ln transformed in order to attain normality.

*Correlation is significant at the 0.01 level.

**Correlation is significant at the 0.05 level.

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**Fig. 5.** (a) Clutch size (eggs per female) of Eurytemora affinis collected at eight different occasions from station B3 in 2003. (b) The four first occasions were pooled into ‘during’ the diatom bloom and the four last were pooled into ‘after’. Values are average (±SE).
the second sample was taken well after the bloom had ended. The hatching frequency towards the end of the diatom bloom was significantly lower (72%) than that after the bloom (92%) (one-tailed t-test, \( P = 0.03 \)) (Fig. 6a). There was also a significant difference between the clutch size measurements, with larger clutch size during low hatching frequency and vice versa (two-tailed t-test, \( P = 0.03 \), not assuming equal variances) (Fig. 6b). Because we only had two sampling occasions from station C14, it was not possible to examine any correlations with environmental factors such as Chl a or diatom abundances.

Laboratory experiments

All S. costatum clones originated from filaments collected on 11 June 2003, when the in situ copepod hatching frequency was the lowest. The N : C values for the different clones, calculated from the particular carbon and nitrogen analyses of the cultures used in the experiments, were quite similar (0.20 ± 0.01). In the first laboratory experiment, clones 6B7, 6C3, 7B2, 14A1, 14A2, 14A4 and 14B1 all resulted in a copepod hatching frequency lower than 25%. Clone 14A3 was somewhat better, and clone 7A3 resulted in the highest average hatching frequency (≈75%) (Fig. 7). The obvious differences between clones (Fig. 7) were not statistically significant, which most likely was due to large variation and too few replicates in some of the clones (one-way ANOVA; \( P = 0.08 \), \( F = 2.10 \), df = 8).

We therefore conducted a second laboratory experiment using a design with a larger number of replicates. Two of the clones that represented extremes in the data set above, i.e. 14A4 and 7A3, were now tested against each other (Fig. 8a). Clone 14A4 exhibited a significantly lower hatching frequency than clone 7A3 (two-tailed t-test, \( P = 0.04 \)), giving further statistical support to the idea of contrasting properties among some of the clones. There was no difference in egg production between the two tested clones (Fig. 8b).

DISCUSSION

Field study

We observed seasonal changes in both hatching frequencies (%) and clutch sizes (eggs per female) of E. affinis from the Gulf of Bothnia. Hatching frequency varied significantly between sampling occasions at both stations, and the lowest hatching frequencies were observed during and towards the end of the diatom spring bloom. At station B3, there was a significant negative correlation between hatching frequency and the total amount of both diatoms and the diatom genus Chaetoceros (Table II). The Chaetoceros community at station B3 was dominated by Chaetoceros gracilis and Chaetoceros wighamii during the spring bloom. Chaetoceros gracilis has previously been shown to reduce both the hatching frequency and the egg production in the copepod Acartia granii (Ban et al., 1997), whereas the effects on E. affinis are still unknown. Any reproductive effects on copepods by C. wighamii have not yet been examined.

For the species mentioned above, and for the other dominating diatom species, further studies are needed in order to understand the possible role of different diatom taxa in observed reductions in hatching frequency during the spring bloom. These studies should be accompanied by actual PUA production measurements, because the ability of diatoms to produce PUA is shown to be both species and strain specific (Wichard

Fig. 6. The (a) hatching frequency (%) and (b) clutch size (eggs per female) of Eurytemora affinis collected at two different occasions from station C14 in 2003. The first occasion was at the end of the diatom bloom and the second was after. Values are average (±SE).
Low hatching frequency has also been shown to be caused by nutritional deficiency in certain diatom species, such as lack or presence of certain fatty acids (Jónasdóttir and Kiørboe, 1996) or poor N-status (Jones and Flynn, 2005). During the spring bloom in the present study, 30–50% of the phytoplankton consisted of Dinophyceae, providing an alternative food source. However, we did not measure the ingestion of the different food items, and therefore selective feeding and its consequence for the nutritional properties of the ingested food items cannot be ruled out as factors affecting the results. Over the growth season, diatom dominance decreased, and during the second phytoplankton bloom when ciliates dominated the food base, hatching success was high.

At station B3, diatoms only accounted for ~50% of the total phytoplankton biomass during the spring bloom, which may explain the less dramatic reduction in hatching frequency than reported from, for example, the Adriatic Sea (Miralto et al., 2003), where diatoms constituted >90% of the phytoplankton. In a set of laboratory studies, a ‘dilution effect’ was found when the copepod *T. stylifera* was fed a mixture of diatom and dinoflagellate diets (Turner et al., 2001). At station C14, the proportion of diatoms was higher (~70%) than at station B3, so one would expect the hatching frequency to be lower, but this was not the case. However, there were only two sampling occasions at station C14, and both were at the end or after the diatom bloom, making a comparison between the two stations difficult.

Clutch size, in contrast to hatching frequency, seemed to increase during and towards the end of the diatom bloom in our study. At both stations B3 and C14, there was a strong tendency towards an inverse relationship between hatching frequency and clutch size, with the largest average clutch size and the lowest average hatching frequency during/towards the end of the diatom bloom. Due to the large clutch sizes, the total number of produced nauplii was higher during the bloom (18 and 21 nauplii per female at stations B3 and C14, respectively) than after (14 and 18 nauplii per female), in spite of the decrease in hatching frequency. However, diatoms may also negatively affect naupliar viability (Halsband-Lenk et al., 2005), and high hatching frequency per se might therefore not indicate a lack of population effects of a diatom diet. The fact that diatoms induce a negative effect on hatching frequency but not on egg production has been shown in laboratory studies.

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considered (Ianora et al., 1997; Turner et al., 2001), indicating that food conditions promoting high egg production may not promote high hatching frequency and vice versa. According to the present study and other studies (Jonasdottir et al., 2002; Miralto et al., 2003; Halsband-Lenk et al., 2005), this appears to be true in the field as well. In population dynamical studies, egg production alone may therefore give misleading results on recruitment, and hatching frequency as well as nauplii survival should always be considered (Ianora et al., 2004).

Depending on historic interactions between diatom and copepod populations, varying levels of local adaptation to diatom inhibitory substances are possible. A prerequisite for adaptation to take place is that there is variation in the trait under selection (Kirkpatrick, 1996). In the present study, there was indeed high variation among females in terms of hatching frequency during the bloom. Provided the ability to produce viable eggs is heritable, this means that copepods could become adapted to the inhibitory substances. However, the occurrence of such processes and their possible implications for the different field scenarios that have been recorded remain to be investigated.

### Laboratory experiments

In more saline waters, *S. costatum* can be one of the dominating species during the spring diatom bloom (e.g. Adriatic Sea; Miralto et al., 1999), whereas in the Gulf of Bothnia, it usually only accounts for a minor part of the total diatom biomass (Swedish Environmental Monitoring Program for the Gulf of Bothnia). Nevertheless, *S. costatum* is a cosmopolitan species, on which data can be obtained from different regions (D’Ippolito et al., 2002; Miralto et al., 2003), and *S. costatum* is hence used here and in future studies as a model species for variation in inhibitory properties.

The response of *E. affinis* in the first experiment appeared to differ among the treatments, but these differences could not be statistically validated. However, the pattern found in the first experiment was supported by the second one, with significant differences in the effects on copepod hatching frequency between the two *Skeletonema* clones. In the second experiment, clone 14A4 resulted in a comparatively higher hatching frequency than in the first experiment, although still significantly lower than clone 7A3. This might be caused by insufficient number of replicates in the first experiment, but in recent studies, we have shown that a clone’s production of PUA may vary over time, which might also explain the difference between the experiments (Fredrik Broms et al., unpublished data). Egg production was virtually identical with the two tested clones, suggesting that reproductive failure can be detected by egg hatching measurements only, as also indicated by the field data (inverse relationship between egg hatching and clutch size). Our findings of large variability within the *S. costatum* community at the clone level, with regard to the effects on *E. affinis*, open up for alternative ways of explaining variable results from the field.

Because diatoms commonly have been considered the major contributor of food energy to higher trophic levels in oceans, the ecological significance of the inhibitory substances found in diatoms is heavily debated. Can the negative effect of monocultures of certain diatom species found on copepod hatching frequency really explain reproductive patterns in natural systems? In this study, hatching frequency was indeed negatively affected during the diatom bloom, which is in agreement with Miralto et al. (Miralto et al., 2003) and Laabir et al. (Laabir et al., 1995), but not with Irigoien et al. (Irigoien et al., 2002). One explanation to this discrepancy can be differences in the species composition of the diatom blooms. Recent studies have shown that there are a variety of deleterious properties amongst different diatom species (Pohnert et al., 2002). In addition, as shown by recent PUA measurements (Wichard et al., 2005) and indicated by our own laboratory results, variation in inhibitory effects of diatoms does occur at the clone level as well. It can be hypothesized, therefore, that differences in the frequencies of diatom strains with different inhibitory properties may cause variation in the responses of copepod populations, even if the species’ composition is similar among sampling sites or occasions. This hypothesis, and also whether the PUA production can vary with diatom nutrient status, remains to be explored in future studies.

Our data suggest intraspecific variation in both the response of *E. affinis* (field study) and the effect of *S. costatum* on hatching frequency (laboratory study). In a predator–prey system, different kinds of chemical defences may be selected for in the prey. In terrestrial systems, predators are known to be able to adapt to such defences by evolving resistance (Geffeney et al., 2002), which may lead to an arms race between the prey and their predators (Brodie and Brodie, 1999). As indicated previously, an arms race can lead to variation between different predator–prey populations in terms of the defence of prey and the resistance of predators. The existence of clones with different inhibitory properties in natural environments, as shown here, may be a mechanism by which the diatom population can rapidly evolve a defence. However, for this to be an effective defence strategy, grazers must select against toxic clones and thereby promote the dominance of them. In recent studies using radiolabelling techniques, we have seen that...
E. affinis selects against the clone with the higher PUA production in a mixture (Fredrik Brums et al., unpublished data). We therefore suggest that future studies also consider food selection in mixtures of diatoms with varying inhibitory properties.

In conclusion, our results support the idea that hatching success can be affected by the diatom bloom and its composition, but further experimental studies are needed to support these findings, especially in the light of actual PUA productions and alternative hypotheses, such as nutritional deficiencies. The importance of inhibitory substances produced by diatoms in natural diatom–copepod systems must also be studied in a broader context, which considers differences on the diatom clone level.

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