Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe

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**Abstract.** Nauplii and adults of the copepod *Eurytemora affinis* Poppe collected from the Multiscale Experimental Ecosystem Research Center (MEERC) mesocosms and from the Choptank River (a subestuary of Chesapeake Bay) reduced ciliate and dinoflagellate microplankton densities significantly during grazing experiments. Protozoan microplankton generally were consumed in proportion to their availability, although both adult copepod and naupliar clearance rates were higher for larger prey (~40 µm). Ingestion of ciliate microplankton was confirmed by examining copepod gut fluorescence after brief (1 h) incubations with ciliates labeled with a fluorescent vital stain (5-chloromethylfluorescein diacetate). In addition, both adults and nauplii of *E. affinis* cleared protozoan microplankton at considerably higher rates than chlorophyll a. Naupliar clearance rates were generally an order of magnitude lower than adult rates, but given naupliar abundances in copepod assemblages, they should contribute substantially to total grazing on protozoan microplankton. Grazing by the total copepod assemblage in mesocosms and in the field may be underestimated if juvenile stages are ignored.

**Introduction**

The objective of this study was to determine the grazing impact of adults and naupliar stages of the calanoid copepod *Eurytemora affinis* Poppe on quasi-natural assemblages of protozoan microplankton and phytoplankton. *Eurytemora affinis* is one of the predominant calanoid copepods found in Chesapeake Bay during spring, when salinities are typically <18 p.s.u. and temperatures are relatively low (Heinle and Flemer, 1975; Bradley, 1991). In addition to temperate estuaries, *E. affinis* is commonly found in a range of other environments, including freshwater (Katona, 1971; Bradley, 1991). Despite its abundance, comparatively few grazing studies have been conducted on this species, and knowledge of its feeding behavior is limited. *Eurytemora affinis* has been shown to graze phytoplankton (Richman et al., 1977; Barthel, 1983), free and attached bacteria at very low rates (Boak and Goulder, 1983), and ciliates (Berk et al., 1977). Heinle et al. (1977) found that ingestion of ciliates by *E. affinis* supported normal egg production rates. However, none of these experiments were designed to address the feeding strategy of *Eurytemora* on natural prey assemblages containing a broad size spectrum of phytoplankton and protozoan microplankton.

Many studies of copepod grazing have utilized simple mixtures of cultured prey as tools to examine grazing selectivity (e.g. Mullin, 1963; Frost, 1977; Stoecker and Egloff, 1987). There has also been considerable work using natural prey assemblages to investigate selectivity (e.g. Huntley, 1981; Gifford and Dagg, 1988; Tiselius, 1989; Gifford, 1993a; Fessenden and Cowles, 1994; Ohman and Runge,
1994; Verity and Paffenhofer, 1996). Predation by copepods is often an important source of mortality for planktonic ciliates (Sanders and Wickham, 1993; Fessenden and Cowles, 1994; Nielsen and Kiørbøe, 1994), and recent studies have documented ingestion of protozoan microplankton by copepod nauplii (Stoecker and Egloff, 1987; Dolan, 1991; Fessenden and Cowles, 1994). However, most copepod grazing studies have concentrated on the feeding and selectivity of adult stages. Research with juvenile stages has seldom been carried out, although nauplii may exhibit different feeding behavior than copepods and adults (Paffenhofer and Lewis, 1989), and their abundances may equal or exceed those of adults and copepods in natural populations (Fulton, 1984). Allan et al. (1977) used an electronic particle counter to investigate selection by adults and nauplii of *E. affinis* and *Acartia* spp. for various phytoplankton based on prey size and relative biomass. This study was innovative, but electronic particle counter results should be interpreted cautiously since they may falsely indicate grazing selectivity when prey of various shapes are used in the same study (Harbison and McAlister, 1980; Gifford et al., 1981). Microscopic enumerations are more labor intensive, but give less speculative results (Huntley, 1981; Verity and Paffenhofer, 1996).

Copepod nauplii have been described as being less selective than more mature stages (Conover, 1982), and also as occupying the same feeding niche as adult stages (Conover, 1982; Berggreen et al., 1988). When naupliar grazing is included, estimates of copepod grazing impact on natural protozoan microplankton populations may be increased by 10–20% (Fessenden and Cowles, 1994). However, nauplii may exhibit different grazing patterns and/or rates with respect to the total numbers of cells grazed and the prey size most frequently ingested (Stoecker and Egloff, 1987; Berggreen et al., 1988; Paffenhofer and Lewis, 1989). At least in some species of calanoid copepods, nauplii may not be able to capture small prey as efficiently as can later stages (Paffenhofer and Lewis, 1989). Additionally, nauplii may not be able to capture particles as large as those captured by copepods and adults (Berggreen et al., 1988). Thus, while the overall diet is expected to overlap with that of copepods and adults of the same species, nauplii tend to utilize a more restricted size spectrum of prey (Paffenhofer and Lewis, 1989). As a result, copepod age class distribution may have a strong influence on the population dynamics of protozoan microplankton assemblages.

**Method**

**Experimental design**

Five grazing experiments were carried out with *E. affinis* adults and nauplii, and natural assemblages of phytoplankton and protozoan microplankton prey. Copepods were collected from the Multiscale Experimental Ecosystem Research Center (MEERC) mesocosms for the two experiments conducted in 1994, and from the Choptank River estuary (12 p.s.u.) for the experiments conducted in 1995. Collections were made by siphoning water from the mesocosms into a submerged 202 μm mesh, or by oblique tows in the Choptank River using a 0.75 m ring net (202 μm). Prior to all experiments, gravid adult females were sorted into several 4 l beakers containing filtered (<64 μm) Choptank River water. In
Grazing impact of *Eurytemora affinis*

addition to the natural prey assemblage remaining in the 64 μm fraction, copepods were fed a mixture of *Thalassiosira weissflogii* and *Isochrysis galbana*. Incubations were maintained until nauplii were observed in subsamples from the beakers. Each beaker was fitted with a removable 202 μm Nitex barrier to separate adult copepods and nauplii. Prior to use in grazing experiments, nauplii were maintained as described above until reaching at least stage N3. Feeding experiments were conducted with a mixture of stage N3-N6 nauplii. Naupliar stages were identified according to Katona (1971).

Methods for the grazing experiments were modified from a protocol developed by Gifford (1993b). Choptank River water was screened gently through a submerged 64 μm Nitex mesh in order to remove metazoans and allow most protozoa and phytoplankton to pass into a clean 20 l polyethylene carboy. The screened water was siphoned slowly through silicone tubing into eight 500 ml polycarbonate bottles. Fifty laboratory-reared nauplii were added to each of four bottles and 10 adult females collected from the mesocosms or recent net tows were added to each of the remaining four bottles. All bottles were topped with 64-μm-filtered water, covered with parafilm, and promptly capped to avoid the formation of air bubbles, which may damage fragile protozoa.

Copepods were acclimated to the experimental conditions of light, turbulence, temperature and food availability by placing bottles on a rotating (~2 r.p.m.) wheel in a temperature-controlled incubator. The temperature was 18°C for both experiments conducted in 1994, 10°C for experiments conducted on 23 March and 12 April 1995, and 15°C for the experiment conducted on 27 April 1995. Irradiance was 100–110 μE m⁻² s⁻¹ on a 12:12 h light:dark cycle. Experimental temperatures were chosen to reproduce ambient temperatures where the copepods and prey were collected. After 24 h, nauplii and adult copepods were recovered, and transferred carefully into new bottles containing a similar prey assemblage. These bottles and eight control bottles, containing the prey assemblage without copepods, were placed on the rotating wheel. Four of the eight control bottles were harvested after 1 h (see Gifford, 1993b), and a subsample from each bottle was preserved with 10% (v/v) acid Lugol's solution (Stoecker et al., 1994). After 24 h, the remaining control and treatment bottles were harvested and sampled as described above.

Preserved samples were concentrated by settling (Utermöhl, 1958) and enumerated at a magnification of 200X with a Zeiss inverted microscope. Ciliates and dinoflagellates were grouped according to size and general cell morphology (Table I). Ciliate taxa were identified according to Small and Lynn (1985). Diatoms and other phytoplankton generally were not enumerated, but dominant species were noted and identified according to Cupp (1977). Settled volumes were either 25, 50 or 100 ml, depending on microplankton densities. In all cases, the entire slide was enumerated, and total protozoan counts always exceeded 200 cells (Venrick, 1978).

The remaining water in each bottle was split for analysis of total and size-fractionated (<10 μm) chlorophyll *a* (Chl *a*). Total Chl *a* samples were filtered onto GF/F filters, ground and extracted in acetone, and measured using a Turner Designs™ fluorometer (Parsons et al., 1984). Size-fractionated (<10 μm) Chl *a* samples were treated as above after passing through a 10 μm Nitex mesh.
Calculations

Ingestion and clearance rates of protozoan microplankton taxa and Chl a by both adult copepods and nauplii were calculated according to a modification of Frost's (1972) equations (see Gifford, 1993b). Rates were calculated only when the difference between treatment and control abundances was statistically significant (Student's t-test, \( P \leq 0.05 \)). When differences were not significant, feeding may or may not have occurred (i.e. undetectable). Ciliate prey carbon content was calculated from cell geometry, using the fixative-specific conversion factor of 0.22 pg C \( \mu m^{-3} \) for ciliates preserved in 10% (v/v) acid Lugol's solution (Stoecker et al., 1994). Total phytoplankton carbon was estimated by assuming a carbon to Chl a ratio of 50:1 (Landry and Lorenzen, 1989). When present, plastidic ciliates such as Myrionecta rubra (formerly Mesodinium rubrum) contributed to Chl a and thus to the estimates of phytoplankton carbon.

In order to obtain a conservative estimate of the naupliar contribution to total copepod grazing in the mesocosms, the lowest observed clearance rates for nauplii and adults were multiplied by the respective densities of nauplii, and copepodids plus adult copepods from the mesocosms (M.Roman, unpublished data), and by the mesocosm volume (i.e. 0.1, 1.0 or 10.0 m\(^3\)). Naupliar grazing was calculated as a percentage of total (adult plus naupliar) copepod grazing. The fraction of the total mesocosm volume that potentially could be cleared of ciliates per day was estimated by dividing total (adult plus naupliar) volume cleared per day by the mesocosm volume.

Fluorescently labeled prey experiments

Ingestion of protozoan microplankton by E.affinis was visualized directly. A laboratory culture of Strombidium sp., a 40-45 \( \mu m \) planktonic ciliate, was incubated in a 1 \( \mu M \) CMFDA (5-chloromethylfluorescein diacetate) solution (Li et al., 1996). After staining for 1 h, the live ciliates were washed gently four times with autoclaved GF/F-filtered water (12 p.s.u.) to dilute the concentration of unabsorbed CMFDA in the culture. Approximately 10 CMFDA-labeled ciliates ml\(^{-1}\) were added to each of three 50 ml beakers containing five adult female E.affinis, and to three beakers each containing 25 nauplii. Six control incubations containing copepods and water from the fourth CMFDA wash were set up simultaneously. Beakers were covered with aluminum foil and incubated in the dark, at 15\(^\circ\)C for 15, 30 and 60 min. After each time interval, copepods from the treatment and control beakers were pipetted onto glass slides, pressed gently with a coverslip, and examined with epifluorescence microscopy for the presence of bright green gut fluorescence from ingested CMFDA-labeled ciliates.

Results

In all five experiments, the protozoan microplankton assemblage was dominated by choreotrich ciliates, with variable species composition and density among dates (Tables I and II). Because of these natural variations in initial density, the
Grazing impact of *Eurytemora affinis*

Table I. General taxonomic category, cell geometry, cell dimensions and cell volume of available ciliate and dinoflagellate prey in the grazing experiments

<table>
<thead>
<tr>
<th>Prey</th>
<th>Taxon</th>
<th>Geometry</th>
<th>Dimensions (µm)</th>
<th>Volume (µm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STROB1</td>
<td><em>Strobilidium</em> sp.</td>
<td>Sphere</td>
<td>15 x 15</td>
<td>1767</td>
</tr>
<tr>
<td>STROM1</td>
<td><em>Strombidium</em> sp.</td>
<td>Cone</td>
<td>30 x 25</td>
<td>4909</td>
</tr>
<tr>
<td>STROB2</td>
<td><em>Strobilidium</em> sp.</td>
<td>Cone</td>
<td>40 x 30</td>
<td>9425</td>
</tr>
<tr>
<td>STROB3</td>
<td><em>Strobilidium</em> sp.</td>
<td>Sphere</td>
<td>40 x 40</td>
<td>33 510</td>
</tr>
<tr>
<td>STROM2</td>
<td><em>Strombidium</em> sp.</td>
<td>Cone</td>
<td>70 x 35</td>
<td>22 449</td>
</tr>
<tr>
<td>HYPO</td>
<td>Unidentified hypotrich</td>
<td>Prolate spheroid</td>
<td>40 x 25</td>
<td>6545</td>
</tr>
<tr>
<td>MRUB</td>
<td><em>Myrionecta rubra</em></td>
<td>Prolate spheroid</td>
<td>20 x 17</td>
<td>3026</td>
</tr>
<tr>
<td>PRORO</td>
<td><em>Prorocentrum minimum</em></td>
<td>Oblate spheroid</td>
<td>17 x 16</td>
<td>1140</td>
</tr>
</tbody>
</table>

*Assuming a cell thickness of 8.5 µm, as in Coats and Harding (1988).

Detectability of grazing on particular taxa varied among experiments. Both adult copepod and naupliar treatments contained significantly lower densities of one or more protozoan microplankton prey than did controls by the end of each of the five experiments (Table II). However, not all ciliate species were present in sufficient densities to be enumerated in all experiments (Table II). Two ciliates, *Strombidium* spp. 1 and 2, were present in densities >1 ml⁻¹ in all natural assemblages used in the experiments. In most experiments, significant reductions in the abundance of these two species occurred in both the adult copepod and naupliar grazed treatments. *Strobilidium* spp. 1, 2 and 3, a hypotrich ciliate and *M. rubra* were present in sufficient densities to be enumerated in a subset of the experiments (Table II). Removal of *Strobilidium* sp. 1 and the hypotrich ciliate by adult copepods was significant in experiments in which these prey types were abundant. Removal of *Strobilidium* sp. 1 by nauplii was significant in one of two experiments. Naupliar removal of the hypotrich was significant in the one experiment in which this prey type was abundant. *Strobilidium* sp. 2 was present in only one experiment, and at low density. As a result, significant reductions in the density of this species were not observed. Significant reductions of *Strobilidium* sp. 3 by adults and nauplii occurred when this species was present at a density >10 cells ml⁻¹, but the differences between control and grazed treatments were usually non-significant when this ciliate was present at lower densities. *Myrionecta rubra* was not grazed significantly by adults or nauplii.

The phytoplankton assemblage in all experiments was dominated by various unidentified nanoplankton and *Rhizosolenia* sp., except for 23 March 1995 when a large (65 x 40 µm) pennate diatom tentatively identified as *Amphiprora* sp. was common. The dinoflagellate, *Prorocentrum minimum*, was present and enumerated in all three experiments conducted in 1995. Significant reductions in *P. minimum* density occurred in the presence of adult copepods in all three experiments, but significant differences were observed only between control and naupliar treatments in two of the three experiments (Table II). Total Chl a was grazed significantly by adult copepods in all except the 27 April 1995 experiment (Table III). Nauplii also grazed total Chl a significantly in three of the five experiments (Table III). Chlorophyll a in the <10 µm size fraction was grazed by adults and nauplii in the two experiments coincident with the highest <10 µm Chl a values.
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Table II. Student's t-test comparisons of mean prey abundance (cells ml⁻¹) after 24 h incubations in control bottles versus grazed bottles containing 10 adult copepods or 50 nauplii. The standard error of the mean (SE) is given in parentheses beside each mean value. For all values, n = 4

<table>
<thead>
<tr>
<th>Prey</th>
<th>Date</th>
<th>Cells ml⁻¹ (SE)</th>
<th>Control t₀</th>
<th>Control t₂₄</th>
<th>Adults</th>
<th>Nauplii</th>
</tr>
</thead>
<tbody>
<tr>
<td>STROB1</td>
<td>24 April 1994</td>
<td>7.07 (0.36)</td>
<td>9.38 (1.04)</td>
<td>4.74 (1.83)*</td>
<td>11.03 (1.55)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06 May 1994</td>
<td>8.52 (0.46)</td>
<td>50.68 (1.25)</td>
<td>31.57 (3.83)**</td>
<td>30.25 (2.29)**</td>
<td></td>
</tr>
<tr>
<td>STROM1</td>
<td>24 April 1994</td>
<td>8.69 (0.33)</td>
<td>5.29 (0.32)</td>
<td>2.87 (0.81)*</td>
<td>9.66 (0.75)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06 May 1994</td>
<td>3.60 (0.18)</td>
<td>8.21 (0.36)</td>
<td>4.53 (0.43)**</td>
<td>5.57 (0.24)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 March 1995</td>
<td>4.34 (0.09)</td>
<td>5.37 (1.05)</td>
<td>1.13 (0.15)**</td>
<td>3.37 (0.33)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 April 1995</td>
<td>93.47 (2.15)</td>
<td>114.36 (4.18)</td>
<td>64.97 (2.05)**</td>
<td>77.11 (2.74)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 April 1995</td>
<td>4.50 (0.31)</td>
<td>15.11 (1.98)</td>
<td>9.33 (1.73)**</td>
<td>6.03 (1.83)*</td>
<td></td>
</tr>
<tr>
<td>STROB2</td>
<td>24 April 1994</td>
<td>4.01 (0.12)</td>
<td>0.24 (0.06)</td>
<td>0.20 (0.06)**</td>
<td>1.33 (0.36)**</td>
<td></td>
</tr>
<tr>
<td>STROB3</td>
<td>24 April 1994</td>
<td>4.27 (0.22)</td>
<td>11.02 (0.36)</td>
<td>1.86 (0.28)**</td>
<td>5.04 (0.61)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06 May 1994</td>
<td>0.90 (0.10)</td>
<td>1.19 (0.27)</td>
<td>0.39 (0.13)*</td>
<td>0.59 (0.11)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 March 1995</td>
<td>0.41 (0.03)</td>
<td>0.51 (0.18)</td>
<td>0.32 (0.28)**</td>
<td>0.57 (0.05)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 April 1995</td>
<td>1.83 (0.12)</td>
<td>3.22 (0.34)</td>
<td>2.48 (0.15)**</td>
<td>3.08 (0.12)**</td>
<td></td>
</tr>
<tr>
<td>STROM2</td>
<td>24 April 1994</td>
<td>40.70 (0.82)</td>
<td>27.44 (2.57)</td>
<td>10.91 (3.67)**</td>
<td>28.80 (3.16)**</td>
<td></td>
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<tr>
<td></td>
<td>06 May 1994</td>
<td>10.82 (0.14)</td>
<td>20.33 (0.64)</td>
<td>9.14 (0.78)**</td>
<td>12.49 (1.68)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 March 1995</td>
<td>3.93 (0.05)</td>
<td>4.04 (0.13)</td>
<td>1.12 (0.20)**</td>
<td>2.67 (0.22)**</td>
<td></td>
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<tr>
<td></td>
<td>12 April 1995</td>
<td>1.25 (0.13)</td>
<td>2.08 (0.18)</td>
<td>1.00 (0.08)**</td>
<td>0.83 (0.06)**</td>
<td></td>
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<tr>
<td></td>
<td>27 April 1995</td>
<td>2.50 (0.33)</td>
<td>5.00 (0.35)</td>
<td>3.11 (0.54) **</td>
<td>3.17 (0.61)*</td>
<td></td>
</tr>
<tr>
<td>HYPO</td>
<td>23 March 1995</td>
<td>4.42 (0.43)</td>
<td>4.34 (0.07)</td>
<td>0.48 (0.07)**</td>
<td>2.27 (0.26)**</td>
<td></td>
</tr>
<tr>
<td>MRUB</td>
<td>24 April 1994</td>
<td>1.34 (0.06)</td>
<td>1.61 (0.18)</td>
<td>1.86 (0.35)**</td>
<td>4.31 (0.37)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06 May 1994</td>
<td>5.08 (0.37)</td>
<td>5.07 (0.32)</td>
<td>4.73 (0.37)**</td>
<td>4.59 (0.31)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 April 1995</td>
<td>7.75 (0.51)</td>
<td>9.81 (0.78)</td>
<td>7.81 (0.29)**</td>
<td>7.97 (0.56)**</td>
<td></td>
</tr>
<tr>
<td>PRORO</td>
<td>23 March 1995</td>
<td>8.13 (0.08)</td>
<td>9.27 (0.05)</td>
<td>5.03 (0.20)*</td>
<td>5.81 (0.44)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 April 1995</td>
<td>69.49 (0.64)</td>
<td>102.61 (1.96)</td>
<td>70.83 (3.51)**</td>
<td>77.78 (1.80)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 April 1995</td>
<td>316.11 (20.7)</td>
<td>414.94 (25.6)</td>
<td>319.33 (16.4)</td>
<td>355.94 (18.7)**</td>
<td></td>
</tr>
</tbody>
</table>

***P < 0.001, **P < 0.01 and *P < 0.05 when grazed < control; ns, non-significant (P > 0.05).

Table III. Student's t-test comparisons of mean Chl a (total and <10 μm fractionated) concentration (µg l⁻¹) after 24 h incubations in control bottles versus grazed bottles containing 10 adult copepods or 50 nauplii. The standard error of the mean (SE) is given in parentheses beside each mean value. For all values, n = 4

<table>
<thead>
<tr>
<th>Chl a</th>
<th>Date</th>
<th>Chl a concentration (µg l⁻¹)(SE)</th>
<th>Control t₀</th>
<th>Control t₂₄</th>
<th>Adults</th>
<th>Nauplii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24 April 1994</td>
<td>15.8 (0.28)</td>
<td>16.35 (0.17)</td>
<td>7.51 (0.24)**</td>
<td>9.58 (0.48)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06 May 1994</td>
<td>6.68 (0.25)</td>
<td>5.55 (0.13)</td>
<td>4.42 (0.08)**</td>
<td>4.04 (0.30)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 March 1995</td>
<td>0.93 (0.04)</td>
<td>1.06 (0.04)</td>
<td>0.68 (0.05)**</td>
<td>1.12 (0.07)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 April 1995</td>
<td>7.49 (0.09)</td>
<td>12.50 (0.20)</td>
<td>8.22 (0.10)**</td>
<td>6.94 (0.32)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 April 1995</td>
<td>4.00 (0.29)</td>
<td>5.86 (0.13)</td>
<td>5.70 (0.53)**</td>
<td>5.65 (0.39)**</td>
<td></td>
</tr>
<tr>
<td>&lt;10 µm</td>
<td>06 May 1994</td>
<td>2.29 (0.08)</td>
<td>2.25 (0.11)</td>
<td>2.28 (0.12)**</td>
<td>2.49 (0.21)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 March 1995</td>
<td>0.63 (0.02)</td>
<td>0.76 (0.05)</td>
<td>0.66 (0.04)**</td>
<td>0.64 (0.02)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 April 1995</td>
<td>3.98 (0.18)</td>
<td>5.26 (0.15)</td>
<td>3.75 (0.49)**</td>
<td>3.31 (0.09)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 April 1995</td>
<td>2.40 (0.05)</td>
<td>3.40 (0.18)</td>
<td>2.85 (0.07)**</td>
<td>2.19 (0.09)**</td>
<td></td>
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</table>

***P < 0.001, **P < 0.01 and *P < 0.05 when grazed < control; ns, non-significant (P > 0.05).
For all grazed ciliate and dinoflagellate prey taxa, the number of cells ingested increased in a generally hyperbolic fashion for both adults and nauplii as the total number of prey available increased (Figure 1; Table II). Clearance rates for adults and nauplii paralleled each other, although naupliar clearance rates were roughly an order of magnitude lower than rates measured for adults (Figure 2). Maximum clearance rates for both adults and nauplii occurred for prey which had a maximum cell dimension of 40 µm (Figure 2). Prey taxa possessing these dimensions had relatively low initial abundances during all experiments. Adult mean clearance rates for ciliate and dinoflagellate microplankton during all five experiments ranged from undetectable to 4.7 ml copepod$^{-1}$ h$^{-1}$, depending on prey type. Naupliar mean clearance rates were roughly an order of magnitude lower, ranging from undetectable to 0.52 ml copepod$^{-1}$ h$^{-1}$. The plastidic ciliate *M*. *rubra* was not grazed significantly by adults or nauplii. Applying the lowest non-zero adult copepod and naupliar clearance rates for ciliate prey to observed densities of adults and nauplii in the MEERC mesocosms suggested that nauplii contributed 2–56% to total copepod grazing. Estimates of combined grazing by adults and nauplii indicated that ciliates could be cleared from 22–300% of the total mesocosm volume per day.

Adult and naupliar clearance rates for *Chl a* were generally lower than rates observed for clearance of ciliates and *P*. *minimum*. Mean adult clearance rates for

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**Fig. 1.** Ingestion rates of various ciliate and dinoflagellate prey versus density of prey. Plotted data show prey from all five experiments that were grazed significantly (see Table II) by adult copepods and nauplii. Each plotted ingestion rate represents the mean of four replicate treatment bottles from one experiment. Each plotted ciliate or dinoflagellate density represents the mean of counts from four replicate initial condition bottles during the same experiment. Data have been fitted with hyperbolic functions. Open circles represent various ciliate prey. Filled triangles denote *Prorocentrum minimum*.
total Chl \( a \) ranged from undetectable to 1.6 ml copepod\(^{-1}\) h\(^{-1}\), while clearance rates for <10 \( \mu m \) Chl \( a \) ranged from undetectable to 0.72 ml copepod\(^{-1}\) h\(^{-1}\). Mean naupliar clearance rates for total Chl \( a \) ranged from undetectable to 0.25 ml copepod\(^{-1}\) h\(^{-1}\), with clearance rates for <10 \( \mu m \) Chl \( a \) ranging from undetectable to 0.19 ml copepod\(^{-1}\) h\(^{-1}\).

Ingestion of ciliate carbon varied in relation to the total phytoplankton carbon available in a particular experiment. Ciliate carbon was most important in copepod diets when phytoplankton carbon was relatively low (Figures 3 and 4). Relative ratios of ciliates and phytoplankton (including \( M.\text{rubra} \) and \( P.\text{minimum} \)) expressed as total prey carbon (Figure 3) revealed that ciliate carbon generally contributed \(~8\text{--}24\%\) to total available carbon, with the exception of the experiment conducted on 23 March 1995, where the contribution was 42%. Ciliate carbon was ingested by both adults and nauplii in all experiments. Adults consistently ingested a higher proportion of ciliate carbon relative to the ration available in the initial prey assemblage (Figure 3). Nauplii followed a similar trend in three of the five experiments. On 23 March 1995, nauplii failed to graze phytoplankton biomass significantly (Table III). Consequently, the carbon ration of nauplii consisted entirely of ciliate carbon in this experiment (Figure 3).

Quantitative data were not obtained from grazing experiments using CMFDA-labeled Strombidium sp. However, using epifluorescence microscopy, it was possible to observe bright green fluorescence in the guts of both adults and nauplii of \( E.\text{affinis} \) that had been incubated with the labeled ciliate prey (Figure 5). Samples examined after a 60 min incubation showed brighter gut fluorescence than did those observed after 15 and 30 min. Eurytemora that were
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Fig. 3. Ratio of phytoplankton (including MRUB and PRORO) carbon to ciliate carbon for all five experiments. Letters above bars denote the following: I, mean initial carbon ration available in four replicate bottles during a particular experiment; A, mean carbon ration removed by adult copepods from four replicate bottles; N, mean carbon ration removed by nauplii from four replicate bottles.

Fig. 4. Contribution of the <10 µm fraction and the >10 µm fraction to total phytoplankton carbon for each experiment (top panel). The phytoplankton fraction >10 µm was not measured directly, but was determined by subtraction. The bottom panel shows total ciliate carbon available for each experiment, except for MRUB which is included as >10 µm fraction phytoplankton carbon (top panel). N/D denotes no data.
Fig. 5. Adult female *E. affinis* (top panel) and stage 3 nauplius (bottom panel) showing gut epifluorescence resulting from ingestion of CMFDA-labeled *Strombidium* sp. Photographs were taken after copepods were exposed for 60 min to labeled prey at a density of ~10 cells ml⁻¹. Gut contents of the adult female (top panel) have been distorted as a result of slide preparation. Size bars equal 250 and 50 μm for the top and bottom panels, respectively.

incubated in control bottles lacking labeled prey did not exhibit bright green gut fluorescence.

**Discussion**

**Prey composition**

Choreotrichs and other protozoan microplankton are generally major contributors to microplankton biomass in natural assemblages (reviewed in Stoecker and Capuzzo, 1990). Our experiments were in agreement, with ciliate biomass contributing roughly 8–42% to available prey carbon. As a result, protozoan microplankton have the potential to comprise a major dietary portion for suspension-feeding copepods, such as *E. affinis* in Chesapeake Bay. In our experiments,
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adults and nauplii of the copepod *E. affinis* grazed both phytoplankton and protozoan microplankton. Although phytoplankton biomass was consistently higher than ciliate biomass in all experiments (Figure 4), *E. affinis* cleared and ingested total and <10 μm Chl a at lower rates than ciliate prey. Similar observations by Verity and Paffenhofer (1996) showed that ciliates were cleared by *Eucalanus pileatus* at higher rates than larger and more abundant diatom prey. On all dates except 23 March 1995, ciliate carbon comprised between 25 and 60% of the daily ration of adult *E. affinis*, while contributing between 7 and 55% to the daily ration of nauplii. Similar contributions of protozoans to copepod daily rations were noted by other investigators (e.g. Gifford and Dagg, 1988, 1990; Tiselius, 1989; Gifford, 1993a; Fessenden and Cowles, 1994). During the experiment conducted on 23 March 1995, the daily ration for nauplii consisted of 100% ciliate carbon. This high ciliate ration was probably observed because phytoplankton biomass was dominated by cells in the <10 μm fraction (Figure 4).

Since nauplii do not develop mature feeding appendages until copepodid stage C1, they may be unable to capture prey effectively that are at the extreme small and large ends of the size spectrum (Marshall and Orr, 1956; Fernández, 1979; Björnberg, 1986; Fryer, 1986; Paffenhofer and Lewis, 1989). In our experiments, the relatively larger ciliate prey may have provided an optimal signal, allowing them to be more easily collected and ingested than cells in the <10 μm size range (Stoecker and Egloff, 1987; Paffenhofer, 1988). Further, unlike the other four experiments in which prey biomass was dominated by phytoplankton, ciliate and phytoplankton carbon were present in nearly equal initial concentrations. Gifford and Dagg (1988) and Kleppel (1993) have reported that copepod grazing on protozoan microplankton tends to increase as the ratio of phytoplankton carbon:protozoan microplankton carbon decreases. In addition, Stoecker and Sanders (1985) and Stoecker and Egloff (1987) have shown that grazing pressure on ciliates may be reduced as algal concentration increases, although clearance rates for the algae do not increase.

**Prey selection**

Our results demonstrate that when large protozoan microplankton are present, they are ingested selectively. Some studies have classified *E. affinis* as feeding on small particles, including detritus and bacteria (Heinle and Fiemer, 1975; Boak and Goulder, 1983), and nanoplanckton (Gasparini and Castel, 1997), which may be supported by its mouthpart morphology (Schnack, 1982). A study by Tackx et al. (1995) generally supports these findings, although feeding selectivity for live phytoplankton or microzooplankton by *E. affinis* was occasionally measured.

The phenomenon of higher clearance rates for protozoan microplankton versus phytoplankton in our experiments might be explained by selection based on increased handling efficiency for larger particles (e.g. Frost, 1972; Donaghay and Small, 1979; Price and Paffenhofer, 1985). However, increased motility, and perhaps chemical cues associated with food quality, may be important factors responsible for higher feeding rates on ciliates than most phytoplankton (e.g. Corner et al., 1976; Fernández, 1979; Stoecker and Egloff, 1987). Zooplankton
which create a feeding current may initially perceive prey at a distance via olfaction, followed by a more qualitative chemoreceptive examination at the mouth (Donaghay and Small, 1979; Koehl and Strickler, 1981; Paffenhofer et al., 1982; Verity and Paffenhofer, 1996). Since large cells create a greater hydrodynamic disturbance, they may be perceived more easily than smaller prey by calanoids that utilize mechanoreception (Légier-Visser et al., 1986; Paffenhofer, 1988; Jonsson and Tiselius, 1990). In addition, ciliate microzooplankton have been described as high-quality food items for copepods (Corner et al., 1976; Stoecker and Sanders, 1985; Stoecker and Egloff, 1987). Perhaps they are preferentially ingested as a result of their favorable chemical properties or 'taste' (Paffenhofer and Van Sant, 1985). Nanoplankton generally dominated the autotrophic biomass in our experiments, but were not cleared at high rates, probably because they were near or below the threshold size at which Eurytemora can perceive and capture individual cells (Price et al., 1983; Paffenhofer, 1984; Price and Paffenhofer, 1986; Price, 1988). Although we are uncertain of the exact mechanism by which E.affinis perceives its prey, our experimental results are compatible with the models described above.

All ciliate and dinoflagellate prey in our experiments were within the size spectrum of ingestible particles reported by other researchers. Berk et al. (1977) found that E.affinis consumed 15 X 20 μm ciliates, while Burkill and Kendall (1982) reported particles up to 40 μm in diameter in the gut of Eurytemora. Ingestion of large cells has been reported previously for calanoid copepod nauplii. Mullin and Brooks (1967) observed that young Rhincalanus nasutus nauplii fed preferentially on large particles. Stoecker and Egloff (1987) found that Acartia tonsa nauplii were capable of ingesting ciliates up to 40 μm in diameter. Paffenhofer and Lewis (1989) observed that late naupliar stages of Eucalanus spp. were capable of ingesting phytoplankton nearly as long as the nauplius itself. Our findings are strikingly similar since Eurytemora nauplii were able to graze even the largest available ciliate prey, a Strombidium sp. (STROM2) which had a diameter of 35 μm (Table 1). Clearance rate data for Eurytemora (Figure 2) showed the highest clearance rates for a hypotrich (HYPO) and a Strobilidium sp. (STROB3), both of which have a maximum cell dimension of ~40 μm. Even though the hypotrich (HYPO) and the Strobilidium sp. (STROB3) had low abundances relative to other ciliate prey in our experiments, they were cleared at higher rates, perhaps because their motility and relatively large size allowed them to be more easily perceived by Eurytemora. The ciliate M.rubra was within the size range grazed by E.affinis, but was not grazed, apparently because of its ability to avoid capture through sudden rapid swimming behavior (Jonsson and Tiselius, 1990). Both adults and nauplii of E.affinis were able to graze the entire size spectrum of prey in our experiments, suggesting comparable food availability for all copepod developmental stages, as indicated by Berggreen et al. (1988).

Direct observations of ingested prey

The results of our short-term (1 h) experiments with CMFDA-labeled Strombidium sp. provide direct evidence that ciliates are ingested at least semi-intact
by both adult copepods and nauplii, although individual prey were not easily recognizable by microscopic observation (Figure 5). The majority of copepod grazing studies have measured ingestion and clearance rates based on disappearance of prey over time. This assumes that all prey losses are attributable to copepod ingestion, with little consideration of other mechanisms of prey disappearance. Fragile prey such as choreotrichs might undergo losses via other means, such as contact with air bubbles, or excessive turbulence (Gifford, 1993b). Since many choreotrichs like *Strombidium* sp. have no hard cell structures, as do tintinnid ciliates or diatoms (Stoecker and Egloff, 1987), analyses of copepod gut contents (e.g. Burkill and Kendall, 1982) or fecal pellets (e.g. Turner, 1984) are poor estimators of ciliate ingestion.

**Relative abundance of copepod developmental stages**

Because naupliar stages are usually more abundant than adults in natural copepod assemblages (e.g. Heinle and Flemer, 1975; Fernández, 1979; Fulton, 1984; White and Roman, 1992), they have the potential to contribute significantly to total grazing impact. Fessenden and Cowles (1994) have suggested that copepod nauplii add 10–20% per day to total copepod grazing impact on ciliates in the Oregon upwelling system. When clearance rate data from our experiments were applied to field abundances of *Eurytemora* from the Patuxent River, Maryland (Heinle and Flemer, 1975), an added grazing contribution of 9–84% by naupliar stages was indicated. When the same clearance rates were applied to the numerical abundance of copepods from the MEERC mesocosms, nauplii contributed 2–56% to total copepod grazing impact. Nauplii <200 µm were not included in the calculations because early naupliar stages (N1, N2) do not feed. Copepodids were assumed to have clearance rates similar to adults, although they may exhibit rates that are substantially lower. This suggests that our estimates of the naupliar contribution to total copepod grazing may be conservative. The lowest observed non-zero adult and naupliar clearance rates (1.02 and 0.16 ml copepod⁻¹ h⁻¹, respectively) were used to estimate total volume cleared per day of ciliates in the MEERC mesocosms. Calculations based on these conservative rates showed that daily clearance rates could range from 22 to 300% of the total mesocosm volume, depending on the relative densities of copepods and ciliate prey, similar to Dolan's (1991) estimate of 35–200% day⁻¹ for copepods grazing on microphagous ciliates in Chesapeake Bay surface waters.

**Summary**

*Eurytemora affinis* is an important grazer of ciliate and dinoflagellate microplankton because of its general abundance and ability to ingest large particles throughout its development. Increased clearance rates for ciliate microplankton relative to phytoplankton probably arise from a combination of a stronger perception of larger (~40 µm) motile particles, enhanced food quality, and better handling efficiency for relatively larger cells. Ingestion rates showed a generally positive increase with increasing prey concentration. Experiments using
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fluorescently labeled ciliates provided direct evidence that ciliates were ingested, and that ciliate losses during grazing experiments generally could be attributed to ingestion by copepods. Estimates of the naupliar contribution to total copepod grazing impact on ciliates reveal that copepod nauplii can contribute substantially to total community grazing. Given the clearance rates of *E. affinis* for ciliates, and the densities of copepods and ciliates present in the MEERC mesocosms, 22–30% of the total mesocosm volume could be filtered per day. This suggests that *E. affinis* is not only capable of ingesting ciliate microplankton as a large portion of its diet, but can potentially control protozoan microplankton populations.

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