Competition between crustacean zooplankton in continuous cultures

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Abstract
It has been proposed that cases of diaptomid dominance in oligotrophic lakes are due to lower food requirements for Diaptomus species than Daphnia species. We test this hypothesis by competing Daphnia pulicaria and Diaptomus oregonensis populations in continuous and semicontinuous cultures.

The threshold food concentrations for population growth of D. pulicaria and D. oregonensis were statistically indistinguishable. The thresholds of both species were within the range of concentrations characteristic of ultraoligotrophic lakes. The dominant species varied across experiments in a pattern consistent with an effect of temporal variation in food concentration. Neither our data nor previously published studies provide support for the hypothesis that cases of Diaptomus dominance in oligotrophic lakes are due to a simple difference in threshold food concentrations.

The taxonomic composition of the zooplankton assemblage has important implications for community and ecosystem processes. In northern hemisphere lakes, that composition is largely a function of the distribution of Daphnia and Diaptomus. On average, 1.6 Daphnia species and 1.4 Diaptomus species occur in 1,168 North American and European lakes of various sizes and trophic statuses (Patalas 1971, 1975; Anderson 1974; Carter et al. 1980; Dodson 1991). In many cases a member of one or the other genus is the dominant herbivore (e.g. Patalas 1975; Sprules 1975).

Differences in foraging selectivity, nutrient recycling, and vulnerability to predators provide a complex variety of opportunities for Daphnia and Diaptomus to differentially affect community and ecosystem processes. For example, Daphnia has a broader diet (e.g. DeMott 1990; Wylie and Currie 1991) and greater potential to regulate phytoplankton abundance (e.g. Knisely and Geller 1986; Vanni and Temte 1990; Sarnelle 1992), although that potential can be suppressed by the presence of algal filaments (Richman and Dodson 1983). On the other hand, nutrients contained in the diffuse fecal material produced by Daphnia may be more readily available to phytoplankton than nutrients contained in the compact fecal pellets produced by Diaptomus (Vanni and Temte 1990).

Meanwhile, because Daphnia tissue has a lower N:P ratio than Diaptomus tissue (Andersen and Hessen 1991; Hessen and Andersen 1992), material excreted by Daphnia is likely to have a higher N:P ratio than material excreted by Diaptomus, and this may affect competitive relationships among the phytoplankton (Elser et al. 1988; Sterner 1990). Finally, vertebrate predators tend to prey more rapidly on Daphnia than on Diaptomus (Zaret 1980; Lazzaro 1987). Thus, energy and material may be transferred to higher trophic levels more efficiently when Daphnia is dominant than when Diaptomus is dominant. Because of their broad distributions and great potential for differential effects on ecological processes, it is important to understand the mechanisms that regulate the abundance of Daphnia and Diaptomus.

Several field examples suggest that the ratio of diapomids to daphnids is an inverse function of lake productivity (e.g. Lake Constance—Elster and Schwoerbel 1970 cited by Muck and Lampert 1984; Einsle 1983; North American Great Lakes—Patalas 1972, 1975; arctic lakes—Hobbie 1973; Lago Maggiore and Lago Lugano—Ravera 1980; Gjersjøen—Faafeng and Nilssen 1981, Quebec lakes—Pace 1986; Esthwaite Water—George et al. 1990). On the basis of such observations and early feeding studies, McNaught (1975) proposed that low food concentration favors calanoid copepods (e.g. Diaptomus) over cladocerans (e.g. Daphnia). It has subsequently become clear that zooplankton are often food limited (e.g. Lampert 1985), and McNaught's hypothesis appears to have gained some acceptance (Richman and Dodson 1983; DeMott 1989; McQueen 1990).

Lampert and Muck (1985; Muck and Lampert 1984) investigated McNaught's hypothesis by comparing the

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ingestion rates, growth rates, productivity (adult growth plus egg production), survivorship, and clutch sizes of individual adult *Eudiaptomus gracilis*, *Daphnia hyalina*, and *Daphnia galeata* fed various species of green algae in a continuous-flow culture system. On the basis of a variety of experiments, they concluded that “...diaptomids do not collect food more efficiently than cladocerans at low food concentrations” (Lampert and Muck 1985, p. 318). Furthermore, Lampert and Muck concluded that the threshold food concentration for maintenance of body mass by adults is around 50 μg C liter⁻¹ for both species, which is within the range of ultraoligotrophic conditions (Wetzel 1975). Lampert and Muck noted that, by focusing on adults, they were unable to determine whether immature instars of *Daphnia* and *Diaptomus* have different food requirements. If one species has a bottleneck at an earlier instar, then its threshold food concentration for population growth (the minimum food concentration that is sufficient for a population to maintain its size, Lampert 1977; Tilman 1982) would be higher than that estimated from studies of the growth and egg production of adult individuals. Our results build upon Lampert and Muck’s studies by comparing food thresholds for population growth and by providing data for a second pair of *Daphnia* and *Diaptomus* species and a different food source.

The specific objective of this study is to test the hypothesis that low concentrations of food favor *Diaptomus oregonensis* over *Daphnia pulicaria*. We present the results of a continuous culture experiment in which we competed *D. oregonensis* and *D. pulicaria* populations and estimated their threshold food concentrations for population growth. Comparisons of threshold resource concentrations for population growth have been informative in studies of phytoplankton (e.g., Sommer 1989), but direct measurements of zooplankton thresholds are rare (Rothhaupt 1990; Sommer 1992). Our simple continuous culture system is suitable for culturing copepods and cladocerans for multiple generations with relative ease and can detect small differences in competitive ability. To help the reader understand these results, we include results from a preliminary continuous culture experiment and from two earlier semicontinuous culture experiments.

**Methods**

*Species*—We studied populations of *D. pulicaria* and *D. oregonensis* isolated from Lake Waynewood, a small lake in northeastern Pennsylvania (41°30'N, 75°15'W) that has experienced cultural eutrophication. We chose *D. pulicaria* because its food threshold for individual growth is lower than those of other species of *Daphnia* that have been examined (Gliwicz 1990), and therefore *D. pulicaria* should provide a conservative test of the hypothesis that *Diaptomus* species have lower food thresholds for population growth than do *Daphnia* species. We chose *D. oregonensis* because it co-occurs with *D. pulicaria* in Lake Waynewood.

Lake Waynewood was our only readily available source of *D. pulicaria*. Both *D. pulicaria* and *Daphnia laevis* are present in the lake. Routine zooplankton sampling shows a spring maximum of 12–24 *Daphnia* liter⁻¹, followed by low densities during summer (2–2 liter⁻¹) and a more modest peak in fall (~12 liter⁻¹). The spring peak tends to be dominated by *D. pulicaria*, with a shift to *D. laevis* during summer and fall. *D. oregonensis* typically shows peak abundances of 7–17 copepods plus adults per liter in September–October and lower numbers during the rest of the year (1–5 liter⁻¹) (R. E. Moeller and C. E. Williamson unpubl. rep.).

We fed the zooplankton the algae *Cryptomonas reflexa* (Williamson et al. 1985). Both zooplankton species grow well for multiple generations (indefinitely) when fed *C. reflexa* in our laboratory cultures. The *C. reflexa* culture was isolated from Whiteacre Pond, Pennsylvania, and batch cultured in a modified MBL medium (Williamson and Butler 1987). Under our growth conditions, the largest cells were ~12 × 32 μm, 2.9 × 10³ μm³, and had a dry mass of 0.6 ng (Williamson et al. 1985). At this volume, 100 cells ml⁻¹ is equal to a volume of 0.29 ml m⁻³, and has a dry mass of 60 μg liter⁻¹. During the week before each experiment, the zooplankton were fed high concentrations of *Cryptomonas* (~1,000 cells ml⁻¹) to ensure that a large proportion of females was carrying eggs at the beginning of each experiment.

**Conditions**—Experiments were performed and stock cultures of phytoplankton and zooplankton were grown in spring water that is piped directly into the environmental sciences building at Lehigh University. This water has a pH of 6.4 and an alkalinity of 450 μeq liter⁻¹. Water was 0.22-μm filtered and stored in sterilized carboys before use. Stock cultures were maintained and experiments were performed at 20°C on a 14:10 L/D cycle. Light intensities were 30 μmol m⁻² s⁻¹ under the stock culture conditions and 5 μmol m⁻² s⁻¹ under the experimental conditions.

**Continuous culture experiments**—Two experiments (*Cₐ*, begun March 1992; *Cₜ*, begun October 1991) were performed in continuous cultures (Fig. 1). (The experience of performing experiment *Cₜ* enabled us to refine our methods before beginning experiment *Cₐ*.) The plankton populations were cultured in 1,200-ml transparent gray polycarbonate cylindrical jars. Water with phytoplankton was supplied through Teflon hose by gravity feed from Mariotte-type reservoir bottles (Kubitschek 1970; Williamson et al. 1985). In experiment *Cₐ*, each 18-liter reservoir bottle supplied water and phytoplankton to six cultures (two replicates of each of three treatments). In experiment *Cₜ*, each 3-liter reservoir bottle supplied water and phytoplankton to four cultures (one replicate of each of four treatments). Elevated Teflon stirring bars were used to keep phytoplankton well mixed in the reservoir bottles. Patchiness in phytoplankton distributions was prevented by also suspending elevated Teflon stirring bars in the culture vessels and spinning them slowly for 10 s every 10 min. This procedure thoroughly mixed the
chamber contents every 10 min. Air bubbles were excluded from the chambers to prevent Daphnia from being caught at the air-water interface.

Reservoir bottles were replaced daily. Experimental chambers and the hoses that connected them to the reservoirs were cleaned weekly. Flow rates were controlled by adjusting the pressure head and the length and diameter of the outflow hoses. The flow rate of this system is not affected by the level of medium in the reservoir because air enters the Mariotte bottle through a tube that terminates below the surface of the medium, thereby keeping the pressure head constant as long as the water level is above the air inlet (Fig. 1). In experiment $C_a$ we measured flow rates of individual replicates daily and adjusted them as necessary. In experiment $C_b$ we measured flow rates daily for each reservoir bottle (each block of four experimental units) and adjusted them as necessary. Outlet hoses were changed whenever maintenance of target flow rates became difficult (about every 2 weeks). To minimize diel periodicity in input phytoplankton concentrations, we mixed log-phase cells with spring water in reservoir bottles 1 d before use. This procedure slowed the growth rate of the phytoplankton, presumably because nutrient concentrations were reduced by dilution.

Under these culture conditions, consumer populations grow until they deplete the resource concentration to the minimum sufficient to maintain the consumer population (the threshold food concentration for population growth).

Resource concentration is therefore a function of the requirements of the consumer. We use mean food concentrations in cultures as estimates of threshold food concentration for population growth (Lampert 1977; Tilman 1982).

**Semicontinuous culture experiments**—Before developing the continuous culture system, we performed two experiments in 3-liter semicontinuous cultures (4-liter aquaria with surface barriers of 100-μm Nitex on acrylic frames that prevented Daphnia from becoming caught at the water surface). Phytoplankton were added either every other day (experiment $S_d$, begun January 1991) or once per week (experiment $S_w$, begun November 1990). The water was changed and the vessels were cleaned weekly. The water was gently stirred once per day, but this was not sufficient to prevent spatial patchiness in Cryptomonas. Cryptomonas is attracted to light and tends to aggregate at the surface above the meshes during the day. A substantial proportion of the food was therefore unavailable to the zooplankton during the day.

**Details of individual experiments**—Table 1 provides the details of the methods and designs of the four experiments. Starting densities of zooplankton were selected to provide approximately equal biomasses of Daphnia and Diaptomus and in continuous culture experiments were chosen to approximate expected equilibrium densities in

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**Fig. 1.** The continuous culture system. We used 3-liter Mariotte bottles in experiment $C_a$ and 18-liter bottles in experiment $C_b$. During the experiments each bottle provided water plus phytoplankton to an equal number of replicates of each treatment.
Table 1. Experimental conditions.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatments</th>
<th>Replicates</th>
<th>Duration (d)</th>
<th>Starting densities</th>
<th>Target flow rate (ml culture⁻¹ d⁻¹)</th>
<th>Input algae (cells ml⁻¹)</th>
<th>Pretreatment of algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_a</td>
<td>Daphnia alone</td>
<td>4</td>
<td>91</td>
<td>15 lg + 5 sm Daphnia, and (or) 20 adult + 25 copepodite + 55 naupliar Diaptomus (10% of Diaptomus from single sp. cultures)</td>
<td>2,500</td>
<td>250</td>
<td>diluted and shaded for 1 d</td>
</tr>
<tr>
<td></td>
<td>Diaptomus alone</td>
<td></td>
<td>134</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daphnia and Diaptomus</td>
<td></td>
<td>134</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4 Diaptomus invasion of Daphnia)</td>
<td></td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_b</td>
<td>Daphnia alone, no predation</td>
<td>4</td>
<td>83</td>
<td>Single sp. treatments: 20 lg + 10 sm Daphnia or 14 adult + 16 copepodite + 80 naupliar Diaptomus; half as many of each in 2 spp. treatments</td>
<td>250</td>
<td>2,000</td>
<td>diluted for 1 d but not shaded</td>
</tr>
<tr>
<td></td>
<td>Diaptomus alone, predation</td>
<td></td>
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<td></td>
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<td></td>
<td>Daphnia and Diaptomus, no predation</td>
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<td></td>
<td>Daphnia and Diaptomus, predation</td>
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</tr>
<tr>
<td>S_a</td>
<td>Daphnia alone</td>
<td>4</td>
<td>55</td>
<td>11 adult Daphnia and (or) 24 adult Diaptomus</td>
<td>every other day</td>
<td>350</td>
<td></td>
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<tr>
<td></td>
<td>Diaptomus alone</td>
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<tr>
<td></td>
<td>Daphnia and Diaptomus</td>
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</tr>
<tr>
<td>S_b</td>
<td>Daphnia and Diaptomus</td>
<td>4</td>
<td>35</td>
<td>11 adult Daphnia and 24 adult Diaptomus</td>
<td>weekly</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

order to minimize an initial period of rapid population growth in both species. Experimental durations varied because experiments ended when the more abundant species exceeded ~90% of total animal biomass in mixed-species cultures.

In two treatments of experiment C_b we simulated a nonselective predator by removing a fraction of the zooplankton each day. We began harvesting on day 5 at an intended rate of 14% per day by scooping animals from opened cultures. This method captured a disproportionately large number of Daphnia, so we changed to a core-type sampler on day 13. This method was nonselective for both species. By day 22 it was evident that 14% removal per day was excessive, so we reduced the predation rate to 7% on day 22 and maintained that predation rate until the end of the experiment.

Measurements—Zooplankton populations were censused weekly by counting all live individuals in deep-welled glass dishes under a dissecting microscope. This procedure caused no detectable harm to the animals. In addition to total numbers, we counted the number of individuals in the following categories: juvenile Daphnia, female Daphnia without eggs, female Daphnia with eggs, Diaptomus nauplii, Diaptomus copepodids, Diaptomus males, nonreproducing Diaptomus females, and Diaptomus females in various stages of reproduction. We use the term "reproductively active" to refer to Daphnia carrying eggs and Diaptomus with full ovaries or an egg clutch. We classified Daphnia smaller than the smallest individuals with eggs as juvenile (the size cutoff declined through each experiment as the size of first reproduction became smaller in the strongly food-limited cultures). All larger Daphnia were classified as mature females; we did not observe any Daphnia males.

Phytoplankton were sampled weekly or more often. After stirring the chambers, samples were collected with a pipette and preserved in Lugol's solution. Subsamples (1 ml) were counted in a Sedgwick-Rafter counting cell at 100 X.

Bacteria samples were collected weekly in experiment C_a and occasionally in experiments C_b and S_b. No bacteria samples were collected in experiment S_a. Samples were collected as for phytoplankton, preserved with Formalin, and stored cold and dark until counted. Bacteria were stained with 4',6-diamidino-2-phenylindole (DAPI) and counted with an epifluorescence microscope at 1,000 X, following the procedure of Porter and Feig (1980). We counted a minimum of 25 fields or 300 cells per sample.

Statistical analyses—Cryptomonas and bacteria counts from the continuous culture experiments were analyzed with one-way repeated-measures ANOVA (Winer 1971). Bacteria counts from one date in experiment S_a were analyzed with one-way ANOVA. Repeated-measures ANOVA were performed with the general linear models.
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(GLM) routine of SAS (SAS Inst., Inc. 1989), using type 3 sums-of-squares and the type 3 mean-square for replicate nested within treatment as the error term for tests of the significance of treatment main effects. Missing data forced us to use this model which assumes that all time points are similarly correlated (Winer 1971). Treatment \times time interactions were omitted from the models when they were not significant. The Ryan-Einot-Gabriel-Welsh multiple comparison test was used to test for significant differences between pairs of treatments in both ANOVA and repeated-measures ANOVA (Day and Quinn 1989). The relationship between zooplankton reproduction and resource supply rate in experiment \( C_p \), was evaluated by testing for correlations between the mean proportions of Diaptomus and Daphnia females reproducing each week and the mean flow rate of the preceding 7 d (or 6 d for the proportion of females reproducing on day 7).

Results

Continuous culture experiment (\( C_o \))—All populations persisted in single-species cultures (cultures with only one zooplankton species) with relatively stable numbers of adults. The populations of both species were characterized by an initial pulse of young individuals (as a result of high concentrations of food prior to the beginning of the experiment) and a low proportion of females reproducing (Figs. 2, 3).

D. oregonensis was dominant in three of four mixed-species cultures (cultures that contained both D. pulicaria and D. oregonensis) (Fig. 4). In addition, when we transferred \( \sim 10\% \) of the D. oregonensis from single-species cultures into counterpart D. pulicaria cultures on day 91 (4 males, 4 females, 7 copepodids, and 2 nauplii were transferred per culture), the colonizing D. oregonensis populations grew rapidly, although there were no corresponding declines in the D. pulicaria populations (Fig. 2). By the end of the experiment, D. oregonensis was more frequent than D. pulicaria in these cultures, which had begun as single-species D. pulicaria cultures.

Population stage structures were not appreciably affected by the presence of a second zooplankton species (cf. Figs. 3 and 4 for D. oregonensis and Figs. 2 and 4 for D. pulicaria). There appear to have been some differences between the stage structures of the D. pulicaria populations in the single-species and mixed-species cultures after about day 90, but there were few individuals remaining in the mixed-species cultures at this time; therefore the stage structure figure is sensitive to a change in the condition of one or a few individuals.

All four mixed-species cultures had similar trajectories for the first 2 weeks of the experiment, and then one diverged from the other three. D. pulicaria became dominant in the divergent culture and remained dominant for > 100 d, until the end of the experiment (Fig. 4). Modest D. oregonensis reproduction occurred near the end of the experiment in this culture, after a hiatus of 8 weeks when no nauplii had been present. This reproduction was coincident with a small increase in the number of adult D. oregonensis females, raising the possibility that recently matured individuals were responsible for this late reproduction and that young D. oregonensis females require less food for reproduction than older females. It is curious that the D. pulicaria abundance in this culture was higher than in the single-species D. pulicaria cultures (cf. Figs. 2, 4). There were no obvious differences between the average sizes of individuals or the stage structures of these D. pulicaria populations, nor do we have any reason to suspect that the experimental conditions differed in this particular culture. Thus, we have no satisfactory explanation for the high abundance of D. pulicaria in the divergent mixed-species culture. This culture is omitted from analyses of treatment effects on Cryptomonas and bacteria concentrations.

Average Cryptomonas densities (pooling all dates preceding the transfer of D. oregonensis into the D. pulicaria cultures) were 100 cells ml\(^{-1}\) in the D. pulicaria cultures, 85 cells ml\(^{-1}\) in the D. oregonensis cultures, and 73 cells ml\(^{-1}\) in the mixed-species cultures dominated by D. oregonensis (Fig. 5). These threshold food concentrations correspond to 0.29, 0.25, and 0.21 ml m\(^{-3}\) and 60, 51,
and 44 μg dry mass liter⁻¹, respectively. They are not significantly different (Table 2) and are well within the range of phytoplankton concentrations characteristic of ultraoligotrophic lakes (<1 ml m⁻³, Wetzel 1975). Standard estimation procedures (Cox 1958) based on average Cryptomonas densities for each replicate over the period preceding the transfer of D. oregonensis into the D. pulicaria cultures suggest that we would have detected a statistically significant difference between the Cryptomonas abundance in the single-species treatments if the measured differences in their mean densities had been >32 cells ml⁻¹.

Bacteria densities were measured five times during this experiment—four times before the transfer of D. oregonensis into the D. pulicaria cultures and once after the transfer. Counts were low and not significantly different across treatments (Table 2) [mean number of bacteria per ml ± SE (sample size) for the period preceding the transfer of D. oregonensis into the D. pulicaria cultures: D. pulicaria cultures 3.2 ± 0.8 x 10⁵ (n = 15); D. oregonensis cultures 3.8 ± 0.8 x 10⁵ (n = 13); mixed-species cultures 2.8 ± 0.2 x 10⁵ (n = 12)]. The bacteria were ~1.0 μm long and 0.6-μm diameter or ~0.3 μm³. At 3 x 10⁵ cells ml⁻¹, this is roughly equivalent to 0.1 ml m⁻³, which is on the order of a half to a third the volume of Cryptomonas in the cultures. Bacterivory may have accounted for a small fraction of the D. pulicaria diet but was probably insignificant in the D. oregonensis diet; D. oregonensis feeds on bacteria at much lower rates than D. pulicaria and apparently gains no nutritional benefit as a result (R.W. Sanders unpubl.).

Flow rates were relatively constant in experiment Cₐ (Fig. 6).

**Earlier experiments**—As in experiment Cₐ, all single-species cultures persisted in experiments C₉ (Figs. 7, 8) and S₉. (Experiment S₉ did not include single-species cultures.) However, unlike experiment Cₐ, D. pulicaria was dominant in the mixed-species cultures of all three earlier experiments (Figs. 9–11). Less time was required for one species to become dominant in the semicontinuous cultures (S₉ and S₉₁) than in the continuous cultures (Cₐ and C₉) (cf. Figs. 4, 9–11). Nonselective predation (harvesting) caused an increase in reproduction rates and a shift to younger, smaller individuals in both zooplankton species but did not have any qualitative impact on the outcome of competition: D. pulicaria was dominant in experiment C₉ both in the presence and absence of predation (Figs. 9, 10).

In one mixed-species culture of experiment C₉, the D. oregonensis population declined more slowly and the D. pulicaria population declined more rapidly. The changes in the proportion of stages in the D. oregonensis population and the proportion of the total population comprised by D. oregonensis are shown in Figure 4.

**Fig. 3**. Experiment Cₐ. The abundance and stage structure of *Diaptomus oregonensis* populations in single-species cultures. Symbols in the top panel are means ± 1 SE.

**Fig. 4**. Experiment Cₐ. The abundance and stage structure of *Diaptomus oregonensis* and *Daphnia pulicaria* in mixed-species cultures. Symbols in the top panel are means ± 1 SE in the cultures dominated by *D. oregonensis*. Dotted line represents the number of *D. pulicaria* in the culture it dominated. Dashed line represents the number of *D. oregonensis* in the same culture. Note difference in scales for *Daphnia* and *Diaptomus*.
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Fig. 5. *Cryptomonas reflexa* abundance in the single-species treatments of experiment *C*. Symbols are means ±1 SE. For clarity, the data for the mixed-species treatment are not included.

*pulicaria* population grew more slowly than in the replicate cultures (Fig. 9). However by the end of the experiment, it appeared that *D. pulicaria* would have eventually dominated this culture as well. This culture is omitted from analyses of treatment effects on *Cryptomonas* and bacteria abundance and from analyses of the relationship between the flow rate and the proportion of females reproducing.

*Cryptomonas* abundance differed across treatments in experiment *C* (Fig. 12, Table 2). Mean cell densities (pooled over the duration of the experiment) were 50 cells ml⁻¹ (mixed-species cultures, no predation), 66 cells ml⁻¹ (*D. pulicaria* alone, no predation), 106 cells ml⁻¹ (mixed-species cultures, with predation), and 143 cells ml⁻¹ (*D. oregonensis* alone, with predation). There was no significant difference in *Cryptomonas* abundance in the two treatments that were not subjected to predation, but *Cryptomonas* abundance in these treatments was lower than in the two treatments subjected to predation. Furthermore, *Cryptomonas* densities were significantly lower in the mixed-species cultures subjected to predation than in the *D. oregonensis* cultures subjected to predation. However in both of these treatments, food concentrations typical of oligotrophic lakes were sufficient to support populations subjected to 7% predation each day.

Threshold *Cryptomonas* concentrations appear to have been higher in experiment *Ca* than in the predation-free treatments of experiment *Cb* (cf. Figs. 5, 12), but this is probably due to methodological differences. We used a wider bore pipette that gives 25% higher counts (Schulze and Zagarese unpubl. data) to sample *Cryptomonas* in experiment *Ca*. This change in pipettes could account for about half of the difference between the threshold concentrations in the two experiments. The remaining difference is probably due to differences in the treatment of the phytoplankton before they were added to the culture vessels. The phytoplankton were exposed to light while in the reservoir bottles that supplied the cultures of experiment *Ca*, but were shielded from light in the reservoir bottles that supplied the cultures of experiment *Cb*. This light limitation was introduced to halt cell division in the reservoir bottles of the latter experiment but may also have resulted in a modest reduction in cell size, which would be expected to increase the density of cells required by the zooplankton. In both experiments, threshold *Cryptomonas* concentrations were within the range of phytoplankton concentrations characteristic of ultraoligotrophic lakes (Wetzel 1975).

Bacteria concentrations in experiment *Cb* were highest in the treatments subjected to predation (Table 2) [mean number ml⁻¹ ± SE (sample size): *D. pulicaria* alone, no predation 2.4 ± 0.4 × 10⁵ (n = 8); mixed-species cultures, no predation 3.0 ± 0.5 × 10⁵ (n = 6); *D. oregonensis* alone, with predation 14.0 ± 3.3 × 10⁵ (n = 8); mixed-species cultures, with predation 11.1 ± 2.7 × 10⁵ (n = 8)]. There was no significant difference between the bacteria densities of the two treatments that were preyed upon or the bacteria densities of the two treatments that were not preyed upon. There was an inverse relationship between the abundances of bacteria and *D. pulicaria*, suggesting

Table 2. Results of repeated-measures ANOVA examining the effect of the experimental treatments on densities of *Cryptomonas* and bacteria in the continuous culture experiments. (Statistical procedures described in the text.) Significance: *—P < 0.05; **—P < 0.01; NS—not significant.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Response variable</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × time</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ca</em></td>
<td><em>Cryptomonas</em></td>
<td>1.6* (2,8)</td>
<td>4.2* (12,108)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>0.5* (2,8)</td>
<td>3.7* (3,29)</td>
<td>NS</td>
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<tr>
<td><em>Cb</em></td>
<td><em>Cryptomonas</em></td>
<td>40.9** (3,11)</td>
<td>4.4* (0,009)</td>
<td>1.6† (27,099)</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>18.4** (3,11)</td>
<td>20.2** (1,11)</td>
<td>4.3* (3,11)</td>
</tr>
</tbody>
</table>

† P = 0.06.

Fig. 6. Flow rates in experiments *Ca* and *Cb* (lines without error bars) and numbers of reproducing zooplankton in treatments of experiment *Ca* that were not subjected to predation (lines with error bars). Flow rate data have been smoothed with a 7-d running mean to highlight the patterns on longer time scales. Number of reproducing *Diaptomus oregonensis*—■; number of reproducing *Daphnia pulicaria*—□. Dotted lines represent data from mixed-species cultures. Solid line represents data from single-species *D. pulicaria* cultures. Note that flow rates of experiment *Ca* were an order of magnitude greater than flow rates of experiment *Cb*. 

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**Figure Captions**

- **Fig. 5.** *Cryptomonas reflexa* abundance in the single-species treatments of experiment *C*. Symbols are means ±1 SE. For clarity, the data for the mixed-species treatment are not included.
- **Fig. 6.** Flow rates in experiments *Ca* and *Cb* (lines without error bars) and numbers of reproducing zooplankton in treatments of experiment *Ca* that were not subjected to predation (lines with error bars). Flow rate data have been smoothed with a 7-d running mean to highlight the patterns on longer time scales. Number of reproducing *Diaptomus oregonensis*—■; number of reproducing *Daphnia pulicaria*—□. Dotted lines represent data from mixed-species cultures. Solid line represents data from single-species *D. pulicaria* cultures. Note that flow rates of experiment *Ca* were an order of magnitude greater than flow rates of experiment *Cb*.
that *D. pulicaria* suppressed bacteria in our culture system.

The flow rate was more variable in experiment *Cb* than in experiment *Ca* (Fig. 6). Furthermore, the temporal pattern in the proportion of *D. pulicaria* reproducing in experiment *Cb* was positively correlated with the mean flow rate of the previous week (*D. pulicaria* alone, no predation $r = 0.59$, $n = 12$, $P < 0.05$; *D. pulicaria* in mixed-species cultures, no predation $r = 0.69$, $n = 12$, $P < 0.05$). There was no correlation between the flow rate of the preceding week and the proportion of *D. oregonensis* females reproducing in the absence of predation or between flow rate and the proportion of either species females reproducing in the treatments that were preyed upon (and therefore had higher *Cryptomonas* densities). In addition, few *D. oregonensis* females reproduced after the first few weeks in the mixed-species cultures that were not preyed upon (Fig. 6).

The semicontinuous culture experiments (*Sa* and *Sb*) were characterized by strong temporal variation in food concentration, with the magnitude of the variation dependent on the period between food additions and the density at which food was added (Fig. 13). Phytoplankton densities in the semicontinuous cultures were often above the threshold densities measured in the continuous cultures. During the day, however, the algae aggregated above the meshes near the surface of the semicontinuous culture vessels and were therefore largely unavailable to the zooplankton (see methods).

Bacteria samples were collected on one date during experiment *Sa*. Bacteria density was significantly higher in the *D. oregonensis* treatment than in the other two treatments, but there was no significant difference between the bacteria densities in the *D. pulicaria* treatment and the mixed-species cultures (one-way ANOVA, $F = 7.2$, df = 2, 9, $P < 0.05$) [mean number per ml ± SE (sample size): *D. oregonensis* $10.0 ± 0.4 \times 10^5$ ($n = 4$); *D. pulicaria* $7.6 ± 0.8 \times 10^5$ ($n = 4$); mixed-species cultures $6.9 ± 0.5 \times 10^5$ ($n = 4$)]. In general, these densities were somewhat higher than those of the continuous cultures that were not preyed upon (*Ca* and *Cb*) but lower than those of the continuous cultures exposed to predation (*Cp*).

Discussion

*Food requirements of Daphnia and Diaptomus—Competition theory (Tilman 1982)* predicts that given a constant environment and the absence of interference com-
Daphnia-Diaptomus competition

Fig. 9. Experiment C,. The abundance and stage structure of Daphnia pulicaria and Diaptomus oregonensis populations in mixed-species cultures in the absence of predation. Top panel: solid lines show means ±1 SE in the three cultures that were dominated by D. pulicaria, dotted and dashed lines show the number of D. pulicaria and D. oregonensis respectively in the culture whose population trajectories differed from its replicates. Middle and bottom panels show stage structure of the D. pulicaria and D. oregonensis populations in the cultures dominated by D. pulicaria.

petition, the species with the lowest requirement for a limiting resource will win in competition with species that have higher resource requirements. Under the relatively constant conditions of experiment C,. D. oregonensis out-competed D. pulicaria in three of four mixed-species cultures and successfully invaded established D. pulicaria cultures in four of four opportunities. If we assume that D. pulicaria does not suffer from interference competition due to D. oregonensis and that the conditions of experiment C,. were effectively constant, the dominance of D. oregonensis in this experiment suggests that D. oregonensis has a lower threshold food concentration than D. pulicaria under these experimental conditions. We suspect that this is indeed the case, even though the repeated-measures ANOVA did not detect a significant difference between the thresholds of the two species. If this interpretation is correct, then these results are nominally consistent with McNaught’s (1975) hypothesis that diaptomids require less food than cladocerans. We cannot conclusively determine whether D. oregonensis has a lower food threshold under the conditions of our experiments, but it is clear that both species have extremely low thresholds and that those thresholds differ little, if at all.

The stage and size structures of the populations of both species were a simple function of food concentration—as
Fig. 11. Experiments $S_a$ and $S_b$. Total numbers of Daphnia pulicaria and Diaptomus oregonensis. Symbols are means ± 1 SE for the mixed-species cultures.

one would expect, given the simple environment of our cultures. When simulated nonselective predation increased food concentrations by reducing zooplankton population sizes in experiment $C_b$, reproduction rates increased and population stage and size structures shifted to younger, smaller individuals. Thus, in this situation where food concentration was closely tied to predation rate, even nonselective predation caused a shift in size and stage distributions to smaller, younger individuals.

**Competition between Daphnia and Diaptomus**—Given their similar food thresholds, it is not surprising that changes in culture conditions caused reversals in the outcome of competition between *D. oregonensis* and *D. pulicaria*. *D. oregonensis* was dominant in experiment $C_a$, but *D. pulicaria* was dominant in the three earlier experiments. An explanation for the reversal in dominance across the experiments might provide clues to the factors that regulate the abundance of daphnids and diaptomids in the field. However, because we compare the results of four separate experiments that represented a series of methodological refinements, we are limited to post hoc analysis of the differences in their results.

Across the four experiments, the relative competitive ability of *D. pulicaria* was correlated with the magnitude of temporal variation in resource supply rate (Table 3). *D. pulicaria* outcompeted *D. oregonensis* after only a few weeks in cultures that were characterized by pronounced temporal variation in resource supply rate ($S_a$ and $S_b$); more time was required for *D. pulicaria* to become dominant in experiment $C_b$, which was characterized by modest temporal variation in resource supply rate; and finally, *D. oregonensis* was dominant in experiment $C_a$—the experiment with the least temporal variation in resource supply rate. The correlation between the reproductive activity of *D. pulicaria* (but not *D. oregonensis*) and the previous week’s resource supply rate in experiment $C_b$ suggests a mechanism that could explain this dominance pattern: *D. pulicaria* used the food pulses to produce eggs, but *D. oregonensis* was not able to do so.

Other possible explanations for the difference in dominance across experiments include changes in the abundance of bacteria (an alternate food for *Daphnia*), changes in the magnitude of interference competition, and effects of spatial patchiness in food distributions. High concentrations of bacteria could give *D. pulicaria* an advantage, but bacteria densities were in the range typical of oligotrophic lakes (Scavia and Laird 1987; Pedrós-Alió 1989) and lower than those that have been shown to augment *Daphnia* performance in life-table experiments (Pace et al. 1983). Wong et al. (1986) observed that *Daphnia pulex*
interferes with *Diaptomus minutus*, causing it to “jump” more often, thereby presumably imposing an energetic cost. If interference was important in our experiments, the competitive ability of *D. pulicaria* across experiments should have been a direct function of *D. pulicaria* density (because higher densities would have caused more interference). However, the dominance pattern across experiments is not consistent with a simple effect of interference competition. *D. pulicaria* densities were higher in the continuous culture experiment in which it was dominant (*C*,) than in the experiment dominated by *D. oregonensis* (*C*), but *D. pulicaria* was also dominant in experiment *S* as, even though *D. pulicaria* densities in that experiment were low and similar to *D. pulicaria* densities in the single-species cultures of experiment *C*, the experiment in which *D. oregonensis* was dominant.

Finally, spatial patchiness of phytoplankton was pronounced in experiments *S* and *S*, and may have contributed to dominance by *D. pulicaria*, but stirring prevented patchiness in experiment *C* and *D. pulicaria* was dominant in that experiment as well. In addition, stirring had no effect on the dynamics of either zooplankton species in a 5-week experiment in which 100 cells ml⁻¹ of *Cryptomonas* were added daily to stirred (10 s every 10 min) and unstirred semicontinuous cultures (H. E. Zagarase unpubl. data).

It may be that several variables contributed to the pattern of dominance across experiments. In our opinion, however, the most parsimonious explanation for that pattern, and for the differences in time required for *D. pulicaria* to become dominant in experiments *C*, *S*, and *S*, is that temporal variation in resource concentration gave *D. pulicaria* an advantage over *D. oregonensis*. This hypothesis is consistent with the results of earlier studies. Lampert and Muck (1985) found that in an environment of low food concentration, increases in food concentration led to greater increases in the mass-specific ingestion rates of *Daphnia* spp. than of *E. gracilis*. Thus, they predicted that under severe food limitation, egg production of *Daphnia* spp., but not *E. gracilis*, would increase in response to food pulses. MacIsaac and Gilbert (1991) observed that *Daphnia ambigua* excluded *Keratella cochlearis* from mixed-species cultures more rapidly as the period between food additions increased. In addition, recent reviews suggest that the competitive abilities of rotifers, cladocerans (DeMott 1989; Rothhaupt 1990), and phytoplankton (Sommer 1989) are generally sensitive to temporal variation in resource abundance.

The hypothesis that temporal variation in food abundance favored *D. pulicaria* over *D. oregonensis* appears inconsistent with one aspect of Muck and Lampert’s (1984) and Lampert and Muck’s (1985) conclusions based on their studies of weight loss of individual adult *Eudiaptomus* and *Daphnia* at low food concentrations. They concluded that “fluctuating food conditions at low levels favour *Eudiaptomus* because *Daphnia* loses weight and reduces its filtering rate during short periods of starvation. The copepod, however, is not affected” (Muck and Lampert 1984, p. 157). However, their assumption that rates of weight loss of adult individuals are predictive of population performance is subject to three important criticisms.

First, *Daphnia* and *Diaptomus* are not closely related, so the consequences of incremental weight loss may differ for individuals of the two genera; if nothing else, *Daphnia* has more weight to lose. Second, *Daphnia* not only lost weight faster when food concentrations were very low, it also had higher mass-specific production rates when food concentrations were higher (Lampert and Muck 1985); the net effect of periods of high and low food should be

![Fig. 13. Experiments S and S. Cryptomonas reflexa abundance. Symbols are means ±1 SE for the cultures with both zooplankton species. In experiment S, the food was returned to 350 cells ml⁻¹ every other day. The actual trajectory is shown for 1 week in the middle of the experiment, but the connections between points are omitted for the rest of the experiment to reduce clutter. The panel for experiment S shows the actual trajectory with the cell density returned to 500 cells ml⁻¹ once per week, when the zooplankton were counted and the cultures cleaned.](image-url)
more useful than the effect of either period alone in attempts to project the population-level effects of fluctuating food regimes. Third, as Lampert and Muck (1985) note, adults may not represent the bottleneck to population growth; earlier life stages may require higher concentrations of food. Experimental studies of populations, rather than of adult individuals, obviate the need to project population dynamics from the behavior or physiology of individuals of a particular life stage. Thus, population level experiments are likely to be more informative than studies of individual behavior or physiology when the objective is to predict population performance under natural conditions.

Whatever the explanation for the difference in outcome of the various experiments, it is clear that subtleties of culture conditions may be critical to competitive ability. One consequence is that continuous cultures are preferable to semicontinuous cultures because pronounced temporal variation in ambient resource concentration and resource supply rate are inherent features of semicontinuous cultures, but these variables may be manipulated as desired (e.g. to reflect field conditions) in continuous cultures.

Field distributions of Daphnia and Diaptomus—Contrary to McNaught's (1975) hypothesis, several lines of evidence suggest that any field pattern in the relative abundance of Daphnia and Diaptomus is unlikely to be a simple function of food quantity. First, both our data and those of Lampert and Muck (1983) show that species of these genera have similarly low food thresholds. Second, both genera are widely distributed across lakes that span a broad range in productivity. Third, relatively modest changes in culture (environmental) conditions caused a reversal in dominance in our experiments. Fourth, differences in life histories, foraging selectivity, and vulnerability to predation provide a variety of opportunities for other sources of environmental variation to differentially affect Daphnia and Diaptomus under natural conditions.

### Table 3. Summary of some results. Final zooplankton densities (No. liter⁻¹) are approximations based on plateaus apparent in the figures (when populations appear to have stabilized during the experiment) or on densities at the end of the experiment (when population sizes were changing at the end of the experiment). The latter are denoted by < or >. Threshold Cryptomonas densities (No. ml⁻¹) are means calculated for the entire data set for experiment Cₚ. and the period preceding the transfer of Diaptomus into the Daphnia treatments in experiment Cₚ. Bacteria densities (×10⁶ ml⁻¹) are means based on all available data except for experiment Cₚ. for which means are presented for counts from the period preceding the transfer of Diaptomus into the Daphnia cultures. Dash—No corresponding treatment; NA—not applicable; ND—no data.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Dominant sp.*</th>
<th>Single species (final)</th>
<th>Mixed species (final)</th>
<th>Threshold Crypt</th>
<th>Bacteria</th>
<th>Variance†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Daph Diap</td>
<td>Daph Diap</td>
<td>Daph Diap Both</td>
<td>Daph Diap Both</td>
<td></td>
</tr>
<tr>
<td>Cₚ</td>
<td>Daphnia (~70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No predation</td>
<td>50 20 - -</td>
<td>50 25 &lt;10 &lt;10</td>
<td>66 50 2.4 3.0</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With predation</td>
<td>- - 100 25 &gt;60 &gt;30 &lt;20&lt;5</td>
<td>143 106 - 14 11</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sₜ</td>
<td>Daphnia (~30)</td>
<td>70 30 110 &gt;50</td>
<td>&lt;1 1</td>
<td>NA NA NA 7.6 106.9 6.9</td>
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<td></td>
</tr>
<tr>
<td>Sₚ</td>
<td>Daphnia (~20)</td>
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<td>&lt;2 &lt;1</td>
<td>- - NA NA ND 7.6 10 6.9</td>
<td>Highest</td>
<td></td>
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* Days to become dominant in parentheses. † Magnitude of variation in resource supply rate.

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