The impact of density-independent mortality on species coexistence: an experimental test with zooplankton

Gary G. Mittelbach, Tara L. Darcy-Hall, Nathan J. Dorn, Erica A. Garcia, Christopher F. Steiner and Jeremy M. Wojdak

Mortality (e.g. predation, disturbance) is often thought to lower the intensity of interspecific competition and thereby promote the coexistence of competing species. However, surprisingly few tests of this idea exist, especially for metazoans feeding on a self-renewing resource. Here we examined the effect of density-independent mortality on the coexistence of four species of pond zooplankton (microcrustacean grazers) in a series of laboratory microcosms. Across the experimental mortality gradient, consumer biomass decreased and resource abundance increased with an increase in mortality. Thus, the treatments resulted in an increase in resource availability per consumer (one measure of reduced competitive intensity). There was no significant effect of mortality treatment on species relative abundances or species evenness, and the zooplankter *Diaphanosoma* dominated community biomass at all mortality levels. Mortality rate did have a marginally significant effect on species richness (p < 0.07), but richness did not increase monotonically with mortality level. Instead, richness tended to be highest in the low- and no-mortality treatments.


How does mortality affect the coexistence of competing species? As Chase et al. (2002) point out in a recent review, there is a mismatch between theory and popular perception on this issue. A commonly held view is that factors that increase overall consumer mortality (e.g. predation, disturbance, environmental harshness) and thereby reduce consumer densities, should lower the intensity of interspecific competition and promote the coexistence of species. These ideas are contained, for example, in a recent meta-analysis of the impacts of predation on experimental studies of competition (Gurevitch et al. 2000). However, as Chase et al. (2002) note, the impact of increased consumer mortality on the outcome of interspecific competition depends strongly on the type of mortality imposed and on the criteria used to measure the intensity of competition (Chesson and Huntly 1997, Abrams 2001). If competition intensity is measured by the short-term response of consumers to resources (Gurevitch et al. 2000), then any factor that increases consumer mortality rate and thereby lowers consumer densities is likely to reduce the intensity of competition as measured by resources available per consumer. However, theory is often directed at understanding the long-term response of consumer populations to variation in mortality rates; e.g. how does predation or disturbance affect the coexistence of...
species? In this case, the impact of varying consumer mortality rates on species coexistence is more complex – an increase in consumer mortality not only increases resources per individual, but it also decreases an individual’s chance of survival (i.e., mortality’s positive effect of lowered competition is countered by its negative effect on population growth).

Chesson and Huntly (1997), Abrams (2001) and Chase et al. (2002) provide excellent summaries of the theoretical impacts of predation (or other sources of mortality) on the coexistence of competing species. They show that species coexistence is enhanced under any of the following conditions: 1) mortality falls selectively on the competitive dominant (Paine 1966, Lubchenco 1978), 2) mortality falls selectively on the more abundant species (i.e., is frequency-dependent, Roughgarden and Feldman 1975, Vance 1978), 3) mortality opens up “patches” and species exhibit a tradeoff between colonizing ability and competitive ability (Connell 1971, 1978, Slatkin 1974, Caswell 1978, Hastings 1980), and 4) mortality impacts species differentially through niche-partitioning and non-linear dynamics (Chesson and Huntly 1997). However, when mortality is non-selective and density-independent, most theory predicts that varying mortality levels will not facilitate the coexistence of consumers (Cramer and May 1971, Abrams 1977, Yodzis 1977, 1986, Holt 1985). This prediction runs counter to the conventional wisdom that non-selective mortality (e.g., disturbance, environmental harshness) makes competition weak and unimportant (Chesson and Huntly 1997).

Given the long history of theoretical interest in this topic, it is surprising that few empirical studies have examined the impact of non-selective mortality on species coexistence. Robinson and Sandgren (1983), Gaedeke and Sommer (1986) and Sommer (1995) examined species coexistence in algal communities grown under semicontinuous culture and subject to periodic mortality (disturbance). Mortality was administered by removing a set volume of media (and algae) at periodic intervals, followed by immediate replacement with fresh media. These studies simultaneously varied the intensity and frequency of mortality, and Sommer (1995) also included a treatment where the intensity of mortality was held constant but the frequency was varied. To our knowledge, no experiments have examined the effects of varying only the intensity of density-independent mortality, and no experiments have studied these effects in an assemblage of metazoa feeding on a self-renewing resource (we discuss the impact of generalized predators in metazoan communities later).

We tested the effects of density-independent mortality on the coexistence of four species of pond zooplankton (microcrustaceans) in a series of replicated, laboratory microcosms. The experiment ran for 104 days (52 days pre-treatment and 52-days post-treatment), encompassing numerous zooplankton and algal generations. We found that zooplankton community biomass decreased and algal abundance increased with an increase in density-independent mortality. Thus, imposing mortality on the consumers had the expected effect of increasing resource availability per individual consumer. Effects on zooplankton species richness, however, were marginal and richness tended to be highest at the two lowest mortality levels. We discuss these results in light of the predicted effects of density-independent mortality on species coexistence.

**Methods**

**Set-up**

The experiment was conducted indoors in 20-l polyethylene containers. There were four mortality treatments, each replicated six times, plus four replicates of a phytoplankton only treatment, yielding 23 total experimental units. Containers were filled on 26 June, 2001 with 16.2 l of well water and covered with nylon window screen. Each container was illuminated with two 55-watt fluorescent lights mounted directly above and received continuous 24-h light. Water temperatures averaged 24.4 ± 0.7°C over the course of the experiment (±1 SE; 5 sample dates). Because nutrient concentrations in the well water were quite low (especially phosphorous; TP = 5.8 μg l⁻¹, TN = 140 μg l⁻¹ initially), we added a solution of NH₄NO₃ and KH₂PO₄ (in 12:1 molar N:P ratio) at the start of the experiment to raise nutrient concentrations to the mesotrophic range (average nutrient concentrations measured on 2-July (1 week after addition) were: TP = 23.6 ± 1.0, TN = 181.2 ± 7.5. ±1 SE, n = 10 randomly selected containers). At the time of nutrient addition, each container was inoculated with 15 ml of a concentrated laboratory culture of *Monoraphidium* (unknown sp.), a unicellular, highly-edible green algae (Steiner 2001). Algal populations grew quickly and concentrations on 2 July (measured as chlorophyll a) averaged 54.3 ± 6.5 μg l⁻¹ (±1 SE, n = 6).

**Zooplankton species**

We used four common species of pond zooplankton (Cladocera), *Daphnia pulex*, *Ceriodaphnia quadrangula*, *Diaphanosoma brachyurum*, and *Chydorus sphaericus*, as our experimental community (we refer to species by their generic names hereafter). Zooplankton were collected in May 2001 from ponds at the Kellogg Biological Station experimental pond facility and cultured in the laboratory as single-species stocks raised on laboratory-cultured *Monoraphidium* for approximately 1 month prior to the start of the experi-
ment. On 3 July, twenty haphazardly selected individuals of each species were stocked into each container (1.23 individuals per species 1\(^{-1}\)). Because of differences in body size, equal species densities resulted in differences in total biomass stocked for each species (initial biomass (\(\mu g\) dry mass 1\(^{-1}\)) based on average zooplankton mass: \(Daphnia = 35.4, Ceriodaphnia = 2.7, Diaphanosoma = 2.2, Chydrive = 1.3\)).

### Sampling

Zooplankton were sampled by gently mixing each container and then withdrawing 1 l of water with a plastic dipper from the container’s center. The water was poured through a 80-µm mesh to remove zooplankton and then the filtered water was returned to the source container. On the final sampling date, we took an additional large (4 l) zooplankton sample from each container to better sample rare species. Care was taken to thoroughly rinse sampling dippers and sieves with fresh water between samples, and separate sets of dippers and sieves were maintained for each treatment. Zooplankton were immediately preserved in acid Lugol’s solution and then counted in their entirety under a dissecting microscope. Zooplankton species biomasses were determined by first estimating the mean body length for each zooplankton species – we measured up to 15 haphazardly chosen individuals per species from each experimental container on two sample dates (28 August and 15 October; located near the beginning and end of the treatment period). Average body length for each species did not change significantly with time or vary significantly among treatments. Therefore, we calculated the total biomass for each species by multiplying the number of individuals present in a container times the average biomass per individual (calculated using mean body length and published length–weight regressions for each species; Burns 1969, Dumont et al. 1975).

Phytoplankton were sampled by withdrawing 500 ml of water with a plastic dipper from each container. Algae were sampled before mixing the containers so as not to include algal contributions from the sediments or wall growth. Approximately half of each sample was filtered onto a Gelman A/E glass fiber filter for subsequent analysis of chlorophyll a (sensu Welchmeyer 1994) as a correlate of total algal biomass. Although we inoculated the experiment with a single species of small, edible algae, other algal species could potentially invade our non-sterile system over time. Therefore, we filtered the other half of each water sample through 35-µm Nitex to remove large, potentially inedible algae. We refer to the < 35-µm chlorophyll a sample as “edible algae”. We recognize that zooplankton species differ in the maximum-sized algae they can consume (Burns 1968, Neill 1975) and that factors other than size may affect algal edibility (Sterner 1989), however, the < 35-µm fraction represents a commonly used measure of “edible algae” (Cottingham 1999). Chlorophyll a was extracted over-night in cold, 95% ethanol and measured using narrow band fluorometry (Welchmeyer 1994).

### Initial zooplankton dynamics and treatment imposition

Our goal was to impose the four mortality treatments after zooplankton community biomass had reached approximate steady state levels. Based on Steiner’s (2001) experiments, where he raised \(Daphnia pulex\) and \(Ceriodaphnia quadrangula\) under conditions similar to ours (but using somewhat higher and lower nutrient levels), we expected zooplankton biomass to stabilize after about 30 days and in roughly the range of 0.08 – 0.30 mg dry mass 1\(^{-1}\). Based on these expectations and the observed initial zooplankton dynamics (Fig. 1), we began the mortality treatments on day 52 of the experiment (24 August) and applied mortality every four days for the remainder of the experiment (an additional 52 days). Twenty individuals of each zooplankton species were added to each container on 27 August to allow each species the opportunity to invade the community under the new mortality conditions. In one container, all species failed to establish after invasion and this container was eliminated from the analysis.

Density-independent mortality was applied to the zooplankton community by gently mixing each container and then removing a fixed volume of water (set by the mortality rate) with a 2-liter dipper. The water was poured through a 80-µm mesh (to remove all zooplankton) and was then returned to the source container. We filtered 0, 26, 45, or 59% of the water from each container every four days to impose estimated daily per capita mortality rates of 0, 0.075, 0.150, and 0.225 d\(^{-1}\). These mortality rates were in addition to the mortality imposed on all containers by zooplankton sampling (1 l, ~6% of total volume was removed every eight days). In order to equalize mixing among treatments, we removed the same total volume of water from each container, but only filtered zooplankton from the volume of water needed to apply mortality. Sampling for zooplankton and phytoplankton resulted in the containers being mixed once every four days. All water was returned to the container from which it was taken. Water levels were maintained at 16 l in the containers by periodically adding a small amount of well water. Zooplankton were heat-killed and then returned to their appropriate containers, so as to not deplete nutrients through time.
Statistical analyses

Density-independent mortality effects were investigated using repeated measures ANOVA (GLM procedure in Systat v.8) on post-treatment total zooplankton biomass, chlorophyll a (total and < 35 μm fractions), species richness and species evenness. Evenness was estimated using Hurlbert’s (1971) metric, which measures the probability that two specimens picked at random from a sample will be different species (Olszewski 2004). Zooplankton biomasses and chlorophyll a concentrations were log10 transformed to achieve homogeneity of variances. We report Greenhouse-Geiser adjusted p-values for time and time × treatment effects to account for potential violations of repeated measures assumptions of sphericity. To test for changes in species relative abundances through time, we used a doubly-multivariate repeated measures MANOVA (SAS v.8) to analyze mortality and time effects (and their interaction) on the log10 transformed biomasses of the four zooplankton species (each species’ biomass was treated as a response variable).

Results

There was a rapid increase in total zooplankton biomass following inoculation and a corresponding rapid decline in algal abundance – zooplankton biomass increased 35-fold in the first 2 weeks (Fig. 1) and edible chlorophyll a declined 130-fold over the same time period (54.3 μg l⁻¹ to 0.41 μg l⁻¹). Zooplankton populations appeared to overshoot the system’s carrying capacity (Fig. 1) and then declined to a lower biomass. Mortality treatments were initiated on day 52 and the intensity of mortality had a significant effect on zooplankton biomass (rmANOVA, treatment effect, F1,21 = 7.51, p = 0.01; time effect, F6,126 = 0.44, p = 0.69; time × treatment interaction, F6,126 = 3.07, p = 0.03). The significant time × treatment interaction was due to the rapid divergence in treatments following the imposition of mortality (Fig. 1). If we consider only the final six sampling dates (after the system had stabilized), rmANOVA shows no significant interaction of treatment with time (p = 0.30), but a highly significant treatment effect (p = 0.01). A post-hoc comparison of means for this period shows that zooplankton biomass in the low- and no-mortality treatments was significantly higher than in the medium- and high-mortality treatments (Tukey HSD multiple comparisons; < p = 0.05).

Varying density-independent mortality on the zooplankton grazers had a significant cascading effect on algal resources as measured by edible chlorophyll a (rmANOVA, treatment effect, F1,21 = 6.37, p = 0.02; time effect, F4,84 = 1.128, p = 0.35; time × treatment interaction, F4,84 = 0.518, p = 0.72). Both edible and total algal abundance increased with an increase in zooplankton mortality rate (Table 1). Also, there was a significant, negative correlation between average zooplankton biomass and increased algal abundance. Edible algae represented 65–80% of the total algae present in the containers (Table 1), which suggests that algal species other than our cultured Monoraphidium invaded the microcosms. However, there were no differences between treatments in the proportion of the algal assemblage that was edible (i.e. < 35-μm) (rmANOVA, treatment effect, F1,21 = 1.06, p = 0.31; time effect, F4,84 = 2.19, p = 0.10; time × treatment interaction, F4,84 = 1.68, p = 0.18).

Diaphanosoma dominated the zooplankton community at all mortality levels, averaging 53–73% of total zooplankton biomass in all treatments from day 64 to day 104. There was no significant difference among

![Fig. 1. Total zooplankton biomass (mg l⁻¹) in each treatment through time (mean ± 1 SE). Vertical arrow indicates when mortality treatments were initiated. “Mean” values are the mean of means for each treatment over the final six sampling dates (± 1 SE).](image)

<table>
<thead>
<tr>
<th>Mortality level</th>
<th>Edible algae (μg l⁻¹)</th>
<th>Total algae (μg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.31 ±0.06</td>
<td>0.51 ±0.06</td>
</tr>
<tr>
<td>Low</td>
<td>0.39 ±0.05</td>
<td>0.61 ±0.09</td>
</tr>
<tr>
<td>Medium</td>
<td>0.78 ±0.20</td>
<td>0.99 ±0.24</td>
</tr>
<tr>
<td>High</td>
<td>0.63 ±0.17</td>
<td>0.91 ±0.31</td>
</tr>
<tr>
<td>Phytoplankton only</td>
<td>0.98 ±0.42</td>
<td>1.03 ±0.43</td>
</tr>
</tbody>
</table>
mortality treatments in the relative abundance (biomass) of the four zooplankton species (MANOVA, treatment effect, $F_{12,54} = 1.60, p = 0.12$; time effect, $F_{2,24} = 2.24, p = 0.10$; time × treatment interaction, $F_{24,42} = 0.07, p = 0.81$). Species evenness also was not affected by mortality rate (Fig. 2b; rmANOVA, treatment effect, $F_{1,11} = 0.67, p = 0.43$; time effect, $F_{6,66} = 0.90, p = 0.46$; time × treatment interaction, $F_{6,66} = 0.28, p = 0.85$). Thus, changes in density-independent mortality rates did not shift community dominance amongst the species. Mortality rate, however, did have a marginally significant effect on the number of coexisting zooplankton species (Fig. 2a, rmANOVA, treatment effect, $F_{1,21} = 3.695, p = 0.068$; time effect, $F_{6,126} = 1.48, p = 0.23$; time × treatment interaction, $F_{6,126} = 1.52, p = 0.22$). Species richness tended to be higher in the low- and no-mortality treatments, as shown by mean species richness averaged over the final six sampling dates (Fig. 2a). However, differences amongst these treatment means are not significant based on post-hoc comparisons (Tukey HSD multiple comparisons; $p > 0.05$).

On the final sampling date, we took an additional, large (4l) sample from each container to better estimate species richness; earlier samples were kept relatively small (1l or ~6% of volume) so as to limit the amount of “background” mortality caused by sampling. There was no significant difference in average species richness measured in the large and small samples on the final date (paired t-test; $t = -1.545; p = 0.14$), which suggests that our routine sampling was adequate. An ANOVA based on the large sample showed no significant effect of mortality on species richness ($F_{1,21} = 0.80; p = 0.38$).

**Discussion**

A number of theories predict that a simple change in density-independent mortality rate will not facilitate the coexistence of consumers (Cramer and May 1971, Abrams 1977, Yodzis 1977, 1986, Holt 1985). However, despite more than 30 years of theoretical work on this topic, there are surprisingly few empirical tests of this prediction. Abrams (2001) cites two experiments that examined the impact of generalist (non-selective) predators on prey species richness; Addicott (1974) and Risch and Carroll (1982). Addicott’s (1974) classic study examined the impact of mosquito larval predation on the protozoan communities of pitcher plants. When he manipulated the densities of a (presumably) non-selective predator, larvae of the mosquito *Wyeomyia smithii*, Addicott (1974) found that the total density of protozoans within a pitcher plant decreased, as did protozoan species richness (protozoan species richness declined monotonically with increased mosquito density). No information was provided on resource abundance in the pitcher plants or on the death rates imposed by the predator. Risch and Carroll (1982) studied the effects of fire ants, *Soenopsis geminata*, on prey (arthropod) species richness and found that prey abundance decreased and prey species richness decreased in the presence of ants compared to ant removal plots. Again, no measures of arthropod resources, ant predation rates, or ant feeding preferences were available. In our study, we simulated the effects of a generalist predator by inflicting different rates of non-selective mortality. We found that increasing mortality decreased consumer biomass and increased resource abundance. Thus, individual consumers experienced higher per capita resource levels at higher mortality rates. Like Addicott (1974) and Risch and Carroll (1982), we found that species richness tended to be lower at higher mortality rates, however,
the overall effect of mortality on species richness was only marginally significant.

How do the mortality rates in our experiment compare to those observed for zooplankton in nature? There are few estimates of zooplankton mortality rates from ponds, but Dudycha (2001) reports *Daphnia* death rates in Michigan ponds to be quite high (0.50–1.50 \( \text{d}^{-1} \)). In natural lakes, zooplankton instantaneous death rates range from 0.05–0.50 \( \text{d}^{-1} \) (Hall 1964, Prepas and Rigler 1978, Taylor 1988, Tessier et al. 1992, Leibold and Tessier 1998, Dudycha 2001) and values between 0.10 and 0.20 \( \text{d}^{-1} \) are common summertime averages. Thus, our experimental removals (calculated to produce instantaneous death rates of 0, 0.075, 0.150, and 0.225 \( \text{d}^{-1} \)) cover the lower end of observed natural mortality rates. However, we caution that our experiment was not designed to test whether varying density-independent mortality rates affect zooplankton coexistence in nature. In most natural systems, zooplankton species face selective mortality based upon their size, habitat use, or behavior (Brooks and Dodson 1965, O’Brien et al. 1979, Mittelbach 1981). Therefore, we expect that many of the mechanisms outlined in the Introduction, whereby selective mortality may affect species coexistence, will operate to influence zooplankton diversity in natural systems. Here, we address the simpler, but relatively unexplored question—does an increase in density-independent mortality lead to a lessening of competition (as measured by per-capita resource availability) and does this lead to increased species richness?

Our results support the conclusions of Chase et al. (2002) that the effects of density-independent mortality on per-capita resource availability do not, by themselves, lead to increased species richness. Species richness in our experiment did not increase directly with mortality rate. Instead, we found that species richness tended to be highest in the low- and no-mortality treatments. Our study was designed to look at the effects of density independent mortality in a simple, homogeneous environment where most theory predicts that density independent mortality will not promote species coexistence. In more complex environments (e.g., with multiple food resources), Abrams (2001) has shown that density-independent mortality may promote species coexistence by reducing apparent competition and increasing resource heterogeneity. Chesson (1994) also found that species may coexist under some mortality levels but not others when mortality modulates the expression of niche differentiation amongst the species. Future empirical studies are needed to test the effects of density-independent mortality in these and other more complex models.

Acknowledgements — We thank Steve Hamilton, Mathew Leibold, Alan Tessier, and Pam Woodruff for generously sharing equipment and advice, and we thank Spencer Hall for comments on the manuscript. This work was supported by NSF grants DBI-9602252 and DBI-9907740 and is KBS contribution #980.

References


