

## TYPE III FUNCTIONAL RESPONSE IN *DAPHNIA*

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**Abstract.** The functional response of *Daphnia*, a common pelagic herbivore in lakes, was assessed with a combination of secondary and meta-analyses of published data and new data from an experiment conducted using very low food levels. Secondary analyses of literature data (28 studies,  $n = 239$ – $393$ ) revealed a significant positive influence of food concentration on *Daphnia* clearance rate at low food levels, i.e., evidence of an overall Type III functional response. This result was not an artifact of including data from *Daphnia* that were exhausted from prolonged food deprivation (more than three hours at very low food). Meta-analysis of *Daphnia* clearance rate vs. food concentration across a range of low food concentrations (eight studies) showed a significantly positive slope across studies, which also supports the presence of a Type III response. Congruent with these analyses of published data, the feeding experiment showed clear evidence of a Type III functional response for *D. pulicaria* feeding on *Ankistrodesmus falcatus*. Food levels at which *Daphnia* clearance rate declined with decreasing food were near the minimum resource requirement for *Daphnia* population maintenance at steady state ( $R^*$ ). We suggest that Type III responses are more common than previously believed, perhaps because of the relative paucity of observations at low food levels, and that reduced prey mortality at low phytoplankton densities could be a stabilizing mechanism for *Daphnia*–phytoplankton systems under resource scarcity.

**Key words:** *Daphnia* feeding; functional response; phytoplankton; population dynamics; population stability; predator–prey.

### INTRODUCTION

The functional response of predators (consumption as a function of prey density) is a fundamental process underlying food-web interactions, and consequently, much of population and community ecology. More specifically, functional response is a major determinant of the strength of predator–prey interactions (Laska and Wootton 1998, Osenberg et al. 1999) and a potentially important factor affecting the stability of predator–prey dynamics (Begon et al. 1998, Murdoch et al. 2003). Given this theoretical and practical importance, many studies have quantified the functional response of a wide variety of taxa (Jeschke et al. 2004), and the effects of different functional response types on population dynamics have been thoroughly examined in dynamic predator–prey models (Murdoch and Stewart-Oaten 1975, Oaten and Murdoch 1975, Hassell and Comins 1978). The Type III functional response has attracted particular theoretical attention because it can potentially stabilize fluctuations of prey and predator populations, and so reduce prey extinction risk (Holling 1959, Murdoch and Stewart-Oaten 1975). The stabilizing effect of the Type III response results from density-dependent prey mortality. Over some range of low prey

densities, the per capita mortality inflicted by individual predators declines with decreasing prey density (Fig. 1). A recent review of functional response studies, however, reiterated the prevailing view that Type III responses are rare (Jeschke et al. 2004, but see Hassell et al. 1977). Thus, it is not surprising that interest in the processes that stabilize predator–prey dynamics and reduce extinction risk has largely shifted to other mechanisms (Murdoch et al. 2003).

The freshwater filter-feeder, *Daphnia*, holds a special position in the study of both the nature and significance of functional responses. More studies have been devoted to the characterization of *Daphnia*'s functional response than any other animal (Jeschke et al. 2004), and the question of whether *Daphnia* reduces its filtering effort at low prey densities has been the subject of careful investigation (Muck and Lampert 1980, Plath 1998). In addition, there has been long-standing interest in the *Daphnia*–phytoplankton interaction as a model system for understanding predator–prey dynamics and stability (Murdoch and McCauley 1985, McCauley and Murdoch 1987, Gurney et al. 1990, McCauley et al. 1990b, Murdoch et al. 2003). Specifically, it remains unresolved why the amplitudes of *Daphnia*–phytoplankton cycles in nature are considerably smaller than predicted by theory (Murdoch et al. 1998, 2003). Attempts to resolve this paradox have reasonably, but almost invariably, assumed that *Daphnia*'s functional response is Type II (McCauley et al. 1990b, Nisbet et al. 1991, Murdoch et al. 1998, 2003), which is inherently destabilizing because

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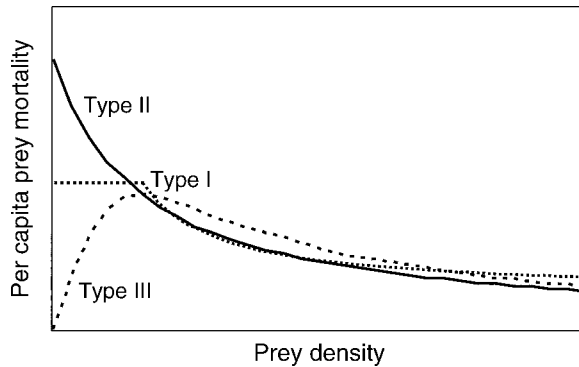


FIG. 1. The relationship between per capita prey mortality and prey density for Type I, II, and III functional responses.

of inversely density-dependent prey mortality (Fig. 1). If *Daphnia*'s functional response is actually Type III, this relationship might contribute to a resolution of this long-standing paradox.

The widely held view that *Daphnia*'s functional response is Type I or II (Lampert 1987) follows, in part, from the conclusions of two widely cited studies of *Daphnia*'s feeding behavior (DeMott 1982, Porter et al. 1982). Further, the most ambitious attempt to test for a Type III functional response in *Daphnia* concluded that any reduction in *Daphnia* filtering effort at low food was an artifact of exhaustion by starved animals (Muck and Lampert 1980). In both widely cited studies, however, *Daphnia* clearance rates were lower at the lowest food concentration than at the next higher food concentration (see DeMott 1982: Fig. 2, Porter et al. 1982: Fig 1), but these differences were not statistically significant. It is important to note that 80% of the data in these two studies were from food concentrations that were far above the minimum resource requirement of *Daphnia* populations at steady state ( $R^*$  [Tilman 1982]). A relative paucity of data at low food concentrations is a major limitation of the existing literature with respect to the detection of Type III functional responses (see *Results: Analyses of literature data*).

Our interest in reassessing the question of whether *Daphnia* has a Type III functional response was stimulated by two relatively recent observations. The first was a carefully designed laboratory experiment demonstrating that *Daphnia* individuals cultured at low food increased the frequency of movement of their filtering appendages when exposed to elevated food (Plath 1998). Plath (1998) was able to rule out starvation-induced exhaustion (Muck and Lampert 1980) as the explanation for reduced appendage movements at low food (Plath 1998). The second observation was of a markedly nonlinear response of phytoplankton biomass to an experimental gradient of *Daphnia* biomass in a field experiment (Sarnelle 2003). In this case, the strength of *Daphnia*'s grazing impact weakened as phytoplankton biomass decreased, and a Type III functional response was suggested as a possible

mechanism underlying this result (Sarnelle 2003). These observations, along with the relative paucity of data on *Daphnia* feeding at low food concentrations in the literature, led us to hypothesize that existing individual studies of *Daphnia* functional response often may lack sufficient power to detect a Type III response. In this paper, we reexamined evidence for the presence of a Type III functional response in *Daphnia* using secondary analysis and meta-analysis of a large data set extracted from published laboratory experiments, and with a new experiment focused on measuring *Daphnia* grazing across a range of low food concentrations.

Given that density-dependent prey mortality is the stabilizing mechanism associated with Type III functional responses (Fig. 1), empirical assessments of functional response should include quantification of per capita prey mortality, not just predator behavior. Demonstrating reduced movements of filtering appendages at low prey density (Plath 1998), while important for assessments of *Daphnia*'s energetics and fitness, does not, by itself, demonstrate that the per capita mortality inflicted by *Daphnia* on phytoplankton is reduced at low prey density. For example, digestive efficiency may increase in response to lowered food concentrations, as a result of increased gut-passage time (Murtaugh 1985), which could offset the reduction in capture rate by increasing the mortality of phytoplankton passing through *Daphnia*'s gut. It is well known that a variable fraction of phytoplankton cells can remain alive after passage through *Daphnia*'s gut (Porter 1977). Consequently, we focus our analyses on the relationship between phytoplankton density and *Daphnia*'s clearance rate, a standard parameter in zooplankton feeding

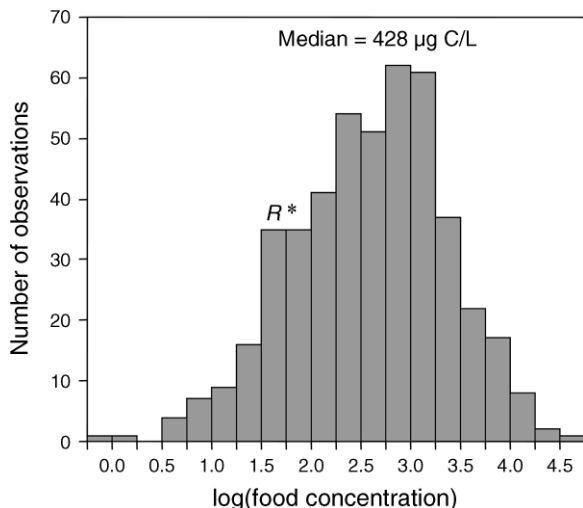


FIG. 2. Frequency distribution of food concentrations for laboratory experiments reporting mass-specific *Daphnia* clearance rates and with acclimation times  $<3$  h (see *Methods* for explanation). The original units of food concentration were  $\mu\text{g C/L}$ . *Daphnia*'s  $R^*$  (Lampert and Trubetskova 1996) is indicated.

studies that is directly proportional to per capita prey mortality (Båmstedt et al. 2000).

Another important issue in the assessment of Type III functional responses is the range of food concentrations over which clearance rate declines as food declines. Clearly, a predator population is not capable of depressing food resources far below its  $R^*$  over the long term, although short-term excursions below  $R^*$  could reasonably be expected. Thus, a reduction in prey mortality that occurs only at prey densities far below *Daphnia's*  $R^*$  would not likely function as a stabilizing mechanism in nature (since the predator would go extinct), and would be more indicative of starvation-induced exhaustion (Muck and Lampert 1980). Plath (1998) has already demonstrated that *Daphnia's* filtering effort declines at food levels near *Daphnia's*  $R^*$ . Our goal was to determine whether the same is true for phytoplankton mortality.

#### METHODS

##### *Analyses of literature data*

We searched the literature for laboratory studies containing data on *Daphnia* feeding or clearance rate and that included at least three food concentrations. We searched for papers by using the Institute for Scientific Information Web of Science and the Aquatic Sciences and Fisheries Abstracts, and the reference lists of two previous surveys of functional response and zooplankton feeding (Peters and Downing 1984, Jeschke et al. 2004). We also searched the reference list of every potentially suitable paper we found in the above searches. Data were excluded from individual experiments that employed heterotrophic bacteria (Urabe and Watanabe 1991), cyanobacteria (Holm et al. 1983), or other poor-quality particles (Sterner and Smith 1993) as the prey source, or only provided feeding or clearance rates for prey size classes (Berman and Richman 1974).

The response variable of interest was *Daphnia* clearance rate (milliliters of suspension cleared of food particles per unit time), because this parameter is proportional to per capita prey mortality, and because statistical analyses based on per capita rates are an effective means of distinguishing among functional responses (Kratz 1996, Juliano 2001, Fussmann et al. 2005). Two general approaches are used to quantify zooplankton clearance rates in the literature. Depletion methods involve measurements of food concentrations in experimental suspensions before and after *Daphnia* feeding (with controls). In depletion studies, clearance rate is estimated directly from control-corrected changes in food concentrations,  $(\ln[N_0] - \ln[N_1])/t$ , the number or mass of animals in the experimental container, and the volume of the suspension (Båmstedt et al. 2000). About 30% of the data we accumulated were from depletion-type studies. In uptake studies, feeding rate (particles eaten per unit time) is estimated directly via the accumulation of food over time by consumers (usually using radiolabeled food particles), and clear-

ance rate is calculated as feeding rate divided by food concentration (Båmstedt et al. 2000). We relied on clearance rates reported by the authors in the majority of cases. When not reported, we calculated clearance rates from reported feeding rates and food concentrations.

Some studies reported clearance rates per individual (milliliters per individual per day), some as mass-specific clearance rates (milliliters per microgram per day). We converted individual rates to mass-specific rates when possible, by either using a *Daphnia* length-mass relationship given in the paper, calculating a length-mass relationship from data given in the paper, or using length-mass relationships from other papers. From these latter studies, we took relationships for *D. galeata*, *D. rosea*, *D. hyalina*, and *D. longispina* (using the *D. galeata* equation from Lynch et al. 1986), *D. pulex* and *D. pulicaria* (using the *D. pulex* equation from Lynch et al. 1986), and *D. magna* (Porter et al. 1982). We created two partially overlapping data sets, a smaller set of individual clearance rates and a larger set of mass-specific clearance rates. We conducted secondary analyses on both sets to examine the robustness of the results (Englund et al. 1999), but emphasize results for mass-specific rates in the interest of brevity.

Food concentrations were reported in a wide variety of units in the original papers. We converted all reported concentrations to carbon units (micrograms C per liter) using conversion factors in the original papers when available. When no conversion factors were provided, we applied these factors: wet biomass to dry biomass = 0.31 (Peters and Downing 1984), dry biomass to carbon = 0.4 (Porter et al. 1982). To save time in collecting data, we ignored observations where food concentrations were >50 000  $\mu\text{g C/L}$ . Such food concentrations are rarely encountered in nature (Bell and Kalff 2001, Hessen et al. 2003) and are irrelevant to determinations of functional response type.

Secondary analyses (Glass 1976) were conducted using mean clearance rate at each food concentration in each study as the unit of observation. Clearance rates and food concentrations were log-transformed to normalize the dependent variable and prevent the many observations at high food from overpowering the phenomenon of interest, namely the functional response at low food levels (Fig. 1). We used general linear models to assess the influence of food concentration and other predictor variables on clearance rates. For individual clearance rates, we considered *Daphnia* length (log-transformed based on millimeters), temperature ( $^{\circ}\text{C}$ ), particle size (equivalent spherical diameter, in micrometers), acclimation time (hours), method (depletion vs. uptake), *Daphnia* species, study, log food concentration, and the square of log food concentration as potential predictors. For mass-specific clearance rates, we considered all of the above predictors except *Daphnia* length. We chose among competing statistical models by minimizing AIC values (Akaike 1974), which

provided results identical to stepwise regression with backward elimination. When two models had very similar AIC values, we chose the model with the fewest predictors. The influences of food concentration and the square of food concentration were always highly significant, so these predictors were included in all models.

A Type I functional response has two components, a constant clearance rate at low food levels and a monotonic decline in clearance rate at food levels higher than the “incipient limiting concentration” (ILC) (Fig. 1, and McMahon and Rigler 1965). For a Type II response, clearance rate decreases continuously with increasing food concentration, whereas for a Type III response there is an initial increase in clearance rate at low food concentrations and then a decrease at higher food levels as feeding saturates (Fig. 1). We regarded a significant positive influence of food concentration on clearance rate at low food concentrations as evidence of a Type III functional response (Fig. 1). A significant positive slope falsifies the null hypothesis that the initial slope is zero (Type I) and the hypothesis that the initial slope is negative (Type II). Failure to reject the null hypothesis would lead us to conclude, as in many previous papers, that a Type I functional response best explains the data. A significant negative influence of the square of food concentration was expected, since food concentrations ranged far above *Daphnia*'s ILC (DeMott 1982, Porter et al. 1982, Peters and Downing 1984).

We addressed the issue of artifactual decreases in clearance rate resulting from starvation-induced exhaustion by running the above analyses for a subset of the data restricted to acclimation times <3 h (the “no exhaustion” data set). The distribution of acclimation times in the literature is roughly bimodal, with most studies having times <3 h and a few studies with acclimation times >10 h. Restricting the data to acclimation times <3 h is conservative with respect to starvation-induced exhaustion because *Daphnia* can survive for up to 8–10 d with no particulate food (Tessier et al. 1983). We present results for both the complete data and the “no exhaustion” subset to ensure that results were robust to these changes in data selection (Englund et al. 1999).

We also conducted a meta-analysis on the few studies ( $n = 8$ ) that were suitable for assessing feeding at low food levels. In contrast to secondary analyses, meta-analysis explicitly examines differences among studies in the functional response, and is often used to conduct a more powerful test of a hypothesis, particularly where each individual study has low power (Gurevitch et al. 1992). None of the individual studies in the meta-analysis provided a powerful test for an increase in clearance rate at low food because each study had only a few (3–5) data points at low food. To be included in the meta-analysis, a study had to have at least three food concentrations <200  $\mu\text{g C/L}$ , and a minimum food

concentration <50  $\mu\text{g C/L}$ . This range is similar to the range at which Plath (1998) observed evidence of Type III behavior in *Daphnia*. For reference, *Daphnia*'s  $R^*$  ranges between 40 and 60  $\mu\text{g C/L}$  when fed cultured algae (Lampert and Trubetskova 1996). The effect size calculated from each study was the log-log slope of the relationship between *Daphnia* clearance rate and food concentration. All but one study in this analysis employed acclimation times less than three hours, and that one study did not report acclimation time (Horn 1981). A mean effect size (slope) across studies that was significantly greater than 0 was taken as evidence for a Type III functional response in the aggregated data. This result would not rule out the possibility that Type I or II was in effect in any individual study. However, if Type I or II was common in many of the studies, meta-analysis would be unlikely to find a significantly positive slope overall.

#### Laboratory feeding experiment

We conducted an experiment to measure the response of *Daphnia* clearance rates to a range of low food concentrations. We chose a range of food levels that was roughly centered about the concentration at which clearance rates were predicted to be maximal from the secondary analyses of literature data (see *Results: Analyses of literature data*). The food particle was *Ankistrodesmus falcatus*, a chlorophyte that we routinely use to culture *Daphnia*. *Ankistrodesmus* was grown in semi-continuous culture in modified WC medium (Stemberger 1981) under cool-white fluorescent lighting and a 16L:8D photoperiod. Cells were centrifuged and resuspended in *Daphnia* medium (Sarnelle and Wilson 2005) before feeding to *Daphnia*.

*Daphnia* used in the experiment were from a clonal culture of *D. pulicaria* that was isolated from Lawrence Lake in July of 2003 (Sarnelle and Wilson 2005). About six weeks before the experiment, fecund *Daphnia* were transferred to 1-L flasks with filtered lake water and a high concentration (>1 mg C/L) of *Ankistrodesmus*. The following day, neonates were collected and added to 1-L flasks with filtered lake water and a high concentration of *Ankistrodesmus* (>1 mg C/L). Neonates were fed daily with *Ankistrodesmus* (>1 mg C/L) and transferred to fresh media with food every two days until the release of their first clutch. This procedure was repeated for several generations until a sufficient number of same-age *Daphnia* ( $n \approx 500$ ) were available for the experiment. Two days before the start of the experiment, *Daphnia* were fed *Ankistrodesmus* at  $\sim 150 \mu\text{g C/L}$  to acclimate them to a low-food environment. Mean length (top of head to base of tail spine) of animals used in the experiment was 1.7 mm.

Food suspensions were prepared on the morning of the experiment from freshly harvested *Ankistrodesmus*. Cells were diluted in *Daphnia* medium to create six food levels using a pre-established conversion between in vivo chlorophyll fluorescence and carbon concentration.

TABLE 1. Statistical results of the secondary analysis of log-transformed individual clearance rates ( $\text{mL}\cdot\text{individual}^{-1}\cdot\text{h}^{-1}$ ).

Independent variable	Complete data set			No exhaustion (acclimation time $\leq 3$ h)		
	Coefficient	SE	P	Coefficient	SE	P
$\log(\text{Daphnia length})$	2.032	0.161	0.001	1.946	0.150	0.001
Temperature	0.032	0.004	0.001			NS
Particle diameter	0.032	0.009	0.001	-0.082	0.015	0.001
Acclimation time	-0.035	0.004	0.001			NS
Method (depletion or uptake)	NA		0.001	NA		0.001
<i>Daphnia</i> species	NA		0.001	NA		0.001
$\log(\text{food concentration})$	0.726	0.095	0.001	0.507	0.095	0.001
$(\log[\text{food concentration}])^2$	-0.229	0.019	0.001	-0.183	0.019	0.001
Full model			0.001			0.001

Notes: Two sets of results are presented: for the complete data set ( $n = 328$ ; full model  $R^2 = 0.80$ ) and for experiments with acclimation times  $\leq 3$  hours ( $n = 234$ ; full model  $R^2 = 0.83$ ) (see *Methods: Analyses of literature data*). NA: coefficients are not meaningful (dummy variables). NS: not statistically significant at  $P < 0.01$ .

Average cell dimensions of *Ankistrodesmus* were: length = 73  $\mu\text{m}$ , diameter = 2.6  $\mu\text{m}$ , volume = 286  $\mu\text{m}^3$ . Experimental food suspensions were prepared in 2-L culture bottles. Half of each suspension was then used to fill 1-L acclimation bottles. The experiment was conducted in three blocks over the course of one day, with a total of six experimental replicates at each food concentration. Experimental temperature was 22°C. At the start of each block, 25 animals were added with a wide-bore pipette to each 1-L bottle and allowed to acclimate for two hours in the experimental suspensions. While animals were acclimating, experimental food suspensions were dispensed into three 220-mL bottles, corresponding to two experimental replicates and one control for each food level in each block. At the end of the acclimation period, the contents of the 220-mL bottles were well mixed and split into two 110-mL bottles, one for the experiment and one containing Lugol's solution as a sample of initial food concentrations. Immediately after filling, 10 animals were pipetted into each experimental 110-mL bottle to start the experiment. Bottles were inverted every 15 minutes to keep food in suspension. After a two-hour feeding period, the contents of each bottle were poured through a 250- $\mu\text{m}$  mesh sieve and into a bottle containing Lugol's for determination of final food concentrations. Samples of the experimental food suspensions were also collected on Pall A/E filters for carbon analysis (Pall Corporation, East Hills, New York, USA). Filters were dried (35°C) and analyzed for organic carbon using a CE Instruments EA 1110 CHN analyzer (ThermoQuest, San Jose, California, USA). Food concentrations reported in the results were based on these carbon measurements.

Initial and final cell concentrations were determined by the inverted microscope technique (Utermöhl 1958). Aliquots (10 mL) from each sample bottle were allowed to settle for two days, after which cells were counted at 400 $\times$ . Counts were made in equal numbers of fields in inner and outer sections of the chamber to avoid bias from nonrandom settling (Sandgren and Robinson

1984). Enough fields were viewed to attain a total count of at least 100 cells for each sample, but no less than 50 fields per chamber were counted. Clearance rates (milliliters per individual per hour) were calculated as  $([\ln(C_0) - \ln(C_1)]V)/tm$ , where  $C_0$  and  $C_1$  are initial and final cell concentrations (cells per milliliter),  $V$  is the volume of the suspension in each bottle (110 mL), and  $n$  is the number of animals in each bottle (10). Correction for changes in the control bottles was not necessary (see *Results: Laboratory feeding experiment*). The shape of the functional response was examined by fitting a polynomial function to clearance rate ( $y$ ) vs. food concentration ( $x$ ) and the square of food concentration ( $x^2$ ). A significant positive influence of food concentration on clearance rate at low food concentrations was taken as evidence of a Type III functional response.

## RESULTS

### *Analyses of literature data*

We obtained data on *Daphnia* clearance rates from 28 studies. The distribution of food concentrations from these studies was centered at  $\sim 430 \mu\text{g C/L}$ , which is  $\sim 10$  times higher than *Daphnia*'s  $R^*$ , and concentrations at or below *Daphnia*'s  $R^*$  were relatively uncommon (Fig. 2). For the secondary analysis of individual clearance rates (milliliters per individual per hour), we found significant positive influences of food concentration ( $P < 0.001$ ) and significant negative influences of the square of food concentration ( $P < 0.001$ ) in both the complete and no-exhaustion data sets (Table 1). Significant influences of *Daphnia* length, temperature, particle diameter, acclimation time, method (depletion vs. uptake) and *Daphnia* species were also obtained for the complete data set (Table 1), but there was no significant influence of study. Given that different methods could give different results, we subdivided the data into depletion vs. uptake studies and ran the above analyses on each subset. Significant positive and negative influences, respectively, of food concentration and the square of food concentration, were found in

TABLE 2. Statistical results of the secondary analysis of log-transformed mass-specific clearance rates ( $\text{mL}\cdot\mu\text{g}^{-1}\cdot\text{h}^{-1}$ ).

Independent variable	Complete data set			No exhaustion (acclimation time $\leq 3$ h)		
	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>
Temperature	0.024	0.004	0.001			NS
Particle diameter			NS	-0.058	0.013	0.001
Acclimation time	-0.036	0.004	0.001			NS
Method (depletion or uptake)	NA		0.001	NA		0.001
<i>Daphnia</i> species	NA		0.001	NA		0.001
Study	NA		0.002	NA		0.002
log(food concentration)	0.572	0.090	0.001	0.517	0.104	0.001
(log[food concentration]) <sup>2</sup>	-0.182	0.018	0.001	-0.172	0.020	0.001
Full model			0.001			0.001

Notes: Two sets of results are presented: for the complete data set ( $N=442$ ; full model  $R^2=0.72$ ) and for experiments with acclimation times  $\leq 3$  hours (no exhaustion,  $N=310$ ; full model  $R^2=0.69$ ) (see *Methods: Analyses of literature data*). NA: coefficients are not meaningful (dummy variables). NS: not statistically significant at  $P < 0.01$ .

both data sets (Appendices A, B), indicating that these influences were not limited to one or the other method.

The results of the secondary analysis of mass-specific clearance rates were similar to those for individual clearance rates (Table 2), as we again found significant positive influences of food concentration ( $P < 0.001$ ) and significant negative influences of the square of food concentration ( $P < 0.001$ ) in both data sets (Table 2). In this case, we also found influences of temperature, particle diameter, acclimation time, method (depletion vs. uptake), *Daphnia* species, and study (Table 2). Subdividing the data set by method again showed that the influences of food concentration and the square of food concentration were not limited to one or the other method (Appendices C, D).

To visualize the influence of food concentration on clearance rate, we fitted mass-specific clearance rate data for the no-exhaustion data set to a model with particle diameter and *Daphnia* species as independent variables (Table 2). The overall influences of method and study were nonsignificant when food concentration was not in the model. We then plotted residuals from this model against food concentration (Fig. 3). We also reanalyzed the mass-specific clearance data after further excluding the three lowest food concentrations (Fig. 3,  $< 4 \mu\text{g C/L}$ ), as these are unlikely values in habitats suitable for *Daphnia*. Without these three data points, the positive influence of food concentration was still highly significant ( $P < 0.002$ ).

Meta-analysis of the slope of clearance rate vs. food concentration was congruent with the secondary analyses. All but one of the eight slope estimates were positive, and the single negative slope had the highest standard error (Table 3). The mean slope across all studies was significantly  $> 0$  (Wilcoxon rank test,  $P < 0.03$ ), again indicating an increase in clearance rate with increasing food concentrations, i.e., a Type III functional response. Statistical significance of the mean slope was robust to whether slopes were unweighted or weighted by  $1/\text{standard error}$ , but weighting did affect the estimate of mean

slope by  $\sim 10\%$ . We conducted the same analysis as above except for using clearance rate scaled to the maximum clearance rate in each study as the dependent variable, with similar results (Appendix E).

#### Laboratory feeding experiment

Initial and final cell concentrations in the control bottles differed by  $< 5\%$ , a difference that was not statistically significant (paired *t* test,  $P > 0.10$ ,  $N = 18$ ). Consequently, no correction for changes in the controls was applied in the calculation of clearance rates. There was also no significant block effect ( $P > 0.50$ ). There was a highly significant positive effect of food concen-

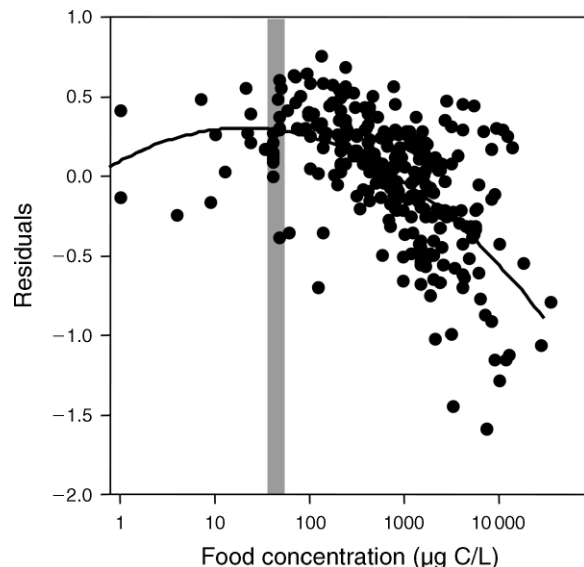


FIG. 3. Plot of residuals (*y*) from a general linear model of mass-specific *Daphnia* clearance rate vs. particle diameter and *Daphnia* species against food concentration (*x*; note log scale) for laboratory experiments with acclimation times  $< 3$  h. The gray vertical bar represents the range of *Daphnia*'s minimum resource requirement,  $R^*$  (Lampert and Trubetskova 1996).

TABLE 3. Meta-analysis of the log-log slope of *Daphnia* clearance rate vs. food concentrations <200  $\mu\text{g C/L}$  from eight studies (see *Methods* for study selection criteria).

Study	Range of food concentrations	Slope	SE	<i>N</i>
Burns and Rigler (1967)	10–120	0.02	0.08	4
DeMott (1982)	24–146	0.01	0.11	3
Henning et al. (1991)	1–123	0.14	0.08	5
Horn (1981)	9–138	–0.02	0.27	4
Horton et al. (1979)	5–123	0.11	0.03	3
Philippova and Postnov (1988)	22–92	0.55	0.19	3
Porter et al. (1982)	21–102	0.08	0.08	3
Wiedner and Vareschi (1995)	1–71	0.03	0.03	3
Mean (weighted by 1/SE)		0.08	0.04	

Note: The weighted mean slope was significantly different from 0 at  $P < 0.03$  (Wilcoxon rank test). *N* = number of food concentrations <200  $\mu\text{g C/L}$  for each study.

tration (*t* test,  $P < 0.003$ ), and a highly significant negative effect of the square of food concentration (*t* test,  $P < 0.003$ ), on *Daphnia* clearance rates (Fig. 4). The food concentration at which clearance rate was predicted to be maximal was 90  $\mu\text{g C/L}$ , based on the regression equation in Fig. 4. Mean clearance rate at the lowest food concentration was  $0.83 \pm 0.20 \text{ mL}\cdot\text{ind}^{-1}\cdot\text{h}^{-1}$  (mean  $\pm$  SE), which was about half of mean clearance rate at 95  $\mu\text{g C/L}$  ( $1.55 \pm 0.27 \text{ mL}\cdot\text{ind}^{-1}\cdot\text{h}^{-1}$ ).

#### DISCUSSION

All three approaches used to assess *Daphnia*'s functional response led to the same conclusion: *Daphnia* can increase clearance rate as phytoplankton abundance increases at low food densities (Tables 1–3, Figs. 3 and 4). This result was particularly clear for our meta- and experimental analyses, although additional observations at very low food densities would have helped the secondary analysis distinguish more clearly between Type III and Type I functional responses (Fig. 3). Each approach has its own well-described strengths and weaknesses, so congruence among all three analyses suggests that *Daphnia* can have a Type III functional response, at least under laboratory conditions of high-quality food. It remains to be determined whether *Daphnia* exhibit a decrease in clearance rates at low concentrations of natural food (but see Chow-Fraser and Sprules 1992).

Our analyses of literature data were robust (Englund et al. 1999) in that we obtained the same basic result for individual and mass-specific clearance rates, and for experiments employing particle-depletion and particle-uptake methodologies (Appendices A–D). We excluded experiments in which *Daphnia* were exposed to low food for more than three hours because of concerns that decreases in *Daphnia* filtering effort at low food might be the result of starvation-induced exhaustion (Muck and Lampert 1980). *Daphnia* can survive for up to 8–10 days with no particulate food (Tessier et al. 1983), so excluding acclimation times of more than three hours seems highly conservative.

We explicitly examined *Daphnia* clearance rate at phytoplankton levels near the range of *Daphnia*'s  $R^*$  (Figs. 3 and 4) to eliminate grazer behavior in systems where populations would not be self-sustaining (i.e., food <  $R^*$ ). The proximity of the peaks in clearance rate to estimates of  $R^*$  (Figs. 3 and 4) indicates that the decrease in clearance rate is likely relevant to *Daphnia*–phytoplankton systems in nature, and so might play a role in stabilizing population dynamics (Murdoch et al. 2003) in systems in which phytoplankton abundance is low. Although detailed theoretical analyses are needed to determine whether the decrease in phytoplankton mortality at low phytoplankton abundance is sufficient to stabilize *Daphnia*–phytoplankton dynamics, we note that potential reductions in prey mortality occur near the prey's equilibrium (the predator's  $R^*$ , Figs. 3 and 4) as required to confer stability (Murdoch et al. 2003). In addition, the decrease in clearance rate that we observed at low food concentrations in our laboratory experiment was not trivial; *Daphnia* clearance rate at the lowest food concentration was about half of the maximum clearance rate at 95  $\mu\text{g C/L}$  (Fig. 4).

Our characterization of *Daphnia*'s functional response provides a provocative contrast to prior literature, although our findings are congruent with several laboratory studies of filter-feeding copepods and one field study of zooplankton grazing (Corner et al. 1972, Gaudy 1974, Paffenhöfer and Stearns 1988, Hansen et al. 1990, Chow-Fraser and Sprules 1992). A recent review of functional response studies across all taxa concluded that filter-feeders typically exhibit a Type I or II response (Jeschke et al. 2004), and reflects the broader view that

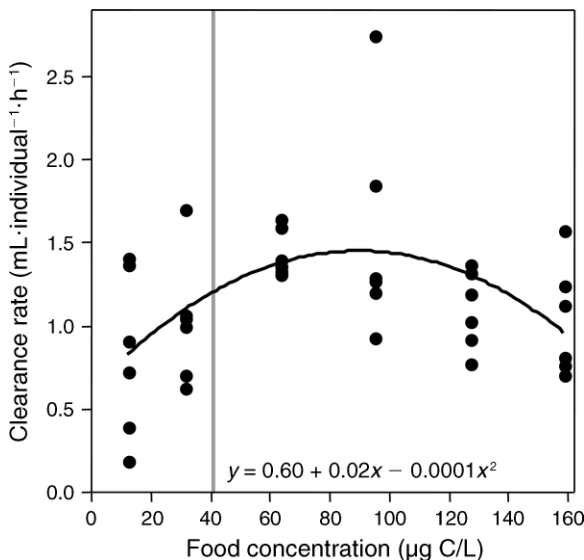


FIG. 4. Effect of food concentration on *Daphnia pulicaria* clearance rate in the laboratory experiment. For the polynomial regression of clearance rate (*y*) on food concentration (*x*),  $R^2 = 0.25$ ,  $P < 0.01$ ,  $n = 36$ . The vertical gray line represents *D. pulicaria*'s minimum resource requirement,  $R^*$  (Lampert and Trubetskova 1996).

Type I or II responses are the norm among invertebrates (Hassell et al. 1977, Walde and Murdoch 1988, Kratz 1996, Jeschke et al. 2002, 2004). Specifically, Jeschke et al. (2004) counted 57 cases of Type I, 19 cases of Type II, and only 2 cases of Type III in branchiopods, the crustacean group to which *Daphnia* belongs. Of these 78 cases, 70 were experiments with *Daphnia*. Given the seeming preponderance of Type I responses noted by Jeschke et al. (2004), we fit a Type I model to the data from the secondary analysis and from our experiment. These analyses indicated a poorer fit for the Type I model relative to models that include an increase in clearance rate at low food density (Appendix F). The latter result is consistent with the experimental findings of Ganf and Shiel (1985), predictions of optimal foraging models (Lehman 1976), and with the observation that *Daphnia* clearance rate appeared to increase with algal density at low food concentrations in two other highly regarded studies (DeMott 1982, Porter et al. 1982).

Type III functional responses may have gone undetected in most previous studies for at least two reasons. First, most studies include relatively few observations at low food density (Fig. 2, Table 3), and so have limited power to detect changes in clearance rate near *Daphnia*'s  $R^*$ . The preponderance of observations at high food in the literature is curious, since in nature *Daphnia* are more likely to experience food concentrations near  $R^*$  than near saturation (DeMott 1989, McCauley et al. 1990a). In situations where individual studies have limited power to detect a particular response, secondary and meta-analysis provide a powerful means of testing general hypotheses (Gurevitch et al. 1992, Osenberg et al. 1999). A previous secondary analysis of zooplankton feeding experiments did not report evidence for Type III functional responses, but the data were restricted to one observation per experiment taken from the midpoint of the food levels employed (Peters and Downing 1984), which would eliminate all observations at low food levels. In contrast, we devoted 18 replicates to food concentrations where we suspected clearance rates might show a decline (Fig. 4), an approach that increased our ability to statistically detect this response in a single experiment. We also acclimated our animals to relatively low food (150  $\mu\text{g C/L}$ ) for two days before the experiment, which may have played a role.

Second, most studies of *Daphnia*'s functional response entail measuring the accumulation of labeled food by the animal after a short feeding period (food-uptake method). Unfortunately, the precision of uptake estimates is partly a function of the total amount of food accumulated; therefore the technique tends to be less precise at food levels needed to identify a Type III response. This limitation can also afflict food-depletion methods, since fewer particles are available for counting in low-density suspensions. We ameliorated this problem in our food-depletion experiment by increasing our counting effort at low food levels (see *Methods: Laboratory feeding experiment*). Experimental error

was fairly similar across food levels (Fig. 4), indicating that this strategy was reasonably successful.

In conclusion, we have provided several lines of evidence suggesting that *Daphnia* can have a Type III functional response. The operation of such feeding behavior is consistent with the unexpectedly small oscillations in *Daphnia* population density observed when zooplanktivores are rare (Murdoch et al. 2003). Given that the functional response of *Daphnia* has been more extensively studied than that of any other animal (Jeschke et al. 2004), yet Type III responses were hitherto rarely identified, we challenge future investigators to conduct analyses specifically designed to evaluate the presence of population-stabilizing feeding behaviors. In particular, we believe that field and experimental analyses should focus on feeding at low prey densities ( $\leq R^*$ ), rather than at high food concentrations where mortality estimates tend to be more precise but inconsequential for detecting Type III behavior. Given the fundamental importance of the functional response in species interactions, new attention to this old issue may be warranted.

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#### APPENDIX A

Statistical results of the secondary analysis of log-transformed individual clearance rates for studies that employed a particle-depletion approach. (*Ecological Archives* E089-103-A1).

#### APPENDIX B

Statistical results of the secondary analysis of log-transformed individual clearance rates for studies that employed a particle-uptake approach. (*Ecological Archives* E089-103-A2).

#### APPENDIX C

Statistical results of the secondary analysis of log-transformed mass-specific clearance rates for studies that employed a particle-depletion approach. (*Ecological Archives* E089-103-A3).

#### APPENDIX D

Statistical results of the secondary analysis of log-transformed mass-specific clearance rates for studies that employed a particle-uptake approach. (*Ecological Archives* E089-103-A4).

#### APPENDIX E

Meta-analysis of the slope of *Daphnia* clearance rate vs. log food concentrations of <200 µg C/L from eight studies. (*Ecological Archives* E089-103-A5).

#### APPENDIX F

Fitting a Type I functional response to literature and experimental data. (*Ecological Archives* E089-103-A6).