



Daphnia performance on diets containing different combinations of high-quality algae, heterotrophic bacteria, and allochthonous particulate organic matter

Anja Wenzel^{1,2} | Tobias Vrede³ | Mats Jansson¹ | Ann-Kristin Bergström¹

¹Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden

²GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

³Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence

Ann-Kristin Bergström, Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden.

Email: ann-kristin.bergstrom@umu.se

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Abstract

1. Filter-feeding zooplankton in lakes feed on a mixture of phytoplankton, bacteria, and terrestrial particles and the proportions and nutritional value of these components can be highly variable. However, the extent to which food quality interacts with food quantity in affecting overall zooplankton performance is not yet fully resolved.
2. Here we performed laboratory feeding experiments to test how the performance of the unselective filter feeder *Daphnia galeata* was affected if various quantities of high-quality food (the phytoplankton *Rhodomonas*) were diluted with low-quality food such as heterotrophic bacteria (*Pseudomonas*) or terrestrial detritus particles (t-POM) from the riparian zone of a boreal forest stream. We hypothesised: that increased proportions of bacteria and t-POM in the diet will lead to decreased survival, somatic growth; and reproduction of *Daphnia* despite the presence of phytoplankton; that these effects are more pronounced for t-POM than for heterotrophic bacteria; and that this response is stronger when phytoplankton availability is low.
3. Increasing the concentrations of *Pseudomonas* affected *Daphnia* survival, growth, and reproduction negatively when *Rhodomonas* was available at intermediate (0.37 mgC/L) and high (0.55 mgC/L) quantities. When *Rhodomonas* quantity was low (0.22 mgC/L), the addition of *Pseudomonas* generally resulted in better *Daphnia* performance except at very high concentrations of the bacterium relative to *Rhodomonas*. In contrast, the addition of t-POM was detrimental for overall *Daphnia* performance at all *Rhodomonas* concentrations.
4. *Daphnia* performance was best described by a model including the interaction between food quality and quantity, with stronger negative effects on *Daphnia* when high-quality food was supplemented with t-POM than with *Pseudomonas*.
5. The results indicate that the ability of zooplankton to use low-quality food is affected by the concurrent availability of high-quality food. Furthermore, food sources that can be used but do not fulfil dietary requirements of grazers (e.g. bacteria), may still provide nutritional benefits as long as other complementary food components are available in sufficient quantities to compensate for biochemical deficiencies.

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6. Therefore, we conclude that heterotrophic bacteria, but not peat layer t-POM, can be an important component of zooplankton diets in boreal lakes, especially if the concentration of phytoplankton is low.

KEY WORDS

boreal forest detritus, *Daphnia galeata*, food quality, *Pseudomonas* sp., *Rhodomonas lacustris*

1 | INTRODUCTION

Zooplankton production in lakes is supported by both autochthonous and allochthonous carbon sources. While the classic concept of the pelagic food web regards phytoplankton (autochthonous) production as the major carbon source (e.g. Nauwerck, 1963; Wetzel, 2001), there are several studies that suggest that terrestrial (allochthonous) carbon is an important subsidy for higher trophic levels (e.g. zooplankton and fish) in unproductive lakes (e.g. Cole et al., 2011; Pace et al., 2004). However, the importance of this allochthonous subsidy has been questioned by studies that show that allochthonous carbon is inefficiently incorporated in the food web (Brett et al., 2017; Lau et al., 2014).

Terrestrial carbon enters lakes in the form of dissolved (t-DOM) or particulate organic matter (t-POM) (Cole et al., 2006). Both DOM and POM can enter the food web, either by uptake of t-DOM by heterotrophic bacteria (Kritzberg et al., 2004; Tranvik, 1988) which are then grazed by other heterotrophic organisms such as flagellates, ciliates, and metazooplankton (Jansson et al., 2007; Jones, 1992), or by direct feeding on t-POM (Cole et al., 2006; Hessen, 1998; Pace et al., 2004). Terrestrial OM is often dominated by recalcitrant dissolved and particulate OM that has been degraded in the catchment before being exported to lakes (Wetzel, 1984). The chemical character of the t-POM varies depending on the characteristics of the terrestrial ecosystem but most of this OM is expected to have a low nutritional value for metazoans due to its high carbon:phosphorus (C:P) stoichiometry and lack of essential biochemicals. This has been shown for different materials, for example grasses and deciduous tree litter (Brett et al., 2009; Taipale et al., 2014, 2016) and t-POM from boreal forest peat layers (Wenzel et al., 2012a).

The magnitude of t-OM inputs into boreal lakes depends both on catchment characteristics and season. Inputs of t-OM are usually high during spring flood and high-flow episodes (Bergström & Jansson, 2000; Hope et al., 1994; Jonsson et al., 2001) and the proportion of t-POM increases with increasing water discharge (Thurman, 1985). Generally, t-POM accounts for <10% of t-OM (Ivarsson & Jansson, 1994; Thurman, 1985). However, as the total input of t-OM can be very high in many boreal lakes, the particulate fraction nevertheless constitutes a large amount compared to other potential food sources. Concentrations of dissolved organic carbon in humic lakes can range between 20 and 50 mgC/L (Thurman, 1985). Hence, particulate organic carbon in those lakes may be as high as 5 mgC/L of which phytoplankton and bacteria only account for a small portion (Jansson et al., 2003). In contrast, boreal lakes with

high inputs of t-OM often only support a low phytoplankton biomass and algal carbon concentrations rarely exceed 0.1 mgC/L (Arvola et al., 1999; Jansson et al., 2003).

The high concentrations of coloured, terrestrial material negatively affect the light climate in humic lakes, resulting in low primary production and biomass of phytoplankton (Deininger et al., 2017; Seekell et al., 2015). As t-OM can support high bacterial production (Hessen, 1985; Jansson et al., 2003), boreal humic lakes are often characterised by high bacteria:phytoplankton biomass ratios compared to other systems (Hessen, 1998) with bacterial biomasses being up to 3-times higher than phytoplankton biomass (Hessen, 1992; Hessen & Andersen, 1990; Jansson et al., 2003). Heterotrophic bacteria are directly consumed by a variety of non-selective filter feeding zooplankton, e.g. *Daphnia* (Wylie & Currie, 1991). Owing to their low C:P stoichiometry, bacteria may be considered a high-quality food for organisms with high somatic P content, e.g. daphnids (Sterner & Elser, 2002). However, bacteria do not meet consumers' requirements in terms of biochemical food quality because they lack essential polyunsaturated fatty acids (PUFAs) (Zelles, 1999) and sterols (Martin-Creuzburg et al., 2011; Volkman, 2003). Consequently, pure bacterial diets cannot sustain *Daphnia* populations (Taipale et al., 2012; Wenzel et al., 2012b).

As phytoplankton, heterotrophic bacteria, and t-POM are present in varying proportions in lakes, zooplankton will encounter fluctuating food qualities and quantities throughout their lives. Therefore, it is necessary to investigate how food quality and quantity interact in the regulation of secondary productivity. Some studies suggest that food quality does not play an important role at low food concentrations when energy limitation dominates (Elser et al., 2003; Persson et al., 2007; Sterner & Robinson, 1994). However, other studies report that food stoichiometry does affect grazer performance irrespective of food quantity (Boersma & Kreutzer, 2002). However, these studies have mainly focused on algal food resources of varying quality, so where the alternative food resource is non-algal it remains unclear how food quality and quantity interact. Wenzel et al. (2012a) concluded that a substantial fraction of algal carbon was needed for *Daphnia* growth and reproduction when the food consisted of algae supplemented with bacteria or t-POM. Although the study clearly showed that bacteria and in particular t-POM are low-quality food resources that cannot sustain *Daphnia* populations alone, the study did not test to the extent to which the quantity of the high-quality algal food can offset the negative impact of the low-quality food.

The main objectives of this study were: (1) to investigate how basic life history parameters of a non-selective filter feeder (*Daphnia*

galeata) are affected if high-quality food (phytoplankton) is diluted with different types of low-quality food (bacteria or t-POM, respectively); and (2) to determine to the extent to which the observed response depends on the absolute quantity and proportion of the high-quality food. In contrast to the majority of food quality studies, we used both non-limiting as well as limiting concentrations of both high- and low-quality food resources. Based on previous results (Wenzel et al., 2012a, 2012b), we hypothesised that increasing proportions of bacteria and t-POM in the diet will lead to decreased survival, somatic growth, and reproduction of *Daphnia* despite the presence of phytoplankton and that these effects will be more pronounced for t-POM than for heterotrophic bacteria. We also hypothesised that this response will be stronger when the concentration of phytoplankton is limiting.

2 | METHODS

2.1 | Experimental design

We conducted experiments with *Daphnia* feeding on different concentrations of high-quality phytoplankton that were mixed with either heterotrophic bacteria or t-POM. In each experimental run, the food suspension contained a constant *Rhodomonas* concentration and a range of concentrations of either *Pseudomonas* or t-POM. The different *Rhodomonas* concentrations were low (0.22 mgC/L), intermediate (0.37 mgC/L), and high (0.55 mgC/L), which is either below (low), at (intermediate), or above (high) the incipient limiting level above which ingestion rate is maximal (0.26–0.36 mgC/L for similar sized *D. longispina*, Lampert, 1987). To each of the three *Rhodomonas* concentrations, we added five different concentrations of either *Pseudomonas* or t-POM (target concentrations 0.15, 0.3, 0.6, 1.2, and 2.4 mgC/L). In each experiment we also ran a treatment with *Rhodomonas* only. This resulted in a total of 33 treatments (three *Rhodomonas* concentrations × two additional food types × five addition levels, plus three treatments with *Rhodomonas* only). Due to space constraints we performed three experimental runs in sequence, one for each *Rhodomonas* concentration.

A clone of *D. galeata* isolated from a boreal mesohumic lake, Nydalasjön (63°49'N, 20°20'E), was grown in 3 × L16 medium (Lindström, 1991) and fed chemostat-grown (see below) *Rhodomonas ad lib.* prior to the experiments. Care was also taken to avoid extensive crowding of *Daphnia* in the cultures. For the experiments, we used neonates from the third clutch or later that had hatched within 24 hr. For each treatment, we used between seven and 11 replicate daphnids that were kept individually in approximately 25 ml feeding suspension in glass scintillation vials (total $n = 384$ individuals). We transferred experimental animals into clean vials with fresh feeding suspension daily using a wide-mouthed pipette. Experimental *Daphnia* were reared under the same conditions, kept in the temperate-controlled rooms with identical conditions during all experimental runs. Survival, occurrence of eggs, and number of neonates hatched were recorded on these occasions. Neonates were then

removed. Each experiment was run for 10 days. Even though care was taken to avoid air bubbles when closing the vials, occasionally bubbles developed overnight and some daphnids died after being trapped in the surface tension. These individuals were randomly distributed across treatments. Because it cannot be assumed that feeding and reproduction is unaffected by being caught in the surface tension, we excluded these individuals from further analysis ($n = 32$ individuals).

Individual daphnids were photographed in a dissecting microscope at day 5 and the body length was measured from the top of the helmet to the base of the caudal spine. Dry mass was then calculated using a pre-determined relationship between dry mass (DM, µg) and body length (L, µm) for this clone ($\ln DM = 3.96 \ln L - 25.4$). Juvenile specific growth rate, r , was calculated as:

$$r = \frac{\ln M_t - \ln \bar{M}_0}{t}, \quad (1)$$

where \bar{M}_0 is the average initial dry mass, M_t is the dry mass of surviving individuals in each treatment, and t is the experimental duration (5 days) for this measurement.

2.2 | Rhodomonas and Pseudomonas cultures

We used the cryptophyte *Rhodomonas lacustris* (strain NIVA 8-82; from the Norwegian Culture Collection of Algae, maintained by the Norwegian Institute for Water Research and the University of Oslo, Oslo, Norway). The genus *Rhodomonas* has been revised and the species *R. lacustris* has been renamed *Plagioselmis lacustris* (Pascher and Ruttner) Javornicky (Javornický, 2001). However, we have chosen to keep the name *Rhodomonas lacustris* here because it has not been renamed in the culture collection. The *Rhodomonas* was cultured in 4-L chemostats in modified 3 × L16 medium adjusted to pH c. 8, containing 100 µl/L Wright's solution, and vitamins and animal trace elements as in COMBO medium (Kilham et al., 1998). We chose *Rhodomonas* as it is a high-quality food for zooplankton, supporting high growth and reproduction rates (Brett & Müller-Navarra, 1997), and because cryptophytes often constitute a large fraction of the phytoplankton biomass in humic lakes (Deininger et al., 2017; Sarvala et al., 1999). Chemostats were run in a 16:8-hr light:dark cycle at an irradiance of approximately 60 µmol m⁻² s⁻¹ (Philips, Master TL5HE, 28W-865 daylight fluorescent tubes, Philips, Kista, Sweden) at 20°C. Cultures were gently stirred with a magnetic stirrer for 10 s every 10 min. *Pseudomonas* sp. (originally isolated from a lake in the Örebro area of Southern Sweden), a common freshwater bacterium, was cultured under the same conditions as *Rhodomonas* but we kept chemostats in the dark and the medium also contained 40 mgC/L in the form of glucose. During the experiment, we measured carbon concentration of *Rhodomonas* and *Pseudomonas* cultures daily with an HSC 1300 high-temperature module connected to an IL 550 TOC-TN analyser (Hach Company, Loveland, CO, U.S.A.) to enable the preparation of the food suspensions.

2.3 | Preparation of t-POM

Terrestrial particulate organic material (t-POM) was collected from the riparian zone of the northern boreal forest stream Degerbäcken ($63^{\circ}59.26'N$, $20^{\circ}22.19'E$) in November 2009. The catchment upstream of the sampling point consists of 53% coniferous forest (spruce and pine), 13% coniferous forest on wetland, 22% open wetland (peat bogs), 7% mixed forest (spruce, pine, birch, and aspen), 3% clear cut forest, and 2% other land use. The total catchment area is 8.0 km^2 . The t-POM was collected from peat layers in the riparian zone of the stream. These layers are subject to freeze-thaw cycles in situ, and peat particles are flushed downstream and into lakes during high flow events such as snow melt. We therefore consider this material to be a suitable model for t-POM produced in boreal forests and transported to lakes.

The t-POM was stored at 4°C . To prepare the material for the experiments, it was suspended in deionised water, sieved through a coarse mesh net to remove large particles and freeze-dried. Small batches (c. 20–25 mg) of the dry material were ground with a sediment grinder (Retsch Mixer Mill MM301, AB Ninolab, Upplands Väsby, Sweden) at 20 Hz for 30 s in plastic scintillation vials. The ground t-POM was then filtered through a 35- μm mesh size nylon net to exclude particles outside the edible size range of *Daphnia* and suspended in P-free 3 \times L16 medium. Visual microscopic inspection of the suspended material confirmed dominance of particles up to 10 μm in diameter, which is comparable to the size of *Rhodomonas* cells. The t-POM suspensions were prepared fresh every second or third day by determining carbon content (as described above) and diluting with 3 \times L16 medium to the desired concentrations.

2.4 | Chemical analyses

Samples for particulate P analysis of *Rhodomonas*, *Pseudomonas*, and t-POM suspensions were collected daily (per batch for t-POM) during the experiments on acid-rinsed GF/F glass fibre filters (25 mm, Whatman, Whatman Arbor Technologies, Ann Arbor, MI, U.S.A.) and analysed with the molybdate-blue method after hot acid digestion with potassium persulfate (Menzel & Corwin, 1965). Samples for particulate C and N analysis were collected at the same time on pre-combusted GF/F filters and analysed on a Costech ECS 4010 elemental analyser (Costech International S.p.A., Milano, Italy).

2.5 | Statistical analyses

Effects of adding increasing amounts of *Pseudomonas*, or t-POM, to three different concentrations of *Rhodomonas* on survival at the end of the experiment were analysed with logistic regression using JMP Pro 14.0.0 (SAS Institute Inc., Cary, NC, U.S.A.). The effects on survival time (measured in days assigning a value of 10 to daphnids surviving until the end of the experiment) by these different treatments were analysed with Spearman rank correlation using IBM SPSS Statistics 19.0 (IBM, Gothenburg, Sweden).

To model juvenile specific growth rate, r , we used three different models, accounting for the effects of food quantity and food quality alone, and the interaction between food quantity and quality:

$$r = a(1 - e^{-btotC}), \quad (2)$$

$$r = a + b\%Rho, \quad (3)$$

$$r = a + b\%Rho(1 - e^{-ctotC}), \quad (4)$$

where a , b , and c are the parameters of the models, $totC$ is the total food concentration (mgC/L), $\%Rho$ is the % carbon contribution of *Rhodomonas* in the diet, which is used as a proxy for food quality. Equation 2 models the growth rate as a positive saturated function of food quantity. Equation 3 models the growth rate as a linear function of the % *Rhodomonas* in the diet. The last model (eq. 4) is a combination of Equations 2 and 3, thus modelling growth rate as a linear interaction between food quantity and food quality. These models were fitted to data using the non-linear platform in JMP Pro 14.0.0 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

We performed an ANCOVA with total food concentration and %*Rhodomonas* as covariates, *Pseudomonas*/T-POC as a categorical factor, including both main effects and first order interactions, and with the total number of eggs and juveniles produced during the experiment as response variable. An analysis of residuals showed that they did not differ from a normal distribution. The effect of experimental run was not included in the growth rate models or in the ANCOVAs. The reason for this is that experimental run is confounded with both total food concentration and %*Rhodomonas*. This weakness of the design is a consequence of our decision to perform the experiment using a large number of single individuals as the replicated unit, which made it impossible to perform all trials simultaneously. To minimise the effects of experimental run, care was taken to grow daphnids, *Rhodomonas*, and *Pseudomonas* under stable conditions prior to the experiments and throughout all experimental runs (see above). When comparing the results of the *Rhodomonas*-only treatments, we observe an increase in both somatic growth rate and reproductive output that is consistent with previously observed responses to increasing food concentration (Wentzel et al. 2012a). We therefore argue that experimental run per se is not the main explanatory factor, but rather that the observed responses can be attributed to the factors %*Rhodomonas* and total food concentration. The ANCOVA analysis was made using JMP Pro 14.0.0 (SAS Institute Inc., Cary, NC, U.S.A.).

3 | RESULTS

3.1 | Food quantity and quality

Rhodomonas concentrations ranged from 0.22 to 0.55 mgC/L, *Pseudomonas* additions ranged from 0.12 to 2.11 mgC/L, and t-POM additions from 0.13 to 2.29 mgC/L (Table 1). This resulted

in *Rhodomonas* percentages between 12 and 81% in the mixtures with *Pseudomonas* and between 9 and 81% when mixed with t-POM (Table 1). Carbon-to-phosphorus ratios were similar for *Rhodomonas* (95 ± 17 , molar ratio, mean \pm SD) and *Pseudomonas* (76 ± 11) and much higher for t-POM (996 ± 192).

3.2 | Survival

Daphnia survival over the 10-day experiment ranged between 0 and 100% across all treatments and was generally higher in treatments with *Pseudomonas* than t-POM when comparing at the same %*Rhodomonas* or at the same food quantity (Figure 1a,b). On 100% *Rhodomonas* diets, all daphnids survived in the intermediate and high *Rhodomonas* treatments while there was 30% mortality in the low *Rhodomonas* treatment. In the *Pseudomonas* treatments, survival decreased significantly with increasing *Pseudomonas* concentration for the low and intermediate *Rhodomonas* concentrations (logistic regression; low: $r^2 = 0.237$, $df = 1$, $\chi^2 = 11.248$, $p = 0.001$, intermediate: $r^2 = 0.217$, $df = 1$, $\chi^2 = 7.765$, $p = 0.005$), while no significant change was found in the high *Rhodomonas* treatment (logistic regression, $r^2 = 0.127$, $df = 1$, $\chi^2 = 2.786$, $p = 0.095$; Figure 1a). In contrast, the decrease in survival with increasing t-POM concentrations was significant in all three *Rhodomonas* treatments (logistic regression; low:

$r^2 = 0.493$, $df = 1$, $\chi^2 = 21.656$, $p < 0.001$, intermediate: $r^2 = 0.622$, $df = 1$, $\chi^2 = 36.177$, $p < 0.001$, high: $r^2 = 0.552$, $df = 1$, $\chi^2 = 26.210$, $p < 0.001$; Figure 1b). Additionally, the survival rate was 0% at the highest t-POM addition in all three *Rhodomonas* treatments, and the survival was 0–33% in the other t-POM additions in the low *Rhodomonas* treatment (Figure 1b).

When *Pseudomonas* was added, *Daphnia* survival time significantly decreased with increasing food concentration in the low and intermediate *Rhodomonas* treatments while there was no significant effect of food concentration on *Daphnia* survival time in the high *Rhodomonas* treatment (Table 2). In the t-POM treatments, survival time decreased significantly with increasing food concentration across all *Rhodomonas* treatments (Table 2).

3.3 | Juvenile specific growth rate

The specific growth rate increased from 0.25 to 0.49 day⁻¹ with increasing *Rhodomonas* concentration in the treatments with *Rhodomonas* only, but showed a more complex response to food quantity when either *Pseudomonas* or t-POM was added, with apparent effects of both food quantity and quality (Figure 2). In the high *Rhodomonas* treatment, addition of *Pseudomonas* initially did not affect the growth rate but at larger additions of bacteria, the growth rate declined (Figure 2a,c). Also, in the intermediate *Rhodomonas* treatment, there was a monotonous

TABLE 1 Concentrations of *Pseudomonas* and terrestrial particulate organic matter (t-POM) and total food concentration (mean \pm standard deviation) and percentage of *Rhodomonas* in the food suspensions with low, intermediate, and high concentrations of *Rhodomonas*

<i>Rhodomonas</i> treatment	<i>Pseudomonas</i>			t-POM		
	<i>Pseudomonas</i> concentration (mgC/L)	Total food concentration (mgC/L)	Rho (%)	t-POM concentration (mgC/L)	Total food concentration (mgC/L)	Rho (%)
Low	0	0.22	100	0	0.22	100
	0.12 \pm 0.02	0.34 \pm 0.04	64	0.13 \pm 0.00	0.35 \pm 0.02	62
	0.24 \pm 0.04	0.46 \pm 0.06	47	0.27 \pm 0.01	0.48 \pm 0.02	44
	0.49 \pm 0.08	0.70 \pm 0.10	31	0.54 \pm 0.01	0.75 \pm 0.02	29
	0.97 \pm 0.17	1.19 \pm 0.18	18	1.07 \pm 0.02	1.29 \pm 0.02	17
	1.70 \pm 0.45	1.91 \pm 0.47	12	2.15 \pm 0.04	2.36 \pm 0.04	9
Intermediate	0	0.37	100	0	0.37	100
	0.13 \pm 0.01	0.49 \pm 0.09	74	0.14 \pm 0.02	0.51 \pm 0.09	71
	0.26 \pm 0.02	0.62 \pm 0.09	58	0.29 \pm 0.03	0.65 \pm 0.10	56
	0.51 \pm 0.04	0.88 \pm 0.10	41	0.57 \pm 0.06	0.94 \pm 0.12	39
	1.02 \pm 0.08	1.39 \pm 0.14	26	1.14 \pm 0.12	1.51 \pm 0.17	24
	2.04 \pm 0.17	2.41 \pm 0.21	15	2.29 \pm 0.23	2.66 \pm 0.27	14
High	0	0.55	100	0	0.55	100
	0.13 \pm 0.02	0.68 \pm 0.03	81	0.13 \pm 0.01	0.68 \pm 0.03	81
	0.26 \pm 0.03	0.81 \pm 0.04	68	0.26 \pm 0.03	0.81 \pm 0.04	68
	0.52 \pm 0.07	1.08 \pm 0.07	51	0.52 \pm 0.06	1.07 \pm 0.06	52
	1.06 \pm 0.13	1.61 \pm 0.13	34	1.04 \pm 0.12	1.59 \pm 0.11	35
	2.11 \pm 0.26	2.66 \pm 0.26	21	2.08 \pm 0.23	2.63 \pm 0.22	21

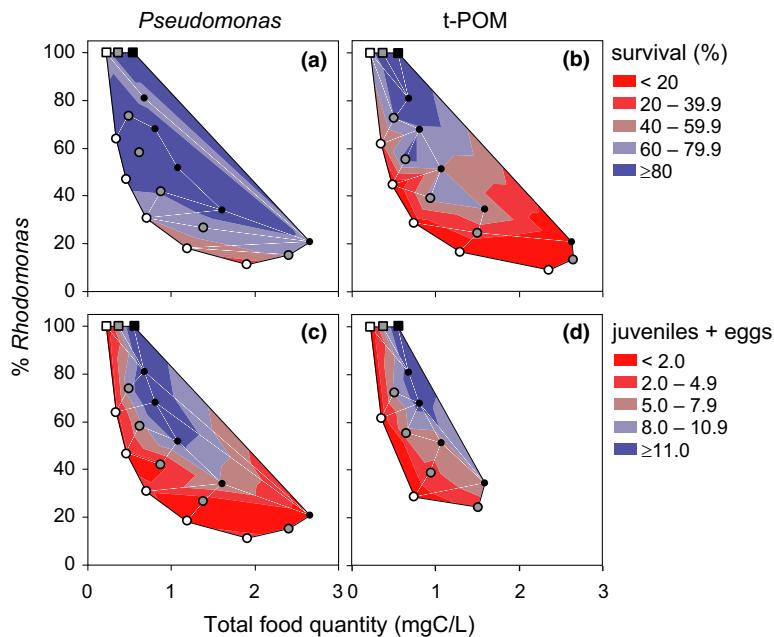


FIGURE 1 Contour plots of the performance of *Daphnia galeata* feeding on mixtures of *Rhodomonas* with different concentrations of *Pseudomonas* (a, c) and terrestrial particulate organic matter (t-POM; b, d). Response parameters are: survival at day 10 (a, b); and cumulative number of eggs plus viable juveniles produced (juveniles + eggs) during the course of the experiment (c, d). Squares: treatments with *Rhodomonas* only; circles: mixtures of *Rhodomonas* and *Pseudomonas* or t-POM, respectively. White symbols: low *Rhodomonas* concentration; grey symbols: intermediate *Rhodomonas* concentration; black symbols: high *Rhodomonas* concentration

<i>Rhodomonas</i> concentration	Additional food type	N	ρ	p
Low	<i>Pseudomonas</i>	60	-0.336	<0.01
	t-POM	60	-0.587	<0.001
Intermediate	<i>Pseudomonas</i>	57	-0.358	<0.01
	t-POM	59	-0.698	<0.001
High	<i>Pseudomonas</i>	50	-0.210	n.s.
	t-POM	50	-0.677	<0.001

Note: n.s.: not significant, $p > 0.05$.

t-POM, terrestrial particulate organic matter

negative effect of the addition of bacteria on the growth rate, but the decline began at small additions of bacteria (Figure 2a,c). In contrast, there was a unimodal response to the addition of bacteria in the low *Rhodomonas* treatment, with the highest growth rates observed at the smaller additions of bacteria (Figure 2a,c). In the t-POM treatments, the growth rate generally decreased with increasing amounts of t-POM added (Figure 2b,d).

The model that best described growth rate response in the experiment was the one including the interaction between food quality and quantity (eq. 4; Table 3; Figure 2; Appendix S1). This model was highly significant and explained 76 and 64% of the variation in the *Pseudomonas* and t-POM treatments, respectively. The models containing only food quality or food quantity (Eq. 2, 3) were mostly non-significant and explained little or nothing of the variation in growth rate (Table 3; Appendix S1). The decline in growth rate with increasing food quantity and decreasing food quality was steeper when adding t-POM than *Pseudomonas* (Figure 2), which is also reflected by a larger value of the parameter c in Eq. 4 for t-POM than for *Pseudomonas* (Table 3).

3.4 | Reproduction

The total production of eggs and juveniles during the experiment was positively affected by the interaction between %*Rhodomonas* in the diet and total food concentration. Both the main effects, %*Rhodomonas* and total food concentration, were positive, whereas the *Pseudomonas*/t-POM treatment was not significant neither as main effect nor in any interactions (ANCOVA with 2nd-degree interaction terms, with total food concentration and %*Rhodomonas* as covariates and *Pseudomonas*/t-POM treatments as categorical variables, $r^2_{\text{adj}} = 0.72$, $F_{6,214} = 95.0$, $p < 0.0001$; Figure 1c,d).

With *Rhodomonas* only, all daphnids in the intermediate and high *Rhodomonas* treatments produced offspring, with the mean number of eggs and juveniles being 8.4 and 13.6, respectively. In contrast, none of the individuals produced eggs in the low *Rhodomonas* treatment. In the intermediate and high *Rhodomonas* treatments, the addition of *Pseudomonas* resulted in a gradual decrease in the production of eggs and juveniles to 0 and 0.4 eggs and juveniles

TABLE 2 Results from Spearman rank correlation tests of survival time of *Daphnia galeata* as a function of total food concentration. N: number of replicate units, ρ : Spearman rho

FIGURE 2 Juvenile specific growth rate of *Daphnia galeata* feeding on mixtures of *Rhodomonas* (Rho) supplemented with different concentrations of *Pseudomonas* (a, c) or terrestrial particulate organic matter (t-POM; b, d). The growth rates are plotted with food quantity (a, b) and food quality (c, d) as independent variables. Squares: treatments with *Rhodomonas* only, circles: mixtures of *Rhodomonas* and *Pseudomonas* or t-POM, respectively. Lines show fitted non-linear models with interaction between food quantity and quality (eq. 4). Error bars show 95% confidence intervals. See Table 3 for statistical details

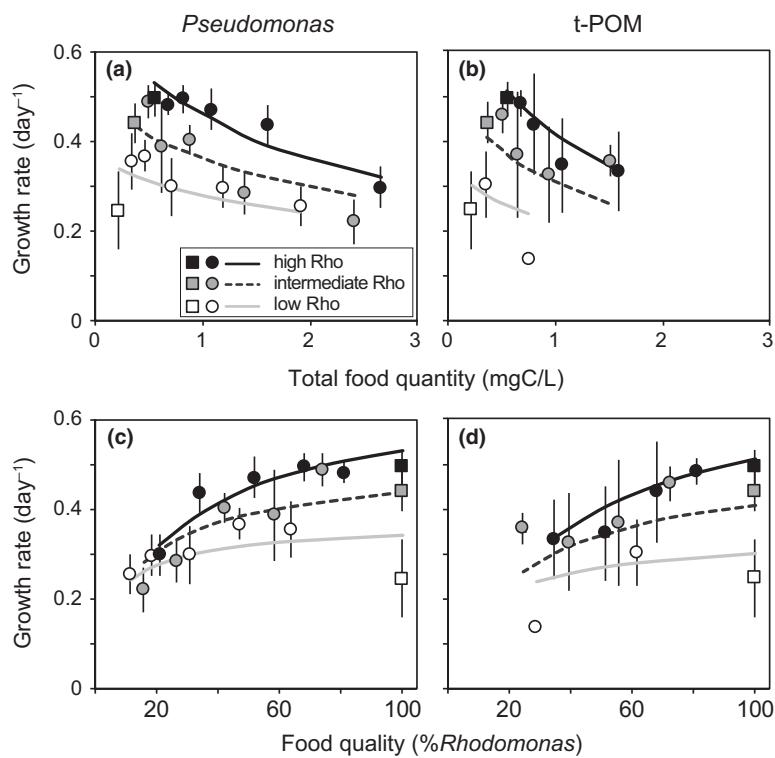


TABLE 3 Statistical results from models of juvenile growth (measured as specific growth rate until day 5 of the experiment) as a function of food quantity and/or food quality (Equations 2–4)

Additional food type	Model	Parameter estimate							
		a	b	c	r² _{adj}	DF _{model}	DF _{error}	p	
Pseudomonas	Quantity (eq. 2)	0.380	8.68		-0.08	2	15	0.40	n.s.
	Quality (eq. 3)	0.277	0.0018		0.25	2	15	3.85	<0.05
	Quality × quantity (eq. 4)	0.165	0.0078	1.15	0.76	3	14	18.96	<0.001
t-POM	Quantity (eq. 2)	0.374	7.82		-0.12	2	10	0.38	n.s.
	Quality (eq. 3)	0.243	0.0019		0.11	2	10	1.76	n.s.
	Quality × quantity (eq. 4)	0.091	0.0079	1.39	0.64	3	9	7.99	<0.01

Note: n.s., not significant, $p > 0.05$. t-POM, terrestrial particulate organic matter

per female, respectively, at the highest food concentration. Adding *Pseudomonas* to low concentrations of *Rhodomonas* resulted in an increased reproductive output of up to 2.3 and 3.0 eggs and juveniles per female at 0.7 and 1.9 mgC/L total food concentration, respectively.

In the t-POM addition treatments, none of the individual produced eggs or juveniles at low *Rhodomonas* concentrations. In the mixtures of t-POM with medium or high *Rhodomonas* concentrations, all surviving individuals produced eggs and/or juveniles during the experiment, but the number of eggs and juveniles declined with increasing food concentration to 7.8 and 3.0 eggs and juveniles per female in the high and intermediate *Rhodomonas* treatments, respectively.

None of the individuals feeding on either pure low *Rhodomonas* diets or mixtures of low *Rhodomonas* with *Pseudomonas* or t-POM

produced any juveniles during the experiment and individuals in these treatments started to develop eggs later than in comparable treatments with intermediate and high concentrations of *Rhodomonas*.

4 | DISCUSSION

Our results suggest that the availability of high-quality phytoplankton biomass is critical for *Daphnia* survival, growth, and reproductive output. As hypothesised, an increasing dilution of a non-limiting concentration of high-quality *Rhodomonas* with low-quality *Pseudomonas* or t-POM led to decreased overall *Daphnia* performance. We also found strong interacting effects between food quality and quantity:

Daphnia performance was best predicted when including the interaction between food quality and quantity in the model, with more pronounced negative effects on *Daphnia* performance when high-quality food was diluted with t-POM than with *Pseudomonas*. At low *Rhodomonas* concentrations, small additions of *Pseudomonas* provided some nutritional benefits to *Daphnia* diets up to a certain threshold above which any further dilution with low-quality *Pseudomonas* outweighed the positive effects of the addition. This is similar to other studies showing poor survival on pure or bacteria-dominated diets supplemented with low phytoplankton concentrations (Taipale et al., 2012; Wenzel et al., 2012b). Likewise, at high *Rhodomonas* concentration, the addition of bacteria could initially sustain a high growth rate, which eventually declined at large additions of bacteria. It has previously been shown that *Daphnia* can readily ingest and assimilate bacterial C and P (Hessen & Andersen, 1990; Hessen et al., 1989; Taipale et al., 2012; Wenzel et al., 2012b) and that dietary P is especially important for high-P requiring organisms such as *Daphnia* (Sterner & Elser, 2002). However, while providing enough energy (organic C) and minerals (P), heterotrophic bacteria in general lack essential fatty acids (FAs) (Zelles, 1999) and sterols (Martin-Creuzburg et al., 2011; Volkman, 2003), which is probably why very high proportions of *Pseudomonas* in the diet negatively affected *Daphnia* performance. Under these circumstances, the positive effects of the addition of *Pseudomonas* P were possibly outweighed by the lack of essential biochemicals. It could also be so that our *Pseudomonas* strain produced toxic compounds (cf. Freese & Martin-Creuzburg, 2013; Martin-Creuzburg et al., 2011), which negatively impacted *Daphnia* performance when *Pseudomonas* proportions in the food suspensions were high.

In contrast, no such positive effects were seen in response to t-POM additions which instead had detrimental effects on *Daphnia* performance at all *Rhodomonas* concentrations. These results are similar to the findings of Brett et al. (2009) who fed *Daphnia* ground alder leaves alone or in combination with different phytoplankton species. They reported a decreased performance of daphnids when the diet consisted of increasing amounts of terrestrial material and concluded that t-POM only played a minor role for zooplankton nutrition while phytoplankton was a much higher quality resource. However, as daphnids did survive and even produced viable eggs on leaf-dominated diets, the authors also concluded that the quality of their t-POM was higher than that of prokaryotic cyanobacteria, which are usually considered a low-quality food (von Elert et al., 2003; Martin-Creuzburg et al., 2008). In contrast to Brett et al. (2009), our peat layer t-POM supported lower *Daphnia* survival, growth, and reproduction than the prokaryote *Pseudomonas* in a previous study, especially at an overall low food concentration (Wenzel et al., 2012a). Likewise, the present study shows that somatic growth responses differed substantially between *Pseudomonas* and t-POM additions in the low *Rhodomonas* treatments. While t-POM additions caused decreased growth, supplementing *Rhodomonas* with *Pseudomonas* initially resulted in an increase in growth rates, followed by a gradual decline in growth rate with increasing addition of *Pseudomonas*. Compounds

that are toxic to daphnids (e.g. phenols; Taylor et al., 1996) may also have been present in our t-POM. Since we did not measure the concentrations of any potentially toxic compounds, we cannot know to what extent it may have contributed to the strong negative response of the daphnids to increasing t-POM concentrations. Nevertheless, the t-POM we used was intentionally selected to mimic a type of t-POM that occurs naturally in boreal lakes. Regardless of the exact mechanism behind the strong negative effect of t-POM, it is safe to conclude that t-POM of this type does not sustain survival, growth, or reproduction.

A positive effect of *Pseudomonas* additions was also seen on egg production even though no viable offspring hatched on any of the low *Rhodomonas* diets. This implies that, in contrast to the conclusions of Brett et al. (2009), allochthonous carbon can play a significant role for zooplankton nutrition when phytoplankton concentrations are low. However, this positive effect is only apparent when the terrestrial carbon is channelled to higher trophic levels (i.e. zooplankton) via the bacterial pathway (Hiltunen et al., 2017) and when the diet still contains a certain proportion of high-quality phytoplankton. In our study, the qualitative differences between *Pseudomonas* and t-POM were especially apparent at the lowest *Rhodomonas* concentration. This can be attributed to the generally poor digestibility of t-POM (Wenzel et al., 2012a), and/or physical interference of t-POM (i.e. the terrestrial material was often stuck to the animals' carapaces in the high t-POM treatments), causing moulting difficulties and clogging of the filtering apparatus, probably reducing the capability in *Daphnia* to ingest phytoplankton. It can also be attributed to the lack of essential minerals (P) and/or biochemicals (FAs). In our previous study (cf. Wenzel et al., 2012b), measured C:P ratios (in molar) in our t-POM (c. 360) were much higher compared to those in *Pseudomonas* (c. 50) and *Rhodomonas* (c. 70), whereas total FA content (mg FA/mg C) in t-POM (16) was lower than in *Pseudomonas* (59) and in *Rhodomonas* (372). In addition, our t-POM contained only saturated and monounsaturated FA, whereas *Pseudomonas* (although containing primarily saturated and monounsaturated FA) had low proportions of PUFA (ω -6 PUFA) in them that is, both in strong contrast to *Rhodomonas* which constituted about 60% of PUFA (ω -3 and ω -6 PUFA). Similar findings have been reported by Taipale et al. (2014) when comparing the nutritional value of phytoplankton, heterotrophic bacteria, and terrestrial particles (although the authors used a different type of t-POM, a riparian grass (*Phragmites australis*)). The authors observed biochemical deficiencies in bacteria while carbon and nitrogen were assimilated efficiently by *Daphnia* in the presence of high-quality phytoplankton that provided essential FAs and sterols. However, the t-POM diets of low ω -3 PUFA content only supported low *Daphnia* growth compared to mixtures of bacteria and phytoplankton. A recent study by Hiltunen et al. (2019) further confirms the poor biochemical quality of t-POM (here originating from leaves and *Sphagnum* moss; i.e. *Daphnia* growth rates did not increase, even under conditions of high t-POM food availability when phytoplankton quantities were low).

We know from previous experiments that a 100% pure diet of our peat layer t-POM, or *Pseudomonas*, cannot sustain survival, growth or reproduction of *D. galeata* (Wenzel et al., 2012a, b). Thus, one could therefore argue from our results in the *Pseudomonas* treatments (i.e. similar to the arguments by Brett et al. (2009) for t-POM; and Taipale et al. (2014) for heterotrophic bacteria and t-POM), that even low levels of phytoplankton in *Daphnia* diets may greatly improve their ability to use low-quality resources. However, for bacteria, it has to be noted that the quality of bacterial strains and taxa may differ substantially. Taipale et al. (2012) compared the nutritional value of three bacterial taxa and reported different growth and reproduction responses of *Daphnia*. A study by Freese and Martin-Creuzburg (2013) showed that some bacterial strains may even be toxic for zooplankton, notably a strain of *Pseudomonas*. The presence of hormesis, where opposite effects in small and large doses of an exposure are seen in organisms due to compensation for an initial disruption/damage caused by a toxin (Stanley et al., 2013), might also be the reason for the beneficial effect in *Daphnia* growth noted when low concentrations of bacteria were added to the low phytoplankton treatment (cf. Figures 1–2). However, we cannot conclude from our experiment whether indigestibility, poor content of essential nutrients and/or biochemicals, or toxicity reduces food quality for our *D. galeata* clone, so this remains to be tested.

The ability to use bacterial carbon in the presence of phytoplankton may also decrease over time. For example, the assimilation of cyanobacterial carbon by *Daphnia* has been shown to be dependent on essential lipid reserves, leading to a decline in carbon assimilation over time when feeding on cyanobacteria-dominated diets (DeMott & Muller-Navarra, 1997). This mechanism may as well apply to diets dominated by heterotrophic bacteria and explain why mortality in our study increased during the second half of the experiment when low amounts of *Rhodomonas* were supplemented with *Pseudomonas*. Thus, bacteria can temporally be an important subsidy to overcome periods of low phytoplankton abundances. However, in the long run, this will not be enough to support population growth.

Somatic growth rates alone are not enough to evaluate food quality as organisms often have different requirements during different life stages (Sterner & Schulz, 1998). In *Daphnia*, juveniles show a higher sensitivity to mineral deficiencies (P) (Urabe & Sterner, 2001), while biochemical limitation plays a more pronounced role for reproductive output in later life stages (Becker & Boersma, 2003). In agreement with these findings, juvenile survival was lower with the addition of t-POM with low P content compared to P-rich *Pseudomonas*. Differences in egg and juvenile production, on the other hand, were less pronounced between t-POM and *Pseudomonas* addition treatments which both are deficient in FAs (Wenzel et al., 2012a) and probably also sterols. This underlines the importance of biochemicals for reproductive success. Polyunsaturated FAs especially may be important for egg production in daphnids, and the minimum PUFA concentrations needed for successful reproductive output in natural systems may be even higher than previously anticipated (Ravet et al., 2012).

While our medium and high *Rhodomonas* experiments may be representatives of productive, nutrient-rich eutrophic lakes, the low *Rhodomonas* concentration tested resembles conditions that zooplankton can experience in humic lakes where phytoplankton biomass seldom exceeds 0.1 mgC/L (Jansson et al., 2003). Here, when feeding on *Rhodomonas* only, daphnids were energy limited and did not perform as well as with moderate additions of *Pseudomonas*. These intermediate additions in the low *Rhodomonas* treatments are similar to conditions in humic lakes where bacteria:phytoplankton biomass ratios of up to 3:1 have been reported (Hessen, 1992; Jansson et al., 2003). Thus, the presence of heterotrophic bacteria may not only increase food quantity but also quality for *Daphnia* by supplying essential minerals such as P as long as a certain concentration of high-quality phytoplankton is still available to meet biochemical needs. In contrast to the positive impact of supplementing *Rhodomonas* with *Pseudomonas*, the addition of t-POM negatively affected *Daphnia* performance in all treatments. Thus, high concentrations of t-POM seem to reduce the capability of *Daphnia* to use phytoplankton. This finding is relevant and has implications for zooplankton population growth in natural systems where t-POM can be present in high concentrations (up to 5 mgC/L in unproductive, humic lakes) (Thurman, 1985).

We used one species of phytoplankton in our study that is known to be a high-quality food for zooplankton (Brett & Müller-Navarra, 1997). In lakes, a mix of different species or taxonomic groups of phytoplankton will always be present and the composition of the algal community may undergo pronounced successional changes across seasons. As different algal taxa contain varying amounts of minerals and biochemicals and differ in their digestibility (Hessen & Leu, 2006; Sterner & Elser, 2002; Taipale et al., 2013), high-quality food may only be available in sufficient quantities during certain periods of the year. Therefore, zooplankton may in general benefit from mixed diets as different food components can constitute complementary resources, as illustrated in experiments feeding copepods (Twombly et al., 1998), cladocerans (Boersma & Vijverberg, 1995), and rotifers (Rothhaupt, 1991) with different phytoplankton. It can be expected that combinations of algal and non-algal food (e.g. heterotrophic bacteria) have similar effects, although it has not yet been thoroughly investigated.

We recognise that there can be considerable variation in the response to various environmental conditions among *Daphnia* species, among clones, and within clones (e.g. Gorokhova et al., 2002; Wolinska et al., 2007), but the response to food quality has been shown to be at least qualitatively similar among clones in a study using mixtures of low- and high-quality phytoplankton (Bednarska et al., 2014). Hence, we propose that our findings for this clone of *D. galeata* are likely to be qualitatively applicable to non-selective filter-feeding cladocerans in general, but not to selective suspension feeders such as diatomid copepods. However, the quantitative response may not be the same across clones or species of cladocerans. This is also supported by stable isotope studies that report the use of more autochthonous food sources by copepods compared

to cladocerans (Berggren et al., 2014; Karlsson et al., 2015). Hence, cladocerans will be probably more prone to food quality limitations than copepods when high-quality phytoplankton is heavily diluted in lake systems with high inputs of terrestrial material. It has also been shown that zooplankton in general show higher allochthonous stable isotope signals in systems with high abundances of heterotrophic bacteria (Karlsson et al., 2003, 2007), which are either consumed directly (Karlsson et al., 2004) or via intermediate trophic levels such as ciliates and flagellates (Hiltunen et al., 2017; Jansson et al., 2007). Hence, direct consumption of bacteria and/or via trophic upgrading via the microbial loop (Hiltunen et al., 2017; Jansson et al., 2007), and not direct t-POC consumption (this study, Taipale et al., 2014) seems to be the major pathway of terrestrial carbon to metazoan zooplankton.

In summary, our results show that the availability and amount of high-quality autochthonous phytoplankton production is a major factor that determines the performance of this clone of *D. galeata*, and we suggest that qualitatively similar patterns may occur in other unselective filtering feeding cladocerans. We found that heterotrophic bacteria, but not peat layer t-POM, can be a temporally important component of *Daphnia* diets in boreal systems where the concentration of phytoplankton alone is limiting. Terrestrial inputs of organic carbon may therefore support higher trophic levels by serving as a potential substrate for bacterial growth, either through direct grazing on heterotrophic bacteria (by cladocerans) or via intermediate bacterivorous consumers such as ciliates or flagellates (by cladocerans and copepods). In more nutrient-rich systems, however, where non-limiting concentrations of phytoplankton are present, both heterotrophic bacteria and t-POM might decrease especially cladocerans performance due to a dilution effect.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

ORCID

Ann-Kristin Bergström  <https://orcid.org/0000-0001-5102-4289>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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