From lethal to beneficial – contrasting dietary effects of freshwater mixotrophs on zooplankton

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Abstract

1. The importance of mixotrophic algae as key bacterivores in microbial food webs is increasingly acknowledged, but their effects on consumers is less understood, with previous studies having revealed contrasting results. In freshwater, this may be related to fundamental differences in the nutritional quality of two major mixotrophic groups. While cryptophytes are generally considered as high-quality food for zooplankton, chrysophytes (golden algae) are often referred to be toxic.
2. Using four chrysophyte species, we performed a comparative study as an attempt to generalize their dietary quality by (1) revealing their stoichiometric and biochemical profiles, and (2) quantifying their dietary effects in feeding bioassays with *Daphnia longispina*. We compared the observed effects to a known high-quality reference food (*Cryptomonas* sp.) and a starvation control as a reference for potential toxicity.
3. We found dramatic differences in survival and growth of *D. longispina* depending on the chrysophyte species provided as food. Even within the same genus, dietary quality ranged from deleterious to high. As this was not reflected in differences in cellular stoichiometry and fatty acid profiles, we suggest that toxicity may be the underlying mechanism.
4. Our results suggest that the dietary effects of chrysophytes cannot be generalised. Besides, the fact that a species previously reported to be deleterious turned out to be a beneficial food source suggests that toxic effects may dynamically vary depending on environmental cues, mode of nutrition or the investigated strain.
5. To fully understand the nutritional value of mixotrophic algae in aquatic food webs, representatives of multiple taxa need to be tested under a range of environmental conditions. This is also needed for a better predictive capability of climate change effects, as it may not only promote the dominance of mixotrophic algae, but also induce functional changes related to their nutritional quality.

1. Introduction

Increasing evidence shows that mixotrophic algae are a dominant component of phytoplankton in the oligotrophic surface waters of oceans (Zubkov & Tarran, 2008; Hartmann *et al.*, 2012) and oligo- and mesotrophic lakes (Watson, McCauley & Downing, 1997; Pålsson & Granéli, 2004; Ptacnik *et al.*, 2008). This is due to their ability to combine photosynthesis and the ingestion of small particles, e.g. bacteria-sized plankton, which can give them a competitive edge over specialized phototrophic and heterotrophic competitors under nutrient limitation (Katechakis & Stibor, 2006; Kamjunke, Henrichs & Gaedke, 2007; Fischer *et al.*, 2017). In these ecosystems, mixotrophs can account for a large fraction of bacterivory (Bird & Kalff, 1987; Domaizon, Viboud & Fontvieille, 2003; Hartmann *et al.*, 2012), and thereby play a key role in carbon cycling (Mitra *et al.*, 2014). While a number of recent studies attempted to quantify their importance as bacterivores (Domaizon *et al.*, 2003; Hartmann *et al.*, 2012; Ptacnik *et al.*, 2016), their impact on higher trophic levels remains rather elusive. As climate change-related processes, such as browning and prolonged summer stratification, are expected to contribute to an increased dominance of mixotrophic algae (Bergström *et al.*, 2003; Wilken *et al.*, 2018), a better understanding of their impact on consumers is critical.

Recent global models on the importance of mixotrophic plankton suggest that mixotrophy increases trophic transfer efficiency in the pelagic food web (Ward & Follows, 2016; Stoecker *et al.*, 2017). This is in line with the observation that cellular stoichiometric ratios of mixotrophs are less variable than that of autotrophs, which in turn may have a stabilizing effect on secondary production (Katechakis *et al.*, 2005; Moorthi *et al.*, 2017). At the same time, it is challenged by the observation that harmful algal blooms are often associated with mixotrophic algae (Burkholder, Glibert & Skelton, 2008; Watson *et al.*, 2015; Flynn *et al.*, 2018).

In lake phytoplankton, there are two widespread groups of nanoflagellates (<20 μm) in which mixotrophy is prevalent: chrysophytes and cryptophytes (Sanders & Porter, 1988). Among them, cryptophytes are widely considered as high-quality food for zooplankton based on their high cellular content of essential fatty acids (Ahlgren *et al.*, 1990; von Elert & Stampfl, 2000). In contrast, surprisingly little is known regarding the dietary effects of chrysophytes on higher trophic levels, despite the fact that they often dominate phytoplankton assemblages. The majority of existing studies reported detrimental effects on zooplankton growth, reproduction and survival suggesting grazing resistance (Vad *et al.*, 2020) and toxicity (Leeper

& Porter, 1995; Boxhorn, Holen & Boraas, 1998; Boenigk & Stadler, 2004; Zhang *et al.*, 2011; Hiltunen, Barreiro & Hairston, 2012) as underlying mechanisms. The biochemical food quality profiles of chrysophytes represent a knowledge gap. Except for sporadic data on individual species (Ahlgren *et al.*, 1990; Boëchat *et al.*, 2007), comprehensive studies on their cellular stoichiometric ratios and fatty acid profiles are largely lacking. Whether this contradiction on the food quality of freshwater mixotrophs is specific to major groups (i.e. chrysophytes vs cryptophytes) or it is related to inter-specific differences, is an important question to be solved to better understand the roles of mixotrophic algae in lake food webs.

Major grazers of mixotrophs, and primary producers in general, are zooplankton. They represent key elements in lakes as they contribute to the structuring of food webs by linking primary production with higher trophic levels (Lindeman, 1942; Carpenter & Kitchell, 1996). There is ample evidence that the efficiency of trophic transfer through zooplankton depends strongly on algal food quantity and food quality (Sterner & Hessen, 1994; Müller-Navarra & Lampert, 1996; Brett & Müller-Navarra, 1997). Assuming predominantly detrimental dietary effects of mixotrophic chrysophytes on consumers, a shift in the phytoplankton assemblage in oligotrophic systems towards these algae may have profound effects on zooplankton secondary production and even fish production.

The aim of this study is to, a) identify the stoichiometric (C:N:P ratios) and biochemical (fatty acid composition) profiles of unicellular chrysophyte species to assess their nutritional quality, and, b) perform a comparative test to quantify their dietary effects on *Daphnia*. We hypothesize that the effects may be species-specific, but in general detrimental compared to *Cryptomonas* used as a high-quality reference food.

2. Material and methods

2.1. Food quality

As a first step in order to assess their stoichiometric and biochemical food quality, three species of chrysophytes, *Ochromonas danica* (SAG 933-7), *O. tuberculata* (CCAP 933/27), *Poterioochromonas malhamensis* and a cryptophyte (*Cryptomonas* sp. SAG 26.80) were cultivated as mono-algal, but non-axenic cultures in a medium with reduced phosphorus concentration to expose them to conditions more similar to the natural environments where mixotrophic algae dominate. It is known that many mixotrophic species

only engage in or have increased rates of bacterivory when nutrients limit their growth (Nygaard & Tobiesen, 1993; Keller *et al.*, 1994). Thus, using a medium with reduced nutrient concentration not only provided more natural conditions, but also likely induced mixotrophic growth. The medium consisted of 90% sterile-filtered lake water from the oligotrophic (5–8 µg total phosphorus L^{-1}) Lake Lunz (situated in the Eastern Alps in Austria, 608 m a.s.l, 47°51.2'N 15°3.1'E) enriched with WEES medium (WEES Medium Recipe vers. 03.2007, Göttingen University, Kies, 1967) modified by leaving out soil extract and with only 10% of the phosphorus concentration relative to the original recipe. Total phosphorus concentration in the medium therefore was ~50 µg L^{-1}. We also applied two different treatments (both in three replicates) to test how nutritional profiles depend on the predominant nutritional mode (i.e. mixotrophy vs photoautotrophy). To induce predominantly mixotrophic nutrition, bacterial growth was stimulated by a daily addition of an allochthonous carbon source (i.e. glucose) at a concentration of 10 mg L^{-1}. The experiment was terminated and samples for analyses (algae retained on pre-combusted and acid-washed Whatman GF/F glass microfibre filters) were taken at the point where algae reached stationary phase. Without glucose addition, the investigated chrysophytes did not show considerable growth resulting in insufficient biomass for further analyses (this was not the case with *Cryptomonas* sp.), therefore we only report their stoichiometric and biochemical profiles resulting from the treatments with an external carbon source.

For obtaining cellular stoichiometric ratios, the amount of particulate organic carbon and nitrogen was determined by an elemental analyser (Elementar vario MICRO cube™). Particulate phosphorus was measured spectrophotometrically using the ascorbic acid colorimetric method (Hansen & Koroleff, 2007) after persulfate digestion (Clesceri, Greenberg & Eaton, 1999).

The analysis of lipids and their fatty acids followed the steps described by Heissenberger, Watzke & Kainz (2010). In brief, samples were freeze-dried and homogenized before analyses. Fatty acid methyl esters (FAME) were analysed by a gas chromatograph (Thermo Scientific TRACE GC Ultra equipped with a flame ionization detector) and separated with a Supelco™ SP-2560 column. FAME were identified using known standards (Supelco™ 37-component FAME Mix). The analyses were done on triplicate samples per each algal species.

2.2. Feeding bioassay

Prior to the experiment, a clone of the cladoceran *Daphnia cf. longispina* (belonging to the *D. longispina* complex, hereinafter referred to as *D. longispina*) was isolated from Lake Lunz. *D. longispina* cultures were kept under constant conditions and fed with *Cryptomonas* sp., which is known to be of high nutritional value for zooplankton (Martin-Creuzburg, Elert & Hoffmann, 2008). Third-brood neonates (<24 hours old) from a previously established F2 generation were used in the bioassays. This procedure aims to achieve a standardized response of juveniles during the experiment and a reduction of maternal effects.

The experiment consisted of six treatments each representing a different algal food source. These were as follows: (1) *O. danica*, (2) *Ochromonas cf. perlata* isolated from Lake Lunz (hereinafter referred as *O. perlata*), (3) *O. tuberculata*, (4) *P. malhamensis*, (5) *Cryptomonas* sp. used as a high-quality reference food and a (6) starvation treatment as control. These nanoflagellates all fall in the size range that can be exploited by *Daphnia*, therefore size-dependent differences in ingestion can be largely excluded as drivers of the observed dietary effects (Table 1). Chrysophytes were cultivated in a medium consisting of 90% lake water (sterile-filtered water of Lake Lunz, filter pore size: 0.2 µm) and 10% WEES medium without soil extract. The phosphorus concentration in the medium was higher than in the cultivation medium for food quality analyses, to obtain sufficient algal biomass in the feeding bioassays. We also stimulated bacterial growth (with glucose at a concentration of 10 mg L^{-1}) to enhance algal growth, but only at the beginning of cultivation (two times in the first week, i.e. well before using them in the experiment) to ensure that results of the feeding bioassay were unaffected by bacteria. This also means that chrysophytes used in the experiment were predominantly growing photo-autotrophically. *Cryptomonas* sp. was cultured in 20% WEES and 80% sterile-filtered lake water (owing to insufficient growth in the more diluted medium). Algae were kept in an exponential growth phase by frequent dilution of the cultures with new medium.

The feeding bioassay was run in sterile-filtered lake water (filter pore size: 0.2 µm) taken from Lake Lunz, enriched with the respective algal species to attain food-saturated conditions of 1 mg carbon L^{-1}. Calculation of algal biomass was based on cell concentrations and species-specific cell biovolumes. Algal biovolumes (Table 1) were calculated using conversion factors for corresponding geometrical shapes of each respective species (Hillebrand *et al.*, 1999), after measuring axial dimensions of at least 30 cells per species

(AxioVision software, Carl Zeiss GmbH). By using a conversion factor of 14% of algal wet weight (Vadstein *et al.*, 1988), carbon biomass was estimated from biovolume. The starvation control consisted of 0.2-µm-filtered lake water, without any food supply.

At the start of the experiment, three *D. longispina* neonates were introduced to glass vials containing the experimental food suspension of the corresponding treatments (30 ml volume). Each treatment was replicated five times. Animals were transferred into a fresh experimental medium every other day to maintain food-saturated conditions. During the preparations and the incubation period, all flasks were kept at 18°C and a 16:8 hours light:dark photoperiod (illumination: ~ 170 µmol photon m^{-2} s^{-1}). Survival and the number of moults, as a proxy for somatic growth (Hessen & Rukke, 2000), were recorded daily. *D. longispina* were considered dead when lying on the bottom of the vial without any movement. During the experiment, vials were gently mixed twice a day to reduce sedimentation and surface accumulation of the algae. The experiment was run for 11 days in total (i.e. the day after *D. longispina* started releasing juveniles).

2.3. Data analysis and statistics

Treatment-specific survival was analysed by (1) testing for differences in life span (i.e. the number of days survived), and (2) following the temporal (i.e. daily) pattern of survival. Given the non-normal distribution of residuals resulting from a one-way ANOVA (tested by Shapiro Wilk test), life span data were analysed by non-parametric Kruskal-Wallis tests followed by Dunn's *post hoc* test (p values were adjusted to account for multiple comparisons with the Holm method) to detect pairwise differences among treatments. Daily pattern of survival (i.e. percentage of living individuals) was analysed by testing for pairwise differences between each treatment and the starvation control via Wilcoxon rank-sum tests. Separate tests were run for each day and treatment. As further indicators of the condition of animals, somatic growth (cumulative number of moults) and the number of mature females of *D. longispina* at the termination of the experiment were also recorded and analysed as described for life span. All statistical analyses were done with the R software (R Core Team, 2020).

3. Results

3.1. Stoichiometric and biochemical profile of chrysophytes

The C:P ratios of all chrysophyte species ranged between 100 and 200, while N:P ranged between 20 and 30 (Table 1) . In *Cryptomonas,* both ratios were dependent on whether it was grown with an external carbon source, with higher values when supplied with glucose.

Considering fatty acid profiles, *O. danica* was outstanding with a higher amount of polyunsaturated fatty acids (PUFA) than *O. tuberculata* and *P. malhamensis*, at the same time comparable to that of *Cryptomonas* sp. (Table 2). Furthermore, all individual PUFA occurred in higher amounts in *O. danica* than in the other two chrysophytes (see Table S1 in Supporting Information).

3.2. Feeding bioassay

The life span of *D. longispina* was significantly influenced by the food source provided (Kruskal-Wallis test: $\chi2 = 24.4$, df = 5, $p < 0.001$; Fig 1.) and was highest when fed on the reference food *Cryptomonas* sp. and the chrysophytes *O*. *perlata* and *O. tuberculata*. All of these treatments resulted in somewhat higher (though not significantly different) values than the starvation control. In contrast, feeding on the other two chrysophytes resulted in shorter life span. *O. danica* had the strongest negative effects, causing significantly lower life span than *Cryptomonas* sp. and the chrysophytes *O. perlata* and *O. tuberculata*.

Within the first three days, a severe drop in survival of *D. longispina* fed on *O. danica* could be observed, resulting in significantly lower survival than in the starvation control (Fig 2). On *P. malhamensis*, high mortalities occurred later in the experiment leading to the death of all neonates by day 10 (significantly lower survival compared to the starvation control on days 8–10; Fig. 2). Survival on *Cryptomonas* sp., *O. perlata* and *O. tuberculata* stayed high throughout the experiment, and was significantly higher compared to the starvation control towards the end of the experiment.

Similar to life span, the number of moults (a proxy for somatic growth) was also significantly influenced by the food source provided (Fig. 3, Kruskal-Wallis test: $\chi2 = 24.2$, df = 5, $p = < 0.001$). Highest numbers of moults were observed on *Cryptomonas* sp., *O. perlata* and O. *tuberculata*, showing no statistical difference among these treatments. Moulting was suppressed on *O. danica* and *P. malhamensis*, and was significantly lower compared to that

on *Cryptomonas* sp.. Somatic growth was furthermore significantly reduced on *O. danica* compared to the other two *Ochromonas* species. The value for moulting success in the starvation control was between the values for the treatments with high and low moulting rates.

Consistent with the results on life span and somatic growth, *D. longispina* reached maturity on *Cryptomonas* sp., *O. perlata* and *O. tuberculata*, but not in the other treatments. Interestingly, the mean number of mature *D. longispina* (i.e. with eggs or embryos) at the time of termination of the experiment was significantly higher on *O. perlata* and *O. tuberculata* compared to the reference food *Cryptomonas* sp. (Kruskal-Wallis test: $\chi 2 = 9.8207$, df = 2, p = < 0.01, Table 3). Within these two chrysophyte treatments, almost every individual carried eggs or had juveniles at the time of termination.

4. Discussion

These feeding bioassays clearly show that dietary effects of mixotrophic chrysophytes can largely vary even among taxonomically closely related species, from beneficial to lethal consequences for *Daphnia*. These results also indicate that the dietary value of freshwater mixotrophs cannot be assigned at the level of major groups (e.g. phylum or class), i.e., chrysophytes do not necessarily represent a suboptimal food source compared to cryptophytes. The fact that species with similar size, cellular stoichiometric ratios and fatty acid composition can differ dramatically in their dietary effects shows that other biological traits, such as toxicity, need to be put in focus of future research to understand the underlying mechanisms.

In the genera *Ochromonas* and *Poterioochromonas*, adverse effects on zooplankton were reported in earlier studies (Katechakis *et al.*, 2005; Zhang *et al.*, 2011; Hiltunen *et al.*, 2012). Moreover, toxic compounds produced by members of these genera were also observed (Reich & Spiegelstein, 1964; Halevy, Saliternik & Avivi, 1971). Our results point out that the negative effects of chrysophytes on zooplankton cannot be generalised. Among the tested taxa, *O. danica* and *P. malhamensis* had clear negative dietary effects on zooplankton, but *O. tuberculata* and *O. perlata* proved to be adequate food largely comparable or, in case of reproductive success, even better than the reference diet *Cryptomonas* sp.

Especially *O. danica* suppressed zooplankton survival and growth. Feeding on this chrysophyte resulted in a severe drop (~50%) in *D. longispina* survival in the first few days of the experiment, which suggests acute negative dietary effects. Although the identification of toxins was not part of the present study, the fact that mortality was higher than in the starvation control during the whole experimental duration suggests toxic effects (Lampert, 1981). Interestingly, after the rapid, immediate decline, survival stabilised. This may be a sign of acclimatization to toxins, which may happen through physiological responses (Schwarzenberger *et al.*, 2010) or changes in the gut microbiota (Macke *et al.*, 2017). However, this was not coupled with somatic growth (indicated by the lack of moults) and most juveniles (except for a single individual) died by the end of the 11-day experimental exposure without reaching maturity. Less acute, but still negative dietary effects were observed on *P. malhamensis*, with mass mortality occurring only in the second part of the experiment. This chrysophyte also suppressed somatic growth and all *D. longispina* died during the incubation period without reaching maturity. These results are altogether consistent with the findings of Hiltunen *et al.* (2012) on *O. danica* and Zhang *et al.* (2011) on *P. malhamensis*. Both studies suggested toxic effects of these chrysophytes on their consumers, however, *O. danica* seemed to be more lethal. These are in line with an earlier observation that *O. danica* contains higher amounts of toxins than *P. malhamensi*s (Reich & Spiegelstein, 1964). Differences in toxin levels may also well explain the less acute effect of *P. malhamensis* on *D. longispina* found in our study, but as this was similar to the starvation control, inadequate nutritional quality cannot be excluded either as the underlying mechanism. Additionally, we could observe a tendency of *P. malhamensis* to form sedimenting aggregates despite regular mixing. Hence, the possibility that an acute toxic effect of *P. malhamensis* was mitigated by lower food ingestion due to sedimentation cannot be excluded.

In contrast, the other two chrysophytes, *O. tuberculata* and *O. perlata,* appeared to be adequate food sources, their dietary effects were largely comparable to the reference food *Cryptomonas* sp. On both of these treatments, only one single *D. longispina* died early in the experiment and all others reached maturity. Interestingly, our results even show that *D. longispina* fed with these two *Ochromonas* strains showed higher reproductive success than on the reference food *Cryptomonas* sp.. This observation on *O. tuberculata* contradicts with previous findings on the species, which reported toxic effects on *Daphnia magna* using the same strain as the present study (Katechakis *et al.*, 2005). Toxin production in 'toxic' algae

often is strain-specific (Otsuka *et al.*, 1999) and often depends on cellular physiological state and environmental cues such as light or nutrient conditions (Watanabe & Oishi, 1985). The same holds for nutritional quality (Hessen, Færøvig & Andersen, 2002). In mixotrophs, cellular stoichiometry was found to be less dependent on environmental conditions than in autotrophs, which may ensure more stable secondary production (Katechakis *et al.*, 2005; Moorthi *et al.*, 2017). However, nutritional mode can modulate dietary quality, and existing studies suggest that osmotrophic nutrition may enhance deleterious effects (Leeper & Porter, 1995; Hiltunen *et al.*, 2012) and modulate fatty acid profiles (Boëchat *et al.*, 2007). These altogether suggest that dietary quality of mixotrophs may dynamically vary through space and time. In the bioassay of the present study, all algae were cultivated under conditions inducing predominantly photo-autotrophic nutrition, and it is possible that the observed effects on *Daphnia* may change with cultivation conditions. However, the dramatic differences in dietary responses resulting from algae grown under standardised conditions suggest that species identity also plays a key role in determining the nutritional value of chrysophytes.

Given the somewhat different experimental conditions, linking the recorded stoichiometric and biochemical profiles to the outcome of the feeding experiments has to be treated with caution. However, it is possible to draw conclusions on the nutritional value of algae and species-specific differences resulting from an experiment with standardised cultivation conditions. The very similar cellular C:P ratios suggest that no species-specific stoichiometric differences can be expected among these chrysophytes under similar environmental conditions. All chrysophytes had C:P ratios (100-200) favorable for *Daphnia* growth (Hessen *et al.*, 2013; Khattak *et al.*, 2018). However, the fatty acid composition of these chrysophytes did not match the anticipated consumer growth response. *O. danica* had higher quantities of essential fatty acids, and PUFA in general, than the other two chrysophytes. This was not reflected in the outcome of the feeding bioassay, where it represented the poorest food source, and altogether suggests that the nutritional quality, as often assessed by fatty acids and PUFA in particular (Mariash *et al.*, 2011; Galloway *et al.*, 2014; Strandberg *et al.*, 2015), may not be the main driver of the observed dietary effects of chrysophytes.

Conclusions and implications

This feeding study suggests that the dietary effects of chrysophytes can vary even in the same algal strain and effects of mixotrophs on consumers may be largely species-specific and are

not a function of major groups. With ongoing global change, it is possible that increasing dominance of mixotrophs will stabilize secondary production in lakes through maintaining relatively stable seston stoichiometric ratios (Moorthi *et al.*, 2017). But this may be dependent on environmental factors e.g. increased input of dissolved organic carbon to lakes may induce a switch in the dominant nutrition mode. In chrysophytes, direct (osmotrophy) and indirect (phagotrophy) utilization of dissolved organic carbon can result in dramatically different consequences on consumers (Leeper & Porter, 1995; Hiltunen *et al.*, 2012). Warming itself modulates mixotrophy by shifting towards heterotrophy with rising temperature (Wilken *et al.*, 2013), but the consequences of this shift on dietary quality is not known. Based on these, we suggest that further studies need to (1) focus on the effects of environmental conditions, and (2) better understand the role of the dominant nutritional mode in determining the dietary quality of mixotrophs. These are needed to understand their roles in freshwater food webs more comprehensively.

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Author contributions

CFV, CS, and RP conceived the study. CS, RF and CFV performed the laboratory experiments. MJK carried out the biochemical analyses. CFV and CS analysed the data. CFV and CS wrote the first draft after which all authors provided critical feedback on the manuscript.

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Tables

Table 1 – Mean (± SD) cell dimensions and biovolumes of all the algae used in the study. For species resembling a 'prolate sphaeroid' shape, biovolume calculations were based on length and width, while for spherical species it was approximated from cell diameter.

Species	**Length (µm)**	**Width (µm)**	**Diameter (µm)**	**Biovolume (μm^3)**
Cryptomonas sp.	14.6 ± 1.7	8.3 ± 0.9	-	537.1 ± 142.4
Ochromonas danica	10.2 ± 1.7	7.8 ± 1.4		345.7 ± 175.2
Ochromonas tuberculata	-	-	11.1 ± 2.1	797.7 ± 519.7
Ochromonas perlata	-	-	6.9 ± 0.6	177.4 ± 49.5
Poterioochromonas malhamensis	-	-	7.2 ± 1.1	207.9 ± 107.8

Table 2 – C:P and N:P elemental ratios of algae along with mass fractions (µg mg dry weight^{-1}) of fatty acid groups, palmitoleic acid (16:1n-7), and the n-3/n-6 PUFA and algal/bacterial fatty acid ratios (data represent mean ± SD of three replicates). For chrysophytes, we present data resulting from the experiment with input of dissolved organic carbon (DOC), i.e. glucose, where they reached biomasses sufficient for the analysis. Fatty acid data on *Cryptomonas* with DOC-input are missing due to a technical error. (SAFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids, used as indicators of mostly algae-produced fatty acids; BFA – bacterial fatty acids).

	***Cryptomonas* sp. -DOC**	***Cryptomonas* sp. +DOC**	***O.danica***	***O. tuberculata***	***P.malhamensis***
C:P	100.9 ± 12.7	244.1 ± 7.7	155.0 ± 27.1	156.4 ± 10.9	155.8 ± 21.0
C:N	16.7 ± 1.6	29.1 ± 1.2	22.5 ± 3.9	21.9 ± 0.7	21.3 ± 3.8
SAFA	27.3 ± 6.9	-	53.6 ± 9.2	39.8 ± 15.6	43.5 ± 6.2
MUFA	14.3 ± 3.4	-	16.3 ± 3.7	10.9 ± 4.2	14.7 ± 1.5
PUFA	31.5 ± 2.2	-	29.8 ± 7.7	6.9 ± 2.6	5.2 ± 0.6
BFA	11.0 ± 1.5	-	12.3 ± 2.8	7.9 ± 3.1	10.6 ± 1.9
ω-3 PUFA	23.3 ± 1.4	-	15.5 ± 4.3	3.9 ± 1.5	3.4 ± 0.2
ω-6 PUFA	8.1 ± 1.0	-	14.3 ± 3.4	2.9 ± 1.1	1.8 ± 0.5
16:1n-7	4.6 ± 2.1	-	1.3 ± 0.3	3.5 ± 1.4	0.6 ± 0.1
n-3/n-6	2.9 ± 0.2	-	1.1 ± 0.1	1.3 ± 0.1	2.0 ± 0.5
PUFA/BFA	2.9 ± 0.3	-	2.4 ± 0.1	0.9 ± 0.0	0.5 ± 0.0

Table 3 – Mean (± SD) number of mature *D. longispina* (i.e. with eggs, embryos or juveniles) on the day of termination in the three treatments in which they were able to reach maturity. Analysis is based on Kruskal-Wallis test and subsequent Dunn's post hoc test (*N*=5). Asterisks indicate significant differences compared to *Cryptomonas* sp. ($p<0.05$)

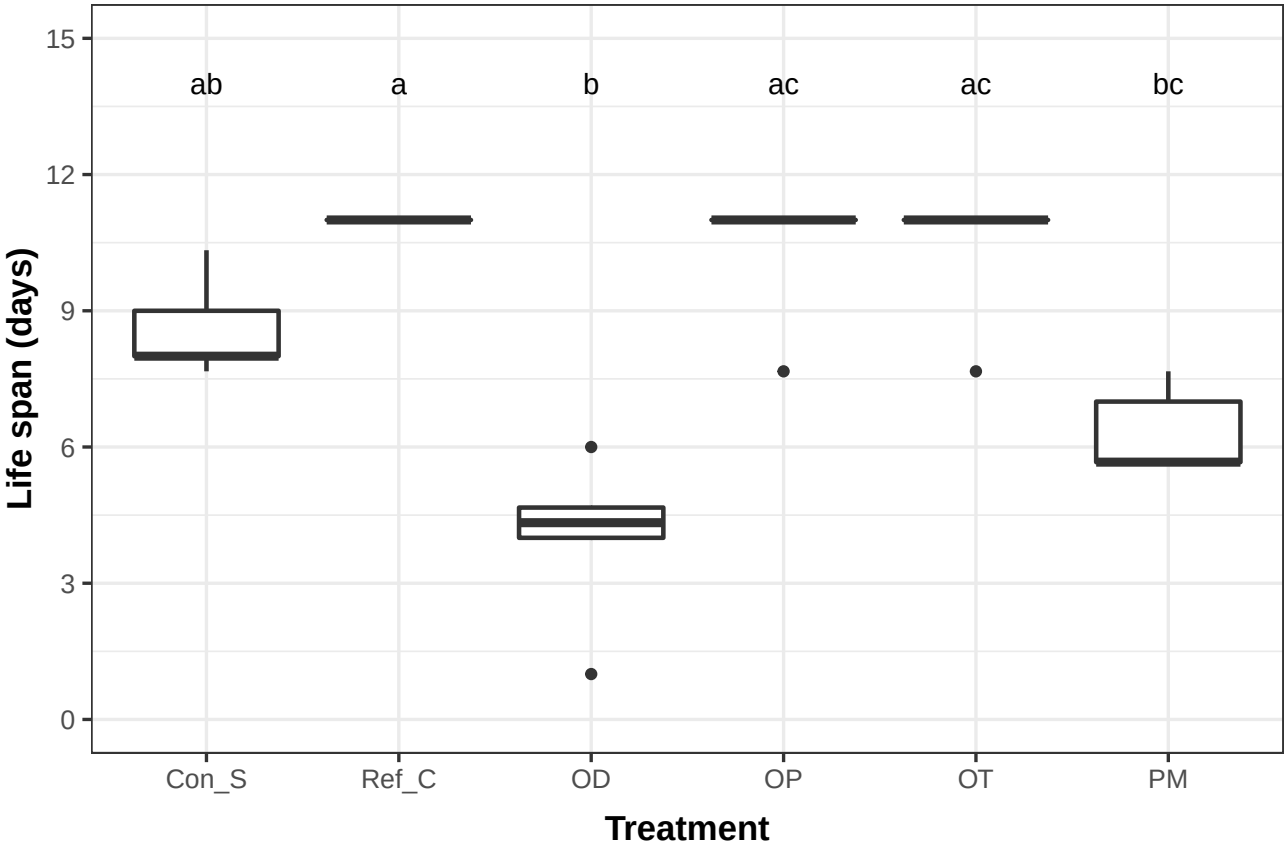
Treatment	No. of females with eggs / juveniles
Cryptomonas sp.	0.26 ± 0.26
Ochromonas perlata	0.90 ± 0.20 *
Ochromonas tuberculata	0.94 ± 0.12 *

Figure captions

Fig. 1 – Life span (days) of *D. longispina* fed by mixotrophic algal species (N=5 per treatment). Letters indicate significant differences between treatments ($p<0.05$) and are based on Dunn's post hoc test performed after a significant Kruskal-Wallis test. Treatments*:* Starvation control (Con_S), the reference food *Cryptomonas* sp. (Ref_C), *Ochromonas danica* (OD), *Ochromonas perlata* (OP), *Ochromonas tuberculata* (OT), *Poterioochromonas malhamensis* (PM).

Fig. 2 – Temporal pattern of *D. longispina* survival (mean ± SD) on the different algae and the starvation control. Asterisks indicate significant differences in comparison to the starvation control based on pairwise Wilcoxon rank-sum tests (significance levels: $p < 0.05$ '*', $p < 0.01$ '**'). Abbreviations: Starvation control (Con_S), the reference food *Cryptomonas* sp. (Ref_C), *Ochromonas danica* (OD), *Ochromonas perlata* (OP), *Ochromonas tuberculata* (OT), *Poterioochromonas malhamensis* (PM).

Fig. 3 – Cumulative number of moults (a proxy for somatic growth) during the incubation period (11 days, *N*=5 per treatment). Letters indicate significant differences between treatments ($p<0.05$) and are based on Dunn's post hoc test performed after a significant Kruskal-Wallis test. Treatments: Starvation control (Con_S), the reference food *Cryptomonas* sp. (Ref_C), *Ochromonas danica* (OD), *Ochromonas perlata* (OP), *Ochromonas tuberculata* (OT), *Poterioochromonas malhamensis* (PM).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Life span (days)
Con_S
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OD
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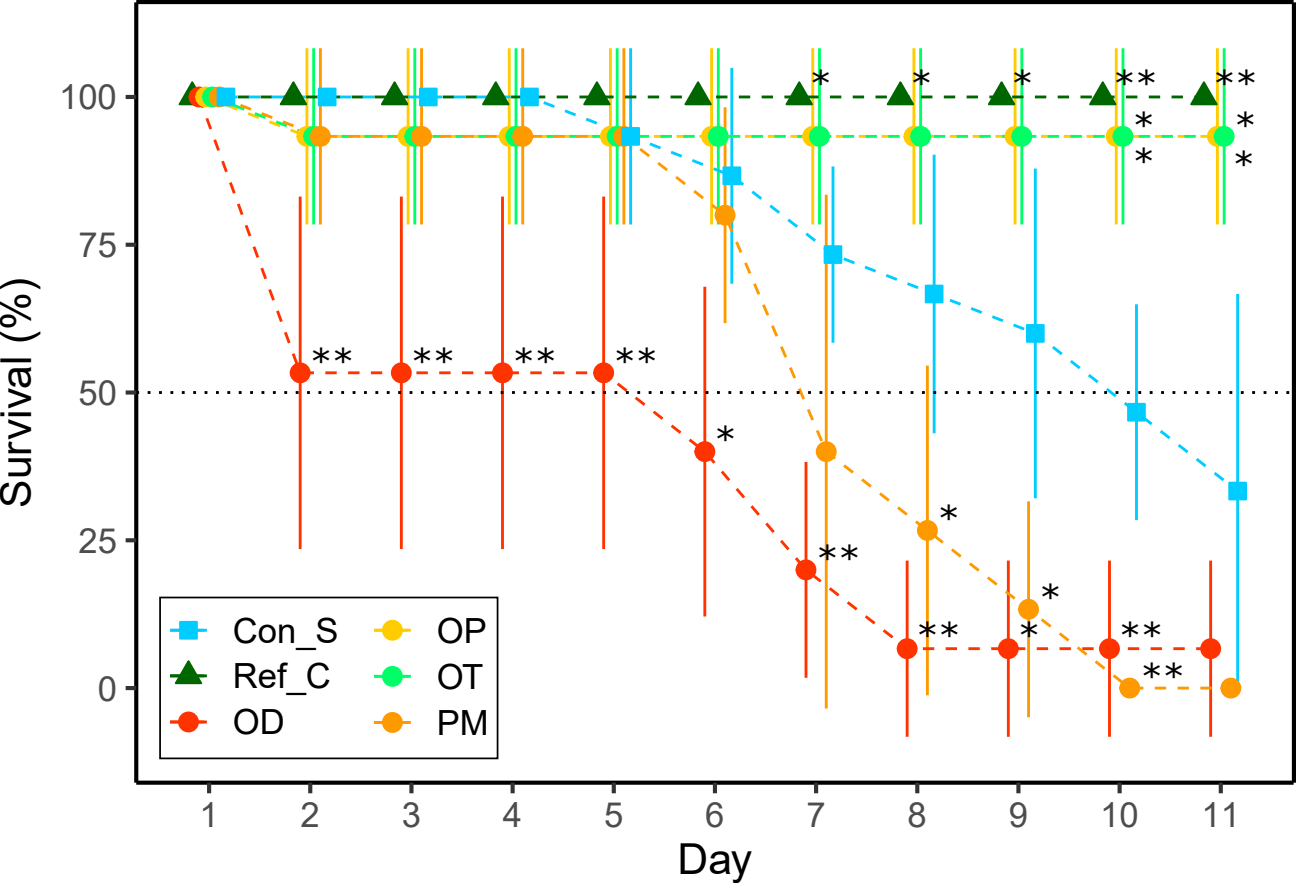
Treatment


Survival (%)
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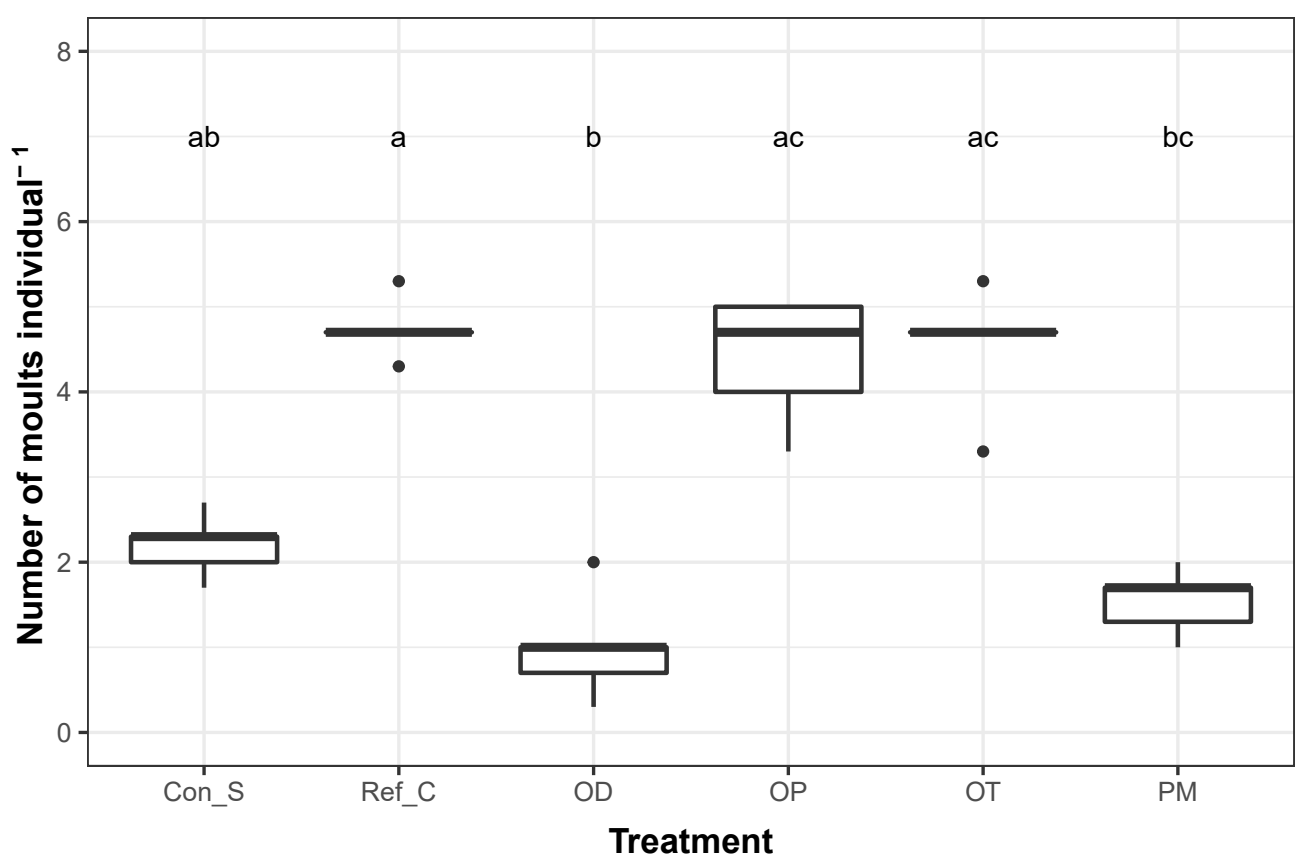
Number of moults individual⁻¹
8
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Treatment