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Article

Complex Trophic Interactions in an Acidophilic Microbial Community

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Abstract: Extreme habitats often harbor specific communities that differ substantially from non-extreme habitats. In many cases, these communities are characterized by archaea, bacteria and protists, whereas the number of species of metazoa and higher plants is relatively low. In extremely acidic habitats, mostly prokaryotes and protists thrive, and only very few metazoa thrive, for example, rotifers. Since many studies have investigated the physiology and ecology of individual species, there is still a gap in research on direct, trophic interactions among extremophiles. To fill this gap, we experimentally studied the trophic interactions between a predatory protist (*Actinophrys sol*, Heliozoa) and its prey, the rotifers *Elosa woralli* and *Cephalodella* sp., the ciliate *Urosomoida* sp. and the mixotrophic protist *Chlamydomonas acidophila* (a green phytoflagellate, Chlorophyta). We found substantial predation pressure on all animal prey. High densities of *Chlamydomonas acidophila* reduced the predation impact on the rotifers by interfering with the feeding behaviour of *A. sol*. These trophic relations represent a natural case of intraguild predation, with *Chlamydomonas acidophila* being the common prey and the rotifers/ciliate and *A. sol* being the intraguild prey and predator, respectively. We further studied this intraguild predation along a resource gradient using *Cephalodella* sp. as the intraguild prey. The interactions among the three species led to an increase in relative rotifer abundance with increasing resource (*Chlamydomonas*) densities. By applying a series of laboratory experiments, we revealed the complexity of trophic interactions within a natural extremophilic community.

Keywords: acid mine drainage; extremophiles; food web; heliozoa; intraguild predation; mining lakes; Rotifera



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1. Introduction

Extreme habitats occur all over the planet from the deep sea to hot deserts, from cold dark caves to hydrothermal springs and many more. They differ in their type and degree of extremeness, but organisms require special adaptations to cope with each [1]. One type of extreme, aquatic habitat is extremely acidic lakes. Most natural water bodies, marine and freshwater, have a circum-neutral pH in the range of 6 to 8.5. Peat bogs are typically acidic (pH around 5–6), but extremely acidic water bodies have a pH < 2.8 (according to the classification of Nixdorf et al. [2]). Extremely acidic lakes can occur naturally, for example, due to volcanic activity [3], or be formed anthropogenically, for example, following the cessation of open-cast mining activities and acid mine drainage. A very low pH is often accompanied by high concentrations of (heavy) metal ions [4]. Under such harsh conditions, prokaryotes (bacteria and archaea) typically dominate the biota, and only a few eukaryotic species contribute to the biotic community [5–9]. Most of these eukaryotes are protists, for example, flagellated mixotrophs from the divisions chlorophyta, euglenophyta and heterokontophyta [10–14] or heterotrophs such as rhizopods, ciliates or heliozoa [15–17]. Acidophilic metazoans such as rotifers and crustaceans are rare, and only a few acidophilic species have been found [18–20].

Whereas the ecology and physiology of archaea, bacteria [4,21,22] and also some autotrophic/mixotrophic protists such as *Chlamydomonas* and *Ochromonas* have been intensively investigated [23,24], studies on their consumers, in particular their trophic interactions, are rare. To fill this gap, we studied the trophic interactions between the protistan top predator *Actinophrys sol* and its prey: the osmo-mixotrophic protist *Chlamydomonas acidophila* (a green phytoflagellate, Chlorophyta), the ciliate *Urosomoida* sp. (Hypotricha) and the metazoa *Cephalodella* sp. and *Elosa woralli* (Monogononta, Rotifera). Specifically, in a micro-scale experiment, we tested the short-term predation impact of *A. sol* on the ciliate and the rotifers depending on the nutritional status of the predator (starved, non-starved) and with and without accompanying algal food. In a population-dynamics experiment (meso-scale), we quantified the impact of *A. sol* on all three prey species. To further study the intraguild predation relationship between the common resource *Chlamydomonas* and the intraguild prey (*Cephalodella* sp.) and predator (*A. sol*), we ran a population-dynamics experiment at various levels of the common resource. Ultimately, we combined these results with those from previous studies [25] to produce two food web scenarios at different levels of productivity.

2. Materials and Methods

2.1. Organisms, Place of Origin and Culture Conditions

All organisms used in this study were isolated from two extremely acidic mining lakes (pH 2.6–2.8): *Actinophrys sol* [25], *Cephalodella* sp. (previously misidentified as *C. hodii*, [26]), *Elosa woralli* [27] and *Chlamydomonas acidophila* [28,29] from mining lake 111 and *Urosomoida* sp. [17] from Lake Goitsche, in the Lusatian region of Eastern Germany. Lake 111 is a small (0.11 km² surface area) brown-coal-mining lake and is located in the East of Germany (Lusatia; 51°29' N, 13°38' E). The mean (maximum) depth is 4.6 (10) m, and the lake is thermally stratified during summer [10]. All organisms were reared in a culture medium simulating the extreme pH (2.65) and the high mineral concentrations of Lake 111 [30]. This medium was particularly rich in iron (2.6 mM) and sulphate (19.74 mM). The nutrient concentrations were 0.44 mM nitrogen as potassium nitrate and ammonium sulphate and 6.5 µM phosphorus as potassium di-hydrogen phosphate. Heliozoan stock cultures were maintained with a mixed diet of *Chlamydomonas acidophila* and the two rotifer species as food sources. The rotifers and the ciliates were also cultured with *Chlamydomonas* as food. The mean individual cell volume of *A. sol* was 34,000 µm³ (±17,600 µm³, standard deviation) and was strongly dependent on feeding history. *Urosomoida* sp. has quite variable cell volume. The mean cell volume in our experiments was 2900 µm³ (±1900 µm³, standard deviation) and was lower than in a previous study 3800 µm³ (±1700 µm³, standard deviation) [16]. The two rotifer species ranged between 50,000 and 100,000 µm³, also dependent on their feeding history [26].

2.2. Experimental Set-Up

We used three experiments to investigate the trophic impact of *Actinophrys* on its prey. All of them were run in a climate chamber at 20 °C and with a light–dark cycle of 16:8 h.

(1) In a micro-scale experiment, we studied the predation using microtitre plates with a volume of 200 µL. Heliozoa were isolated from healthy stock cultures. Four treatments were applied: (i) a control, i.e., *A. sol* without any further treatment, (ii) *A. sol* plus 100,000 cells mL⁻¹ *Chlamydomonas* (~2.1 µg C mL⁻¹), (iii) *A. sol*, which were starved for 48 h prior to the experiment, and (iv) starved *A. sol* as in (iii) plus 100,000 cells mL⁻¹ *Chlamydomonas* (~2.1 µg C mL⁻¹). For each treatment, 24 to 36 *A. sol* were transferred separately into the wells of a microtitre plate, either filled with sterile medium or with the *Chlamydomonas* suspension. Subsequently, two individual prey items were added from a single prey species (either *Cephalodella* sp., *E. woralli* or *Urosomoida* sp.). After 24 and 48 h, the microtitre plates were examined under a binocular microscope, and the survival of the rotifers and *Urosomoida* sp. was recorded. Since some rotifers either produced an egg or died after 24 h, and since *Urosomoida* sp. grew during the experimental period, results are

shown only for the first 24 h time interval. Additionally, in the *Urosomoida* sp. experiments, 12 control treatments were set up with two ciliates and no Heliozoa to quantify the growth without predation. For the analysis, the difference between the number of ciliates in treatments containing *A. sol* and the mean of the treatments without *A. sol* was calculated. For all three predator–prey pairs, three independent sets were run.

(2) A second, meso-scale experiment was run to investigate the impact of *A. sol* on their prey using natural *A. sol* densities [16,24]. One problem encountered during the experiments was our ability to provide sufficient *Chlamydomonas* food to promote rotifer growth without negatively influencing *A. sol*, since the growth rate of *A. sol* is known to decrease when food particle densities are too high [25]. To address this, 500 Heliozoa plus 500 rotifers were added to a 100 mL *Chlamydomonas* suspension with a density of 8000 cells mL⁻¹ (~0.22 µg C mL⁻¹). This density was found to be suitable for the growth of *A. sol* and supported the growth of *E. woralli*. However, it was below the resource density threshold for *Cephalodella* sp. [26,31]. Three parallel flasks were set up with and without Heliozoa to compare the response of the prey to their predator. In the analogous meso-scale experiment with *Urosomoida* sp. (starting with 5000 animals per 100 mL), glucose (20 mg C L⁻¹) was added to the medium to support bacterial growth to provide food for the ciliates. Previous experiments showed that single-celled bacteria do not inhibit or promote the growth of *A. sol* [25]. This experiment was run in conical flasks that were gently shaken. Every two days, 12 mL were removed from each flask, fixed with Lugol's iodine and acidified with sulphuric acid to avoid iron precipitation. The volume removed was replaced with fresh medium. The sub-samples were subsequently examined by inverted light microscopy (Thalheim, Pulsnitz, Germany).

(3) Since we did not control for the resource level in the second experiment, a third experiment was run in which we used five different resource levels and kept them constant. As the intermediate species, we chose *Cephalodella* sp., because it was affected more by the predation of *A. sol* than *E. woralli*. In six-well microtitre plates, we filled three parallel wells with food suspensions of 5000, 10,000, 20,000, 40,000 and 100,000 cells mL⁻¹ *Chlamydomonas* and added 10 individuals of *A. sol* and 10 of *Cephalodella* sp. Each day, most of the food suspension was carefully removed with a fine glass pipette, the heliozoa and rotifers were counted in the remaining medium, and then fresh food suspension of the target concentration was resupplied. After eight days, the experiment was terminated by adding Lugol's iodine, and the final densities of the heliozoa and rotifers were determined.

2.3. Statistical Analysis

For the statistical analysis of the micro-scale experiments with rotifers, the proportion of *A. sol* having eaten a rotifer in relation to the total number of *A. sol* was calculated for each of the three runs separately. These proportions were arcsin-transformed and then analysed with an ANOVA, with treatment and species as independent factors. For the experiments with the ciliate, a different procedure had to be applied. The ciliates grew during 24 h, so we compared the number of ciliates without *A. sol* with the ciliate numbers with the predator. Therefore, we calculated the mean number of ciliates from the 12 predator-free controls and subtracted it from the mean number of ciliates with the predator. Mean differences among treatments were compared using a univariate ANOVA. To compare prey abundances with and without Heliozoa in the meso-scale experiments, a general linear model (ANOVA) with repeated measures was applied. All analyses were performed using SPSS, version 26 (IBM, New York, NY, USA).

3. Results

3.1. Micro-Scale Experiment

3.1.1. Rotifers

Actinophrys sol preyed on both rotifer species in all four treatments; however, predation varied among treatments and species (Figure 1): The proportion of heliozoa having eaten an individual *Cephalodella* sp. was higher than those having eaten an *Elosa woralli* individual

($F = 89.4$, $df = 1$, $p < 0.001$), suggesting a higher predation pressure on *Cephalodella* sp. For both rotifer species, a clear treatment effect was found ($F = 57.5$, $df = 3$, $p < 0.001$), and the proportion of heliozoa having eaten a rotifer was lowest in the treatment with non-starved *A. sol* plus *Chlamydomonas* (Tukey post-hoc test $p < 0.001$). The effect of the additional food source *Chlamydomonas* was overruled by pre-starvation of the animals, because predation on rotifers was high in the presence of *Chlamydomonas* when *A. sol* was starved prior to the experiment. Despite the similarities in the effect on the rotifers, the overall pattern differed among the two species (interaction of treatment * species, $F = 11.6$, $df = 3$, $p < 0.001$), for example, in the response to starvation.

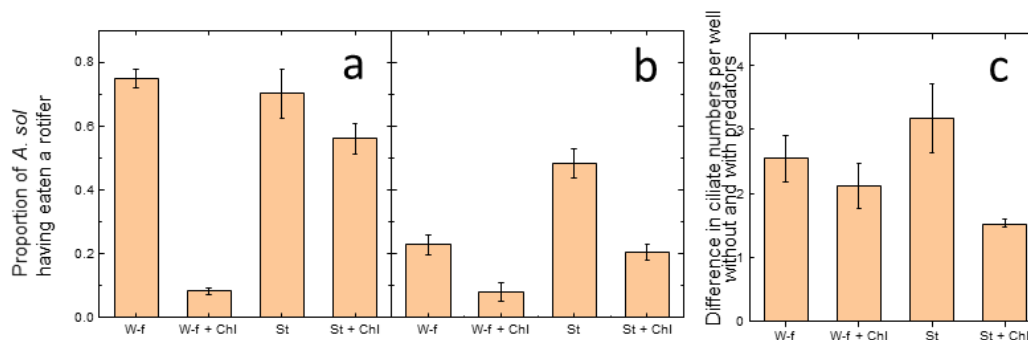


Figure 1. Feeding of *Actinophrys sol* on rotifers and ciliates after 24 h. Proportion of *A. sol* having eaten (a) a *Cephalodella* sp. individual or (b) an *Elosa woralli* individual. (c) Difference in ciliate numbers without and with predators. W-f, well-fed; St, starved; + Chl, 100,000 cells mL⁻¹, *Chlamydomonas* added. Mean \pm standard error, $n = 3$ sets of 24–30 individual wells. For rotifers, both the treatment and the species effect were significant, $p < 0.001$, as was the species \times treatment interaction ($p < 0.001$). For ciliates, no treatment effect was found ($p = 0.07$).

3.1.2. Ciliates

Overall, the variation within treatments in the response of the ciliates to heliozoan predation was high, and no significant differences among treatments were found ($F = 3.5$, $df = 3$, $p = 0.07$). On average, over all treatments, two more ciliates were found in the predator-free environment, suggesting a strong predation pressure on the ciliates (Figure 1c and Supplementary Materials, Table S1).

3.2. Meso-Scale Experiment

3.2.1. Rotifers

The results from the meso-scale experiment were strongly species-specific (Figure 2). Although the abundance of *Cephalodella* sp. was significantly different between treatments with and without *A. sol* ($F = 11.37$, $df = 1$, $p = 0.03$), their abundance in the treatment with *A. sol* over the experimental period was sometimes higher and sometimes lower than in the treatment without *A. sol*, showing no clear predation effect. In neither of the *Cephalodella* sp. treatments did the rotifers grow positively after day six, likely due to low *Chlamydomonas* densities. In contrast, *E. woralli* grew in both treatments or remained relatively constant when taking the experimental dilution into account. *E. woralli* abundances were lower when *A. sol* was present ($F = 10.05$, $df = 1$, $p = 0.03$), indicating a significant predation pressure on *Elosa* (Figure 2b). The growth of *A. sol* was on average lower in the treatment with *E. woralli* (Supplementary Materials, Table S2) than in the *Cephalodella* sp. Thus, *E. woralli* seems to be the less favourable food source.

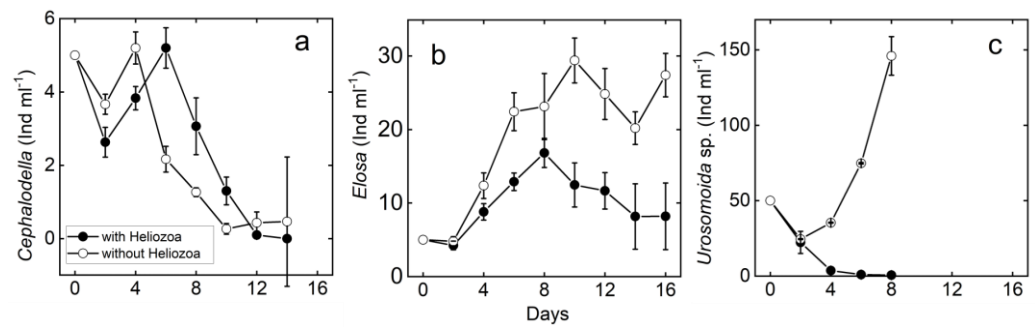


Figure 2. Time course of Heliozoan prey (**a**, *Cephalodella* sp.; **b** *Elosa woralli*; **c**, *Urosomoida* sp.) with and without *A. sol*. Mean \pm standard error ($n = 3$). Abundance of both rotiferan prey differed between treatments ($p = 0.03$ for both) and also for ciliates ($p < 0.001$). Note different y-axis scales.

3.2.2. Ciliates

The most pronounced predation pressure observed was by *Actinophrys sol* on *Urosomoida* sp. ($F = 284.09$, $df = 1$, $p < 0.001$). Within 6–8 days, *A. sol* drove the *Urosomoida* sp. population almost to extinction (Figure 2). In the absence of *A. sol*, *Urosomoida* sp. grew at a rate of approximately 0.36 d^{-1} . Thus, the results from the ciliate micro-scale experiments were reinforced with the meso-scale experiments (Figures 1c and 2c).

3.3. Resource Level Experiment

Increasing productivity applied as increasing numbers of *Chlamydomonas* significantly enhanced the growth of consumers. Mean consumer (*A. sol* + *Cephalodella* sp.) abundance increased from 3.1 mL^{-1} in the $5000 \text{ cells mL}^{-1}$ treatment to 402 mL^{-1} in the $100,000 \text{ cells mL}^{-1}$ treatment. This increase was almost exclusively due to the increase in *Cephalodella* with increasing productivity (from 1.5 mL^{-1} at $5000 \text{ cells mL}^{-1}$ to 382 mL^{-1} at $100,000 \text{ cells L}^{-1}$), whereas the abundance of the heliozoa exhibited a moderate peak at $10,000 \text{ cells mL}^{-1}$ and ranged between 1.4 and 2.9 mL^{-1} over the whole productivity gradient (Supplementary Materials, Table S3). These different numerical responses led to a pronounced increase in the relative contribution of *Cephalodella* sp. to total consumer numbers (Figure 3).

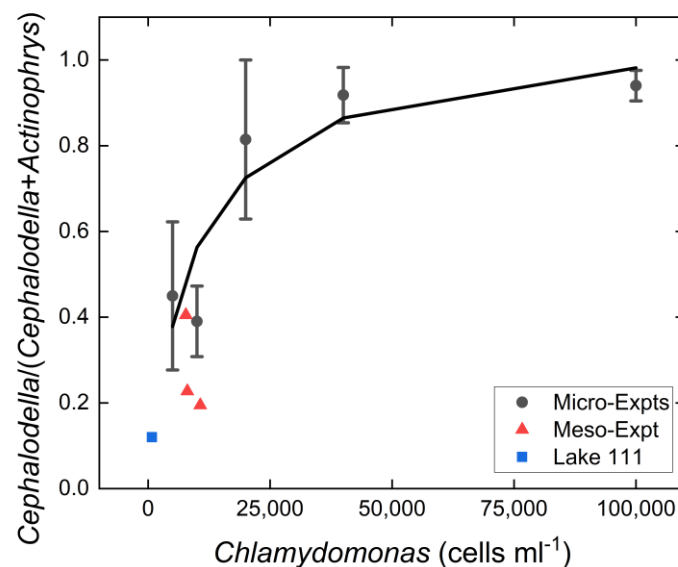


Figure 3. Relative contribution of *Cephalodella* sp. to total consumer abundance at varying productivity levels. Data were combined from the micro-scale experiments (Micro-Expts) mean \pm standard error, the meso-scale experiments (Meso-Expts) and field data for mean abundances over a three-year observation period (field data from [25]). Solid line, trend curve fit.

Combining these findings with those from the meso-scale experiment, as well as mean seasonal abundance data for *Cephalodella* sp. and *A. sol* in their lake of origin, the extremely acidic Lake 111, [25], a consistent pattern occurs: a higher contribution of *A. sol* to total consumer numbers at low resource concentrations and a higher contribution of *Cephalodella* sp. at high resource concentrations. Summarizing these results, two scenarios are apparent: a low-productivity scenario with a dominant intraguild predator and a high-productivity scenario with a dominant intraguild prey species (Figure 4).

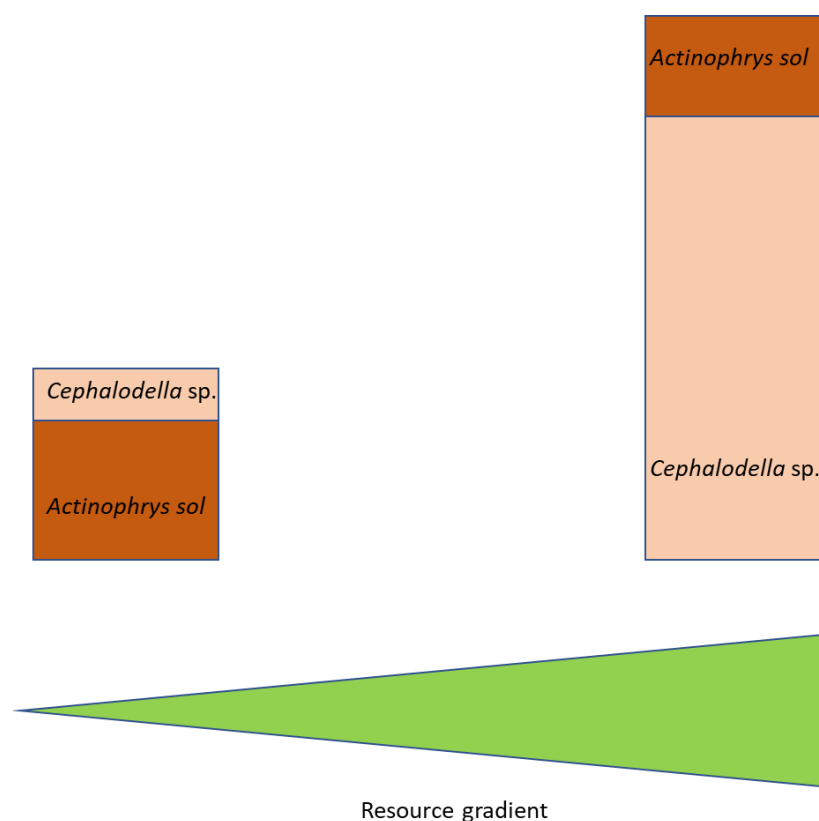


Figure 4. Schematic representation of the relationship of resource availability and consumer and predator abundance.

4. Discussion

We observed clear predation pressure on all three natural prey species tested, emphasising the status of *A. sol* as top predator in the planktonic food web in an extremely acidic lake, Lake 111 [25]. Whereas the ciliates were substantially smaller than the Heliozoa (~10% of *Actinophrys sol*), the rotifers were larger in volume than their predator. *A. sol* manage to ingest prey larger than themselves by their particular feeding mode: when large prey particles attach to the axiopods, they are immobilised [32,33], transported to the cell body, enveloped and digested [34–36]. Despite similarities in prey capture mechanism for each of the three prey species, the predator–prey interactions are complex and lead to context-dependent community compositions.

4.1. Predation on Rotifers

Both rotifer species were eaten by *A. sol*, but the predation pressure was higher on *Cephalodella* sp. than on *E. woralli*. Since heliozoa are non-motile predators, the encounter probability mainly depends on the movement behaviour of their rotiferan prey. From live microscopical observations, it is difficult to pinpoint the differences in movement behaviour between the two rotifer species, but one may speculate that *E. woralli* lowered its capture probability because of a slightly different swimming behaviour. In the micro-scale experiment, the density of heliozoa (1 individual in 200 μL , which equals to 5000 ind L^{-1})

was comparable to the maximum densities observed in Lake 111 in summer of 4000 to 7000 ind L⁻¹ [25]. However, the densities of both rotifer species used in our experiment were much higher than those observed to occur naturally in Lake 111 [27]. This may have led to an overestimation of the absolute predation pressure. Nevertheless, the relative relationships among prey species and treatment are likely consistent. Estimations of natural predation pressure in the field are difficult to make. The abundance maxima in Lake 111 differ between the two rotifers; *Elosa woralli* is dominant in the upper part of the water column and *Cephalodella* sp. in the lower part. Thus, the maxima of *E. woralli* (lower predation risk) and *A. sol* overlap in the field [25,27]. However, the vertical distribution of the three species cannot be explained by a single factor, because resource quantity and quality and temperature all play a role [37–39].

The strength of the predation pressure on the rotifers decreased when *Chlamydomonas* was supplied at a density of 100,000 cells mL⁻¹. This additional food reduced the predation on rotifers not only by “diluting” them, but also by clogging the heliozoa’s axiopods and reducing their total food uptake. This mechanism has been described by Bell et al. and can lead to reduced growth of *A. sol* [25].

The interplay between resource supply and predation was further supported by the meso-scale experiment. For *E. woralli*, the resource level was obviously above the threshold for growth, since in both treatments, *E. woralli* increased in number at the start of the experiment. With predators, *E. woralli* declined after a few days, and their abundance was controlled at a lower level. For *Cephalodella*, the outcome was different: without heliozoa, the abundance of *Cephalodella* was higher than with their predator during the initial days of the experiment, which led to a faster depletion of their algal food source and the decline of *Cephalodella*. With heliozoa, the overall pattern was similar, but initially lower *Cephalodella* abundances likely led to a slower decline of their algal food source and consequently of *Cephalodella* themselves. To further elucidate the relation between heliozoa, *Cephalodella* and their common food resource, *Chlamydomonas acidophila*, we performed the third, intraguild predation experiment (see Section 3.3).

4.2. Predation on Ciliates

Urosomoida sp. is a fast-growing ciliate that grew during the experiment and compensated partly for its predation losses. Consequently, in the micro-scale experiment, the difference in ciliate numbers with and without the heliozoan predator was quantified. Although the overall pattern resembled that of the rotifer experiment, differences among treatments were not significant ($p = 0.07$). Nevertheless, in both ciliate experiments, a clear and substantial predation pressure was found, demonstrated by the differences in ciliate abundances with and without predators. Thus, the ability of ciliates and rotifers to thrive under extremely acidic conditions does not release them from predation pressure. Acidophilic or acidotolerant ciliates have been found in other extreme habitats, but they are typically not the dominant heterotrophs and play a minor role in the food web [9,10,17,40].

4.3. Food Web Implications

The trophic relationship between the heliozoa, the ciliates/rotifers and their common prey, the phytoflagellate *Chlamydomonas acidophila*, represent a special case of intraguild predation. The term intraguild predation refers to a three-way trophic relationship between a shared resource species (in this case *Chlamydomonas acidophila*); a consumer of this resource, the intraguild prey (the ciliate and/or the rotifers, in particular *Cephalodella* sp.); and an omnivorous consumer, the intraguild predator (*Actinophrys sol*) feeding on both the shared resource and the intraguild prey. Results from mathematical modelling and laboratory experiments have demonstrated that enrichment of resources facilitates the intraguild predator and reduces the intraguild prey or even drives the intraguild prey to extinction [41,42]. These interactions result from the specific traits of the species, when the intraguild prey is the superior competitor over the intraguild predator at low productivity, but enrichment of their common resource allows the intraguild predator to feed upon both

the resource and the prey and thereby exert a higher predation pressure on the intraguild prey [41]. In the present case, these relationships are different. A high abundance of the shared prey, *C. acidophila*, reduces the growth rate of the intraguild predator (*A. sol*) by clogging their axiopods and increases the growth rate of the intraguild prey (the ciliate and/or rotifers), leading to the dominance of *Cephalodella* sp. Based on these results, we can predict two scenarios, one for low- and one for high-productivity situations (Figure 4):

- (1) At low productivity, the intraguild predator can suppress the intraguild prey and simultaneously make use of the shared resource.
- (2) At high productivity, the growth of the intraguild predator is reduced due to clogging of the axiopods by too many small prey items. This overload releases the intraguild prey (here: *Cephalodella* sp.) from predation pressure, allowing it to grow to high abundances.

This example of naturally occurring intraguild predation can only be explained when taking into account the specific nature of the interacting species.

4.4. Comparison with Circum-Neutral Habitats

In circum-neutral habitats (pH 6–8.5) such as lakes and oceans, the relative roles of ciliates, rotifers and heliozoa differ from those in acidic lakes. Ciliates contribute little to the food web in extremely acidic lakes [9], but they are important players in the microbial loop in lakes [43–45] and oceans [46,47]. There, they are consumed mainly by crustaceans (copepods and cladocerans) and to a lesser extent by rotifers. Rotifers in turn are preyed upon by a wide variety of invertebrates ranging from predatory rotifers (*Asplanchna*) and crustaceans to insects but also by fish larvae. The mean abundance of *A. sol* in Lake 111 is in the range of the abundance of heliozoa in lakes and coastal oceans [48,49], but episodic higher abundances in mining lakes have been observed [50]. Since the other groups of micro-zooplankton increase in abundance in circum-neutral lakes, the relative importance of heliozoa in these food webs decreases [51]. Thus, the trophic interactions described here differ from what is known from circum-neutral habitats and are of specific relevance for understanding the food web structure in such extreme habitats.

Supplementary Materials: Supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms10071340/s1>, Supplementary_Data_Weithoff&Bell.xlsx (containing Table S1, microscale experiment; Table S2, mesoscale experiment and Table S3, resource level experiment).

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References

1. Bell, E.M. *Life at Extremes—Environments, Organisms and Strategies for Survival*; CABI: Wallingford, UK, 2012; 554p.
2. Nixdorf, B.; Lessmann, D.; Deneke, R. Mining lakes in a disturbed landscape: Application of the EC Water Framework Directive and future management strategies. *Ecol. Eng.* **2005**, *24*, 67–73. [[CrossRef](#)]

3. Pedrozo, F.; Kelly, L.; Diaz, M.; Temporetti, P.; Baffico, G.; Kringel, R.; Friese, K.; Mages, M.; Geller, W.; Woelfl, S. First results on the water chemistry, algae and trophic status of an Andean acidic lake system of volcanic origin in Patagonia (Lake Caviahue). *Hydrobiologia* **2001**, *452*, 129–137. [[CrossRef](#)]
4. Bowers, K.J.; Wiegel, J. Temperature and pH optima of extremely halophilic archaea: A mini-review. *Extremophiles* **2011**, *15*, 119–128. [[CrossRef](#)]
5. Amaral-Zettler, L.A.; Gómez, F.; Zettler, E.; Keenan, B.G.; Amils, R.; Sogin, M.L. Eukaryotic diversity in Spain's River of Fire. *Nature* **2002**, *417*, 137. [[CrossRef](#)]
6. Amaral-Zettler, L.A. Eukaryotic diversity at pH extremes. *Front. Microbiol.* **2013**, *3*, 441. [[CrossRef](#)]
7. Packroff, G.; Woelfl, S. A review on the occurrence and taxonomy of heterotrophic protists in extreme acidic environments of pH values ≤ 3 . *Hydrobiologia* **2000**, *433*, 153–156. [[CrossRef](#)]
8. Bedogni, G.L.; Massello, F.L.; Giaveno, A.; Donati, E.R.; Urbietta, M.S. A deeper look into the biodiversity of extremely acidic Copahue volcano-RioAgridio region system in Nequén, Argentina. *Microorganisms* **2019**, *8*, 58. [[CrossRef](#)]
9. Mesa, V.; Gallego, J.L.R.; González-Gil, R.; Lauga, B.; Sánchez, J.; Méndez-García, C.; Peláez, A.I. Bacterial, archaeal, and eukaryotic diversity across microhabitats in an acid mine drainage. *Front. Microbiol.* **2017**, *8*, 1756. [[CrossRef](#)]
10. Kamjunke, N.; Gaedke, U.; Tittel, J.; Weithoff, G.; Bell, E.M. Strong vertical differences in the plankton composition of an extremely acidic lake. *Arch. Hydrobiol.* **2004**, *161*, 289–306. [[CrossRef](#)]
11. Aguilera, A.; Souza-Egipsy, V.; Gómez, F.; Amils, R. Development and structure of eukaryotic biofilms in an extreme acidic environment, Río Tinto (SW Spain). *Microb. Ecol.* **2007**, *53*, 294–305. [[CrossRef](#)]
12. Aguilera, A. Eukaryotic organisms in extreme environments, the Río Tinto case. *Life* **2013**, *3*, 363–374. [[CrossRef](#)] [[PubMed](#)]
13. Weithoff, G.; Moser, M.; Kamjunke, N.; Gaedke, U.; Weisse, T. Lake morphometry and wind exposure may shape the plankton community structure in acidic mining lakes. *Limnologica* **2010**, *40*, 161–166. [[CrossRef](#)] [[PubMed](#)]
14. Weisse, T.; Berendonk, T.; Kamjunke, N.; Moser, M.; Scheffel, U.; Stadler, P.; Weithoff, G. Significant habitat effects influence protist fitness: Evidence for local adaptation from acidic mining lakes. *Ecosphere* **2011**, *2*, 134. [[CrossRef](#)]
15. Packroff, G. Protozooplankton in acidic mining lakes with special respect to ciliates. *Hydrobiologia* **2000**, *433*, 157–166. [[CrossRef](#)]
16. Bell, E.M.; Weithoff, G. Benthic recruitment of zooplankton in an acidic lake. *J. Exp. Mar. Biol. Ecol.* **2003**, *285–286*, 205–219. [[CrossRef](#)]
17. Weisse, T.; Moser, M.; Scheffel, U.; Stadler, P.; Berendonk, T.; Weithoff, G.; Berger, H. Systematics and species-specific response to pH of *Oxytricha acidotolerans* sp. nov. and *Urosomoida* sp. (Ciliophora, Hypotricha) from acid mining lakes. *Eur. J. Protistol.* **2013**, *49*, 255–271. [[CrossRef](#)]
18. Deneke, R. Review of rotifers and crustaceans in highly acidic environments of pH values ≤ 3 . *Hydrobiologia* **2000**, *433*, 167–172. [[CrossRef](#)]
19. Hrdinka, T.; Šobr, M.; Fott, J.; Nedbalová, L. The unique environment of the most acidified permanently meromictic lake in the Czech Republic. *Limnologica* **2013**, *43*, 417–423. [[CrossRef](#)]
20. Weithoff, G.; Seiferth, J.; Neumann, C.; Weisse, T. Living on the edge: Reproduction, dispersal potential, maternal effects and local adaptation in aquatic, extremophilic invertebrates. *Aquat. Sci.* **2019**, *81*, 40. [[CrossRef](#)]
21. Canganella, F.; Wiegel, J. Extremophiles: From abyssal to terrestrial ecosystems and possibly beyond. *Naturwissenschaften* **2011**, *98*, 253–279. [[CrossRef](#)]
22. Dhakar, K.; Pandey, A. Wide pH range tolerance in extremophiles: Towards understanding an important phenomenon for future biotechnology. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 2499–2510. [[CrossRef](#)] [[PubMed](#)]
23. Spijkerman, E.; Weithoff, G. Acidic Environments. In *Life at Extremes—Environments, Organisms and Strategies for Survival*; Bell, E.M., Ed.; CABI: Wallingford, UK, 2012; pp. 364–379.
24. Schmidtke, A.; Bell, E.M.; Weithoff, G. Potential grazing impact of the mixotrophic flagellate *Ochromonas* sp. (Chrysophyceae) on bacteria in an extremely acidic lake. *J. Plankton Res.* **2006**, *28*, 991–1001. [[CrossRef](#)]
25. Bell, E.M.; Weithoff, G.; Gaedke, U. Temporal dynamics and growth of *Actinophrys sol* (Sarcodina: Heliozoa), the top predator in an extremely acidic lake. *Freshw. Biol.* **2006**, *51*, 1149–1161. [[CrossRef](#)]
26. Weithoff, G. On the ecology of the rotifer *Cephalodella hoodi* isolated from an extremely acidic lake. *Freshw. Biol.* **2005**, *50*, 1464–1473. [[CrossRef](#)]
27. Weithoff, G. Vertical niche separation of two consumers in an extreme habitat. *Oecologia* **2004**, *139*, 594–603. [[CrossRef](#)]
28. Tittel, J.; Bissinger, V.; Gaedke, U.; Kamjunke, N. Inorganic carbon limitation and mixotrophic growth of *Chlamydomonas* from an acidic mining lake. *Protist* **2005**, *156*, 63–75. [[CrossRef](#)]
29. Spijkerman, E. Inorganic carbon acquisition by *Chlamydomonas acidophila* across a pH range. *Can. J. Bot.* **2005**, *83*, 872–878. [[CrossRef](#)]
30. Bissinger, V.; Jander, J.; Tittel, J. A new medium free of organic carbon to cultivate organisms from extremely acidic mining lakes (pH 2.7). *Acta Hydroch. Hydrob.* **2000**, *28*, 310–312. [[CrossRef](#)]
31. Weithoff, G. Dietary restriction in two rotifer species: The effect of the length of food deprivation on life span and reproduction. *Oecologia* **2007**, *153*, 303–308. [[CrossRef](#)]
32. Patterson, D.J.; Hausmann, K. Feeding by *Actinophrys sol* (Protista, Heliozoa). 1. Light microscopy. *Microbios* **1981**, *31*, 39–55.
33. Grebecki, A.; Hausmann, K. Motor behaviour of prey during first steps of food capture by *Actinophrys sol*. *Acta Protozool.* **1993**, *32*, 157–164.

34. Suzaki, T.; Shigenaka, Y.; Watanabe, S.; Toyohara, A. Food capture and ingestion in the large heliozoan *Echinospaerium nucleofilum*. *J. Cell Sci.* **1980**, *42*, 61–79. [[CrossRef](#)] [[PubMed](#)]
35. Sakaguchi, M.; Hausmann, K.; Suzaki, T. Food capture and adhesion by the heliozoon *Actinophrys sol*. *Protoplasma* **1998**, *203*, 130–137. [[CrossRef](#)]
36. Hausmann, K.; Patterson, D.J. Pseudopod formation and membrane production during prey capture in by a heliozoan (Feeding by *Actinophrys*. II). *Cell Motil.* **1982**, *2*, 9–24. [[CrossRef](#)]
37. Weithoff, G.; Wacker, A. The mode of nutrition of mixotrophic flagellates determines the food quality for their consumers. *Funct. Ecol.* **2007**, *21*, 1092–1098. [[CrossRef](#)]
38. Hartwig, M.; Weithoff, G.; Wacker, A. Changes in the competitive abilities of two rotifers feeding on mixotrophic flagellates. *J. Plankton Res.* **2010**, *32*, 1727–1731. [[CrossRef](#)]
39. Wacker, A.; Weithoff, G. Carbon assimilation mode in mixotrophs and the fatty acid composition of their consumers—three rotifers. *Freshw. Biol.* **2009**, *54*, 2189–2199. [[CrossRef](#)]
40. Méndez-García, C.; Peláez, A.I.; Mesa, V.; Sánchez, J.; Golyshina, O.V.; Ferrer, M. Microbial diversity and metabolic networks in acid mine drainage habitats. *Front. Microbiol.* **2015**, *6*, 475. [[CrossRef](#)]
41. Diehl, S.; Feissl, M. Effects of enrichment on three-level food chains with omnivory. *Am. Nat.* **2000**, *155*, 200–218. [[CrossRef](#)]
42. Diehl, S.; Feissl, M. Intraguild prey suffers from enrichment of their resources: A microcosms experiment with ciliates. *Ecology* **2001**, *82*, 2977–2983. [[CrossRef](#)]
43. Beaver, J.; Crisman, T. The role of ciliated protozoa in pelagic freshwater ecosystems. *Microb. Ecol.* **1989**, *17*, 111–136. [[CrossRef](#)] [[PubMed](#)]
44. Müller, H. The relative importance of different ciliate taxa in the pelagic food web of Lake Constance. *Microb. Ecol.* **1989**, *18*, 261–273. [[CrossRef](#)] [[PubMed](#)]
45. Lischke, B.; Weithoff, G.; Wickham, S.A.; Attermeyer, K.; Grossart, H.-P.; Scharnweber, K.; Hilt, S.; Gaedke, U. Large biomass of small feeders: Ciliates may dominate herbivory in eutrophic Lakes. *J. Plankton Res.* **2015**, *38*, 2–15. [[CrossRef](#)]
46. Azam, F.; Fenchel, T.; Field, J.G.; Gray, J.S.; Meyer-Reil, L.A.; Thingstad, F. The ecological role of water-column microbes in the sea. *Mar. Ecol. Progr. Ser.* **1983**, *10*, 257–263. [[CrossRef](#)]
47. Pomeroy, L.R. The Ocean's Food Web, A Changing Paradigm. *BioScience* **1974**, *24*, 499–504. [[CrossRef](#)]
48. Zimmermann, U.; Müller, H.; Weisse, T. Seasonal and spatial variability of planktonic heliozoa in Lake Constance. *Aquat. Microb. Ecol.* **1996**, *11*, 21–29. [[CrossRef](#)]
49. Pierce, R.W.; Coats, D.W. The feeding ecology of *Actinophrys sol* (Sarcodina: Heliozoa) in Chesapeake Bay. *J. Eukaryot. Microbiol.* **1999**, *46*, 451–457. [[CrossRef](#)]
50. Woelfl, S. Limnology of sulphur-acidic lignite mining lakes. Biological properties: Plankton structure of an extreme habitat. *Verh. Internat. Verein. Limnol.* **2000**, *27*, 2904–2907. [[CrossRef](#)]
51. Arndt, H. A critical review of the importance of rhizopods (naked and testate amoebae) and actinopods (Heliozoa) in lake plankton. *Mar. Microb. Food Webs* **1993**, *7*, 3–29.