**Supplemental material**

**Table S1. Strains of phytoplankton used in our experiments and their associated traits.** Cell morphology and colonial status of our cultures. For strains capable of colonial forms, these were found both as single cell and colonial forms throughout the experiment. Cell size ranges reported elsewhere for the species or the specific strain; L: length, D: diameter, V: volume. Medium refers to the nutrient medium on which we grew the phytoplankton. Phytoplankton polyunsaturated fatty acid (PUFA) content is reported from the literature (References) as the percent of all fatty acids; it provides an established index of nutritional quality. Documented secondary metabolites are reported from the literature (References). Means and standard deviations of cyanobacterial toxin production were measured directly during our experiment.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phytoplankton species | Morphology | Cell size range | Medium | PUFAs  (%) | Secondary Metabolites | Toxin Concentration (ppb) | References |
| *Anabaena flos aquae* | Filamentous | L: 5-100μm  D:5μm | MLA | 12.8 | Anatoxin-α | 0.165±0.218 | (Ahlgren et al., 1992; Galhano et al., 2011) |
| *Ankistrodesmus falcatus* | Single-celled | L:30-36μm  D:2.5-4.5μm | COMBO | 25-27.9 | NA | NA | (Jayanta et al., 2012) |
| *Chlamydomonas reinhardtii* | Single-celled | D:~10μm  V: 75-150μm³ | Bristols | 40.7 | NA | NA | (Ahlgren et al., 1992) |
| *Chlorella* sp*.* | Single-celled | D:6-8μm  V:110-270μm³ | Z8 | 35-40.3 | Chlorellin | Unknown | (Ratha et al., 2012; Ahlgren et al., 1992; Pratt et al., 1944) |
| *Microcystis aeruginosa* | Single-celled or colonial | D:2.61-5.40μm | Z8 | 11.5 | Microcystins | 0.123±0.088 | (Ahlgren et al., 1992) |
| *Nodularia spumigena* | Filamentous | D:8-12μm | MLA | 10.6-36.7 | Nodularin | 1.627±2.946 | (Engström-Öst et al., 2002) |
| *Scenedesmus* sp*.* | Single-celled or colonial | L:10-26μm  D:4-7μm | COMBO | 23.3-40.8 | NA | NA | (Ahlgren et al., 1992) |
| *Ulothrix* sp*.* | Filamentous | D:2-4μm  L:17-1030μm | Bristols | 9.9-68.9 | NA | NA | (Sushchik et al., 2010) |

**Table S2. Summary of findings from statistical models investigating effects of phytoplankton diet and parasite infection on performance of *Daphnia* hosts and their parasites.** The block term was omitted from the table since it was not significant in any of the models (abbreviations: Inf.-Infection, Ind.-Individuals). “Parasite” is used to indicate comparisons of the fungus (*Metschnikowia*) vs. the bacterium (*Pasteuria*). “Co-infection” is used to indicate comparisons of hosts exposed to one parasite vs. both parasites. “Infection treatment” is used to indicate comparisons of hosts infected with the fungus vs. those infected with the bacterium vs. those that were coinfected vs. uninfected controls. “Failed Inf” refers to hosts that were exposed to, but resisted, parasite infection.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model Comparison | Predictor variables | DF | Test-Statistics | P-value |
| Proportion of Individuals Infected (Figure 1) | | | | |
| Prevalence model for single fungal and bacterial exposures | Diet | 8 | χ² = 26.01 | 0.0010 |
| Parasite | 1 | χ² = 23.11 | <0.0001 |
| Diet\*Parasite | 8 | χ² = 28.61 | 0.0004 |
| Difference in prevalence for single fungal exp. vs fungal co-exp. | Diet | 8 | χ² = 44.52 | <0.0001 |
| Co-infection | 1 | χ² = 5.00 | 0.0254 |
| Diet\*Co-infection | 8 | χ² = 22.41 | 0.0042 |
| Difference in prevalence for single bacterial exp. vs bacterial co-exp. | Diet | 8 | χ² = 7.55 | 0.4788 |
| Co-infection | 1 | χ² = 0.20 | 0.6568 |
| Diet\*Co-Infection | 8 | χ² = 8.82 | 0.3581 |
| Spore Yield (Figure 2) | | | | |
| Differences in spore production from single exposures | Diet | 8 | F = 1.14 | 0.3464 |
| Parasite | 1 | F = 0.94 | 0.3368 |
| Diet\*Parasite | 5 | F = 2.51 | 0.0384 |
| Differences in spore production of single fungal inf. vs fungal co-infections | Diet | 8 | χ² = 43.68 | <0.0001 |
| Co-Infection | 1 | χ² = 10.96 | 0.0009 |
| Diet\*coinfection | 5 | χ² = 11.47 | 0.0428 |
| Differences in spore productions of single bacterial inf. vs bacterial co-infections | Diet | 8 | χ² = 23.52 | 0.0028 |
| Co-Infection | 1 | χ² = 22.90 | <0.0001 |
| Diet\*Co-Infection | 8 | χ² = 7.68 | 0.4650 |
| Fungal spores prod. | Diet | 5 | χ² = 3.44 | 0.6328 |
| Bacterial spores prod. | Diet | 8 | χ² = 27.06 | 0.0007 |
| Lifetime Offspring Production (Figures 3, S1 & S3) | | | | |
| Full model | Diet | 8 | F = 100.25 | <0.0001 |
| Infection treatment | 3 | F = 213.16 | <0.0001 |
| Diet\*Infection treatment | 21 | F = 10.83 | <0.0001 |
| Fitness cost of resistance to disease model (Failed Inf. vs controls) | Diet | 8 | F = 113.81 | <0.0001 |
| Exposure treatment | 3 | F = 29.17 | <0.0001 |
| Diet\*Exposure treatment | 23 | F = 5.83 | <0.0001 |
| Fungus single inf. vs bacterial single inf. (Interaction effect of full model) | Infection | 1 | χ² = 18.24 | <0.0001 |
| Diet | 8 | χ² = 492.05 | <0.0001 |
| Diet\*Infection | 5 | χ² = 29.15 | <0.0001 |
| Fungus single inf. vs controls | Infection | 1 | F = 269.14 | <0.0001 |
| Diet | 8 | F = 58.40 | <0.0001 |
| Diet\*Infection | 5 | F = 6.60 | <0.0001 |
| Bacterial single inf. vs controls | Infection | 1 | F = 257.47 | <0.0001 |
| Diet | 8 | F = 86.52 | <0.0001 |
| Diet\*Infection | 8 | F = 6.55 | <0.0001 |
| Growth (length at death; Figure S2) | | | | |
| Full model | Diet | 8 | F = 10.41 | <0.0001 |
| Infection treatment | 3 | F = 44.46 | <0.0001 |
| Diet\*Infection treatment | 21 | F = 3.00 | <0.0001 |
| Bacterial single inf. vs controls | Infected | 121 | T = 2.614 | 0.0101 |
| *Anabaena* Infected | 9 | T = 1.032 | 0.329 |
| *Microcystis* Infected | 12 | T = 2.568 | 0.0246 |
| *Microcystis* & Microcystin Infected | 9 | T = 3.400 | 0.00787 |
| *Nodularia* Infected | 10 | T = 2.125 | 0.0595 |
| *Ankistrodesmus* Infected | 11 | T = 1.320 | 0.214 |
| *Chlamydomonas* Infected | 10 | T = 1.840 | 0.0956 |
| *Chlorella* Infected | 10 | T = 0.600 | 0.562 |
| *Scenedesmus* Infected | 15 | T = 1.580 | 0.135 |
| *Ulothrix* Infected | 11 | T = 2.735 | 0.0194 |
| Fitness cost of resistance to disease (Failed Inf. vs controls) | Diet | 8 | F = 18.90 | <0.0001 |
| Exposure treatment | 3 | F = 3.62 | 0.0149 |
| Diet\*Exposure treatment | 23 | F = 1.71 | 0.0324 |
| Mortality (Figure S4 & S5) | | | | |
| Full model | Diet | 8 | χ² = 11.43 | 0.1784 |
| Infection treatment | 3 | χ² = 105.60 | <0.0001 |
| Diet\*Infection treatment | 21 | χ² = 34.96 | 0.0285 |
| Fitness cost of resistance to disease model (Failed Inf. vs controls) | Diet | 8 | χ² = 28.32 | 0.0004 |
| Exposure treatment | 3 | χ² = 10.75 | 0.0131 |
| Diet\*Exposure treatment | 23 | χ² = 29.42 | 0.1669 |
| Fungus single inf. vs bacterial single inf. (Interaction effect of full model) | Diet | 8 | χ² = 5.85 | 0.6643 |
| Infection | 1 | χ² = 71.26 | <0.0001 |
| Diet\*Infection | 5 | χ² = 6.09 | 0.2976 |
| Fungus single inf. vs controls | Diet | 8 | χ² = 11.82 | 0.1593 |
| Infection | 1 | χ² = 78.12 | <0.0001 |
| Diet\*Infection | 5 | χ² = 4.73 | 0.4498 |
| Bacterial single inf. vs controls | Diet | 8 | χ² = 13.89 | 0.0846 |
| Infection | 1 | χ² = 0.27 | 0.6003 |
| Diet\*Infection | 8 | χ² = 7.74 | 0.4594 |

**Figure S1. Lifetime offspring production of individual *Daphnia* infected with (a) the fungus *Metschnikowia*, (b) the bacterium *Pasteuria*, (c) both the fungal and the bacterial parasites, or (d) uninfected controls.** Each point represents the number of offspring produced by an individual. Note that there are no data for *Daphnia* exposed to the fungus *Metschnikowia* and reared on *Anabaena*, spiked *Microcystis* or *Chlorella* diets, because no individuals became infected. (Abbreviations for phytoplankton names: Ana-*Anabaena*, Mic-*Microcystis*, Mic+ - spiked *Microcystis*, Nod-*Nodularia*, Ank-*Ankistrodesmus*, Chla-*Chlamydomonas*, Chlo-*Chlorella*, Sce-*Scenedesmus*, Ulo-*Ulothrix*).

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**Figure S2. Time series of mean offspring production during the experimental trial.** Each point represents the average number of live offspring counted on a given specific day. Note that there are no data for *Daphnia* exposed to the fungus *Metschnikowia* and reared on *Anabaena*, spiked *Microcystis* or *Chlorella* diets, because no individuals became infected. (Abbreviations for phytoplankton names: Ana-*Anabaena*, Mic-*Microcystis*, Mic+ - spiked *Microcystis*, Nod-*Nodularia*, Ank-*Ankistrodesmus*, Chla-*Chlamydomonas*, Chlo-*Chlorella*, Sce-*Scenedesmus*, Ulo-*Ulothrix*).

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**Figure S3.** **Size of *Daphnia* infected with (a) the fungus *Metschnikowia*, (b) the bacterium *Pasteuria*, (c) both the fungal and the bacterial parasites, or (d) uninfected controls.** Growth is represented by the length of individuals at day 30 of the experiment or at time of death. Note that there are no data for *Daphnia* exposed to the fungus *Metschnikowia* and reared on *Anabaena*, spiked *Microcystis* or *Chlorella* diets, because no individuals became infected. (Abbreviations for phytoplankton names: Ana-*Anabaena*, Mic-*Microcystis*, Mic+ - spiked *Microcystis*, Nod-*Nodularia*, Ank-*Ankistrodesmus*, Chla-*Chlamydomonas*, Chlo-*Chlorella*, Sce-*Scenedesmus*, Ulo-*Ulothrix*).

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**Figure S4. Growth of *Daphnia* infected with (a) the fungus *Metschnikowia*, (b) the bacterium *Pasteuria*, (c) both the fungal and the bacterial parasites, or (d) uninfected controls.** Growth was calculated as the length/size of the animal at day 30 of the experiment or at time of death divided by the animal’s lifespan. Note that there are no data for *Daphnia* exposed to the fungus *Metschnikowia* and reared on *Anabaena*, spiked *Microcystis* or *Chlorella* diets, because no individuals became infected. (Abbreviations for phytoplankton names: Ana-*Anabaena*, Mic-*Microcystis*, Mic+ - spiked *Microcystis*, Nod-*Nodularia*, Ank-*Ankistrodesmus*, Chla-*Chlamydomonas*, Chlo-*Chlorella*, Sce-*Scenedesmus*, Ulo-*Ulothrix*).

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**Results**

**Costs of resistance**

*Daphnia* that were exposed to parasites but resisted infection varied in size depending on both the treatment and the diet to which they were exposed. On some phytoplankton diets, parasite-exposed individuals were larger than were controls, while the opposite was observed on other diets (Figure S1, Manuscript Table 1). Since there was no clear pattern to the results, we conclude that growth costs of parasite resistance were affected by the interaction between phytoplankton diet and parasite exposure (Figure S1). Additional costs of resistance to the bacterial parasite, *Pasteuria*, were expressed in both offspring production (Figure S2) and mortality (Figure S3). *Daphnia* that were exposed to *Pasteuria* but that did not become infected produced fewer offspring (all diets) and experienced higher mortality (most diets) than did unchallenged controls (Figures S2, S3, Manuscript Table 1). Additional costs of resistance to the fungus *Metschnikowia* were observed only on a diet of *Nodularia*, which caused reduced offspring production (Figure S2) and increased mortality (Figure S3) among *Daphnia* that resisted this fungus.

**Figure S5. Cost of resistance; host length.** The size of *Daphnia* that were exposed to parasites but that remained uninfected (“resistant”), and of unexposed controls (None), at time of death or at 30 days. Note that there are no data for *Daphnia* fed *Scenedesmus* that were exposed to both parasites, since all individuals became infected in this treatment. (Abbreviations for parasite names: Metsch-*Metschnikowia*, Past-*Pasteuria*).

KFS%20Stuff/New%20Shit/Length.pdf

**Figure S6. Cost of resistance; offspring production per host.** Lifetimenumber of offspring produced by *Daphnia* that were exposed to parasites but that remained uninfected (“resistant”), and of unexposed controls (None). Note that there are no data for animals fed *Scenedesmus* that were exposed to both parasites since all individuals became infected in this treatment. (Abbreviations for parasite names: Metsch-*Metschnikowia*, Past-*Pasteuria*).

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**Figure S7. Cost of resistance; host mortality.** Proportion of individuals (means SE) that died by day 30 among *Daphnia* that were exposed to parasites but that remained uninfected (“resistant”), and among unexposed controls (None). Note that there are no data for animals fed *Scenedesmus* that were exposed to both parasites since all individuals became infected in this treatment. (Abbreviations for parasite names: Metsch-*Metschnikowia*, Past-*Pasteuria*).

../../cost_mortality_dietbyinf.pdf

**Figure S8. Proportion of infected *Daphnia* that died before day 30 after exposure to parasites on different phytoplankton diets.** Mortality of individuals (means SE) exposed to (a) *Metschnikowia*, (b) *Pasteuria*, (c) both *Metschnikowia* and *Pasteuria*, and (d) no parasites. Note that there are no data available for the *Metschnikowia* treatment for *Daphnia* fed *Anabaena*, spiked *Microcystis*, and *Chorella* diets since no individuals became infected. (Abbreviations for phytoplankton names: Ana-*Anabaena*, Mic-*Microcystis*, Mic+ - spiked *Microcystis*, Nod-*Nodularia*, Ank-*Ankistrodesmus*, Chla-*Chlamydomonas*, Chlo-*Chlorella*, Sce-*Scenedesmus*, Ulo-*Ulothrix*).

**../Mortality2.pdf**

**Figure S9. Lack of correlation between anatoxin-α concentrations and the growth (size at day 30) of *Daphnia.*** The average anatoxin-α concentrations to which hosts were exposed were correlated against length at the time of death (or at day 30, if still alive) to evaluate any effects of anatoxin on *Daphnia* growth. The lack of significant correlation below suggests that the negative relationship between anatoxin-α concentration and bacterial spore yield (Manuscript Figure 4a) did not emerge from effects of anatoxin-α on *Daphnia* size. Rather, there may be direct effects of anatoxin-α on bacterial fitness.

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