Consumer-resource dynamics is an eco-evolutionary process in a natural plankton community

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When traits affecting species interactions evolve rapidly, ecological dynamics can be altered while they occur. These "eco-evolutionary dynamics" have been documented repeatedly in laboratory and mesocosm experiments. We show here that they are also important for understanding community functioning in a natural ecosystem. *Daphnia* is a major planktonic consumer influencing seasonal plankton dynamics in many lakes. It is also sensitive to succession in its phytoplankton food, from edible algae in spring to relatively inedible cyanobacteria in summer. We show for *Daphnia mendotae* in Oneida Lake, USA, that within-year ecological change in phytoplankton (from spring diatoms, cryptophytes and greens to summer cyanobacteria) resulted in consumers evolving increasing tolerance to cyanobacteria over time. This evolution fed back on ecological seasonal changes in population abundance of this major phytoplankton consumer. Oneida Lake is typical of mesotrophic lakes broadly, suggesting that eco-evolutionary consumer-resource dynamics is likely common.

Temporal eco-evolutionary dynamics[1-3] have been documented in laboratory microcosms[4-6] and field mesocosms[7-9], but there have been very few studies in unconfined natural systems. Eco-evolutionary dynamics will have the greatest ecological importance if the evolving organism plays a central role in the functioning of its ecosystem. *Daphnia* is a good candidate for exploring the importance of eco-evolutionary dynamics in nature[10,11] because it is a significant consumer of phytoplankton with effects on phytoplankton community composition[12], nutrient availability and stoichiometry[13], and food for zooplanktivorous predators[14], hence playing a central role in the trophic cascade in lakes[15].

In many lakes *Daphnia* faces the challenge that the summer phytoplankton community is dominated by cyanobacteria, which are poor food nutritionally, often contain toxins, and can have colony shapes difficult to capture and ingest[16]. Because *Daphnia* is poor at discriminating among particles when feeding, cyanobacteria dominance typically results in low *Daphnia* growth rate[17-20]. When this occurs, natural selection should strongly favor *Daphnia* genotypes that are relatively insensitive to dietary cyanobacteria. Consistent with this expectation, clonal lineages of *Daphnia*, hatched from dormant eggs laid before and after the appearance of summer cyanobacterial blooms in Lake Constance, Europe, showed evolution of increased tolerance over the course of a decade[18]. Similarly, *Daphnia* clones from lakes with cyanobacteria have been found to be more tolerant than clones from lakes without cyanobacteria[19]. Furthermore, feedback from evolution to ecology has been documented in the lab: tolerant clones suppressed the growth of cyanobacteria in nutrient-enriched mesocosms, while those sensitive to cyanobacteria did not[20].

To investigate whether this adaptive evolution occurs rapidly enough to affect seasonal consumer-resource dynamics in a natural system, we studied evolutionary changes in *Daphnia mendotae* in Oneida Lake, New York, USA, through a single season during which the phytoplankton community underwent typical succession, including a summer cyanobacterial bloom. Of the three species of *Daphnia* present in Oneida Lake over the past four decades, *D. mendotae* (hereafter, *Daphnia*) has been dominant since 2006[36], including in 2015, the year of this study, when it comprised >70% of the daphniid biomass. Because these *Daphnia* only reproduce parthenogenetically in summer[21], with a generation time of approximately one week, we documented evolution as changes in frequency of clones identified using microsatellite DNA genotyping. We then assayed the performance of seven representative clones with peak

abundances at different times of year, by measuring their juvenile growth rate (a fitness correlate)[10,24] when fed 1 mg C L^{-1} (i.e., *ad libitum*) of either spring phytoplankton (diatoms, cryptophytes and chlorophytes) or summer phytoplankton (cyanobacteria and chlorophytes). From this we calculated how mean *Daphnia* growth rate and population dynamics changed seasonally, affected both by succession in phytoplankton quality as food as well as by evolution of *Daphnia* sensitivity to that changing diet.

Results and Discussion

Ecological plankton dynamics in Oneida Lake. Seasonal phytoplankton-zooplankton dynamics in Oneida Lake in 2015 were consistent with the generally accepted pattern in eutrophic lakes depicted in the plankton ecology group "PEG" model[10,22,23] (Fig. 1). Specifically, a spring diatom bloom ended in late May, as grazing by *Daphnia* caused a spring clear-water phase (CWP) dominated by small edible cryptophytes, euglenophytes, diatoms, and chlorophytes that comprised 85–100% of the biomass. The CWP was followed by a crash and then resurgence of the *Daphnia* population, accompanied by an increase in cyanobacteria abundance (75–90% of biomass). Filamentous forms dominated first (*Dolichospermum* then *Aphanizomenon*), followed by colonial *Microcystis* and a late August return of diatoms.

Ecology affecting evolution. We identified 124 unique *D. mendotae* clones which varied seasonally in frequency (Fig. 2a). We chose seven clones with peak abundance at different times during the study period (Fig. 2b; see Methods). These represented on average 48% of the *D. mendotae* isolated on each date (range: 22-84%). Whereas all seven clones had lower JGR on the summer diet containing cyanobacteria than on the spring diet (Fig. 3a; ANOVA food effect, $df = 1$, $F = 174.1$, $p \ll 0.001$, Supplementary Table 1), they differed in their overall performance (clone effect, $df = 7$, $F = 4.86$, $p < 0.001$) and in their sensitivity to diet (food×clone effect, $df = 7$, $F = 11.51$, $p \ll 0.001$). In pairwise comparisons of reaction norm slopes, we found that Clones 2, 8 and 11, which all have relatively shallow reaction-norm slopes, did not differ significantly from each other (Supplementary Table 2), so we designate them "resistant" clones (Fig. 3a). Similarly, Clones 1, 19, 38 and 169, which all have relatively steep slopes, did not differ from each other, and we call them "non-resistant" clones. The reaction norm slopes of clones from resistant and non-resistant categories differed significantly in 13 of 16 pairwise comparisons, and

93 we use these two groupings to simplify discussion of our results. Clone19 is intermediate but
94 closer to the non-resistant clones, so we include it in that group.
95 All resistant clonal lineages, with shallow reaction norms (Fig. 3a), had higher JGR on
96 summer cyanobacteria diet than all except one of the non-resistant clones, but also lower JGR on
97 spring algal diet. This apparent tradeoff in performance on the two diets suggests there is a cost
98 to tolerance of cyanobacteria, although we have not identified it. The two independent isolates
99 of Clone 11 performed very similarly (no difference in ANOVA post-hoc comparison;
100 Supplementary Table 2) indicating that the dietary-performance phenotype was consistent with
101 microsatellite-clone identity. The seven clones had seasonal patterns of frequency change
102 consistent with their performance on summer (cyanobacteria) diet: there is a positive relationship
103 between date of maximum rate of frequency increase and clone growth rate on summer
104 phytoplankton (Fig. 3b; $r^2 = 0.64$, $p = 0.034$). Clones that were more resistant to cyanobacteria
105 generally increased in frequency later in the season as cyanobacteria became dominant, and vice
106 versa. The effect of evolution is reflected in the seasonal change in clonal diversity (Simpson's
107 Index, see Methods) which dropped from 0.88–0.98 in May to mid-August to between 0.58–0.77
108 in late-August and September driven primarily by the increasing dominance of Clone 11, which
109 went from $\leq$ 10% of the population to over 60% during this period (Fig. 2a; Supplementary Fig.
110 1).
111 The *Daphnia* population evolved by clonal succession within a single season (Fig. 2), and
112 our results suggest that this was due to natural selection imposed by change in food quality (Fig.
113 3b). To evaluate further the action of natural selection on each of the seven clones, we estimated
114 how each clone's juvenile growth rate would have changed over the course of the season as
115 phytoplankton composition changed (Fig. 1c). We know the JGR of each clone when fed either
116 0% cyanobacteria (spring food) or 50% cyanobacteria (summer food), and using this information
117 we projected JGR at other frequencies of cyanobacteria in the lake assuming a monotonic
118 relationship given by the reaction norms in Fig. 3a (see Methods and Supplementary Fig. 2).
119 During the spring diatom bloom and the flagellate-dominated CWP, the JGR of the resistant
120 clones was below the JGR of the non-resistant clones (Fig. 4a) showing selection generally
121 against resistant clones at the start of the season, whereas in summer the JGRs of the resistant-
122 clones were above those of the non-resistant-clones showing a selective advantage when
123 cyanobacteria dominated. The opposite is true for the non-resistant clones. The actual mean JGR

for the evolving frequencies of the seven clones on each date was often, but not always, greater than that for a calculated (see Methods) hypothetical no-evolution case of clone frequencies staying constant from the start of the season (Fig. 4b). Through the season, the observed, evolving, mean JGR became significantly progressively better than the no-evolution case so that there was a 60% gain in performance by the end of the season (Fig. 4c).

Evolution affecting ecology. To demonstrate eco-evolutionary dynamics, we must "close the loop" by showing that the rapid evolution of resistance to cyanobacteria in Oneida Lake *Daphnia*, driven by seasonal phytoplankton succession, had an important effect on ecological dynamics. First, through regression analysis we found that the ecological process of per capita *Daphnia* population rate of change in the lake, r (day^{-1}), is best predicted by a combination of changing total phytoplankton biomass (chlA, an ecological consumer-resource interaction) and the rapidly evolving mean adaptation of *Daphnia* clones to the presence of dietary cyanobacteria (frequency-weighted mean $\log_e$JGR; hereafter "mean JGR") (Table 1). This model explains 71% of the variation in *Daphnia* population r, while chlA alone and *Daphnia* mean JGR only account for 38% and 21% of the variation respectively. The importance of the combination of both factors for *Daphnia* population dynamics in the lake is confirmed by AIC analysis (Supplementary Table 3) with a ΔAIC of 8.85 comparing the combined model with the next best model of chlA alone or with the model of mean JGR alone (ΔAIC = 12.13). We chose the 4-day JGR measurement as the quantitative measure of clonal differences in sensitivity to dietary cyanobacteria based on the original study[24] showing that it is highly correlated with laboratory life-table measurements of r in another *Daphnia* species (*D. magna*) when food concentration was varied ($r^2 = 0.88$). Its relevance in our study is confirmed by its importance as a predictor of changes in realized r in the lake population.

Second, we used the regression model to calculate the impact of *Daphnia* evolution (clone-frequency change) on consumer population dynamics in the lake, by comparing observed temporal changes in *Daphnia* population size with projections of what those changes would have been in the absence of evolution. The mean JGR variable in the regression model (Table 1) combines ecology (cyanobacteria abundance) and evolution (clone frequencies). To isolate the contribution of evolution, we used our regression model and the clone-specific responses to dietary cyanobacteria (Fig. S2) to project how much *Daphnia r* would have differed on each day

155 if the *Daphnia* clonal frequencies had not evolved since the early samples when cyanobacteria
156 were absent. These projected daily *r* values then let us contrast observed *Daphnia* seasonal
157 dynamics with the hypothetical no-evolution dynamics that would have resulted if clone
158 frequencies had not changed since the start of the season (see Methods).
159 The result shows a consistently lower projected population size of *Daphnia* throughout the
160 season in the absence of adaptive evolution, compared with the abundance actually observed
161 (Fig. 5a). The effect of this loss of fitness can be seen in the ratio of the projected population
162 size without evolution relative to that observed in the lake (i.e., with evolution). By the end of
163 summer, the loss of fitness that would have accrued over four months of not evolving in response
164 to changing food quality would have resulted in a population of about a quarter the size of that
165 actually observed (Fig. 5b). We thus project, from the observed short-term effects of clone-
166 frequency change, that evolution markedly affected the ecological dynamics of a major
167 consumer.
168
169 **Conclusion.** We have shown that while plankton dynamics in Oneida Lake are typical of those
170 seen in moderately productive lakes as described by the "PEG model"[10,22,23], the patterns of
171 abundance observed in 2015 were underlain by rapid evolution of the dominant consumer
172 population while quality of the phytoplankton taxonomic composition as food for *Daphnia*
173 underwent typical seasonal succession. This result suggests that although the authors of the PEG
174 model did not invoke *Daphnia* evolution as a component of the typical seasonal pattern they
175 described for moderately productive lakes[22,23]; evolution may nevertheless have been present in
176 the lake communities upon which they based their description.
177 The seasonal phytoplankton succession depicted in the PEG model has been documented for
178 the past 40 years in Oneida Lake[25], so the cycle of evolution that we have shown for a single
179 season is very likely to have been an annual occurrence. The genetic variation required for the
180 *Daphnia* population to evolve was present in spring 2015 after what was surely four decades of
181 seasonally fluctuating selection. It is likely that this genetic variation has been maintained year
182 after year through a combination of (i) selection fluctuating in direction and intensity in all or
183 most years (Fig. 4), (ii) the tradeoff we observed among clones in performance (JGR) on spring
184 versus summer phytoplankton (Fig. 3a), and (iii) generation overlap created by annual
185 production of diapausing eggs and their hatching, spread out over multiple years, from the

sediment egg bank[26] which *D. mendotae* has in Oneida Lake[21]. These combined processes can be a potent force maintaining genetic variation in nature[27-31].

The net result is rapid evolution – on the time scale of weeks – of *Daphnia*'s performance on the dominant phytoplankton assemblage, while the annual reversals in food quality mean that there is very likely no long-term directional evolution as long as phytoplankton succession is repeated every year. This is an example of a more general pattern of how natural selection in nature often fluctuates in direction and intensity, with evolutionary change rapid in the short term, but slow on average over longer periods[3,32,33]. Our demonstration of a feedback between ecological change, rapid adaptive evolution, and genetic change affecting the nature and strength of ecological interactions, shows that eco-evolutionary dynamics can have important consequences for ecological processes, even when there is no long-term net evolutionary change.

Methods

Study system. We studied *Daphnia* clonal evolution and its effect on planktonic consumer-resource dynamics in 2015 at Oneida Lake, New York State (43° 13' 3" N, 75° 55' 22" W), a large (surface area: 206.7 km^2), relatively shallow (maximum depth 16.8 m, mean depth 6.8 m) lake that freezes in winter and has multiple brief periods of thermal stratification in summer (i.e., it is cold polymictic). Oneida Lake has been the subject of long-term monitoring by the staff of the Cornell Biological Field Station (CBFS), with sampling typically having occurred weekly from spring to autumn, for zooplankton starting in 1964, and for phytoplankton and nutrients starting in 1975 with additional data available from the 1960s[34]. These data show that at the start of this record, total phosphorus (TP) concentrations exceeded 100 µgP/L but decreased steadily following watershed nutrient management starting in the 1970s. By 2015, the year of our study, summer phosphorus was 20-30 µg TP/L and phytoplankton was $\leq$8 µg Chla/L[25,35]. The phytoplankton community is largely comprised of the five major taxonomic groups typical of North Temperate Zone lakes including Bacillariophyta, Cryptophyta, Chrysophyta, Chlorophyta, and Cyanobacteria. In most years, diatoms are the dominant taxon in early spring and fall, small flagellated cryptophytes and chlorophytes dominate the assemblage (though at low densities) during the clear water phase, and cyanobacterial blooms taking place between July and October, including in 2015 (Fig. 1c).

The Oneida Lake zooplankton community is comprised mainly of seasonally abundant *Daphnia* species[36], several calanoid and cyclopoid copepod species[37,38] and a diversity of rotifers[39]. While three daphniid species, *D. pulicaria*, *D. mendotae* and *D. retrocurva*, have dominated in different years, with at least the first two being present in the sediment record[21], the latter two have been the seasonally dominant *Daphnia* in the water column since 2006, likely due to a disproportionate increase in predation on *D. pulicaria* by planktivorous fish[14,36]. For this study, we focused on *D. mendotae* because it dominated the *Daphnia* assemblage in summer 2015, and because published microsatellite markers are available for closely related *D. galeata*[40] for clonal identification and tracking evolutionary changes in population genetic composition.

Cyanobacteria typically dominate the phytoplankton in most years from late July through October[25], and the blooms that have occurred since 2000 have been dominated by taxa capable of producing toxins, including *Dolichospermum* (formerly *Anabaena*), *Aphanizomenon*, and *Microcystis*[41]. During 2015, sampling for zooplankton abundance was carried out weekly at a 12 m deep central lake site (CBFS "Shackelton Point") as a part of the field station's long-term monitoring program[34]. Zooplankton were collected by a vertically towed 153 µm mesh plankton net with flow meter, and phytoplankton by 10 m integrated tube sampler[25,34]. Zooplankton, including *D. mendotae*, were identified, counted and biomass estimated by CBFS staff. From Samples for phytoplankton were collected weekly May through October. The data use here are averages over four stations along the mid-line of the lake (CBFS Shackelton Point, Three Mile Bay, Buoy 109 and Buoy 125). Phytoplankton were identified, measured, and counted, and biovolumes calculated by PhycoTech Inc. Chlorophyll-a was measured using a calibrated Turner fluorometer in the laboratory. Detailed methods and data are available on the Knowledge Network for Biocomplexity and in eCommons of Cornell University's Mann Library in the Cornell Biological Field Station collection[34].

Identifying seasonal clonal frequencies of *Daphnia mendotae*. In 2015 live *Daphnia* were collected weekly between May 10 and August 31, and biweekly during September. Animals were obtained by vertical plankton tow at a 7 m deep site (mean lake depth) near the "Shackelton Point" standard station. Live plankton samples were held at ca. 15 °C and processed within 2 h. On each date, the first 40-48 female *D. mendotae* encountered under a dissecting microscope were used to establish parthenogenetic isofemale (clonal) lines in culture. By the end of

September 2015, 768 clonal isolates had been established, though some isolates did not survive, and genotyping did not yield usable results for others. In some instances we were nevertheless able to extract usable DNA for genotyping females isolated from the field that died without reproducing. In the end we genotyped animals from 546 isofemale lines with 20-40 (median 37) clonal isolates per date. DNA was extracted from individuals using the HotSHOT protocol[42], and genotyped using seven microsatellite loci (SwiD4, SwiD5, SwiD10, SwiD14, SwiD15, Dp512 and DaB10/14) described for European *Daphnia*[40], closely related to our North American *D. mendotae*. Three additional loci (SwiD2, SwiD12, and SwiD1)[40] were unusable for our animals due to poor peak amplification.

We refer here to each *Daphnia* isofemale line that shared common alleles for all seven loci as a "clone." In a few instances, for which one of the variable loci did not amplify successfully, if the other six loci matched a lineage for which all seven amplified, these two lines were considered to be a single clone. However, any lineage for which two or more of the seven loci failed to amplify was removed from further analysis. We assigned allele identities for the 546 clonal isolates using GenoDive (version 2.0b23)[43]. Each locus was visually inspected to ensure proper peak identification by the software. We reassigned peaks that were misidentified by the software, and then used a distribution code in R version 3.5.1[44] to determine what round number allele values were present in the population. It is, of course, possible that any "clone" identified by our procedure may actually represent several clones. We note, however, that two of the loci we used had highly variable allele frequencies and so provide strong differentiation among our clonal isolates. Clone numbers were assigned in the order that we encountered unique genotypes but have no other meaning.

Using these protocols, we identified 124 unique *D. mendotae* clones in Oneida Lake between May and September 2015. Of these, 16 clones made up at least 10% of the population on at least one date (Supplementary Fig. 3); the rest were rarer, many represented by only a single female on a single date. For each of those 16 clones, temporal changes in frequency (Supplementary Fig. 3) were fitted with a spline generalized additive model (function gam() from the mgcv package in R, using method=REML and family=binomial)[44]. From among the 16 clones, we chose seven with different seasonal maximum frequencies (Supplementary Fig. 3) on which to measure *Daphnia* performance when fed either spring "good" food or summer "poor" food. For one clone (Clone 11), which came to dominate the *Daphnia* population in late summer (frequency > 60%),

we measured performance on two separately isolated isofemale lines to evaluate the consistency of the phenotypic response among clonal replicates identified via matching microsatellite multilocus genotypes. The seven clones chosen made up an average of 48% of the animals sampled on each date, with a range across all dates of 22–84%.

Simpson's Diversity Index applied to *Daphnia* clones. Clonal diversity was calculated using Simpson's Index (1-D) corrected for sampling without replacement[45],

$D = \sum_{i=1}^{R}[n_i\ (n_i - 1)/(N(N-1)]$, where n_i is number of individuals of Clone i for a total of R clones, and N is the total number of individuals sampled. The seasonal pattern of clonal diversity is presented in Supplementary Fig. 1. Our ability to identify rare clones was quite limited, given that we genotyped a maximum of 40 individuals on any given date out of a population size lake-wide on the order of 10^{12} individuals. This population size approximation is based on the product of the mean *Daphnia* density in Oneida May-Sept 2015 of 3,700 m^{-3} (Cornell Biological Field Station data archive) and a lake volume[46] of 1.40×10^9 m^3 so mean total *D. mendotae* population size in Oneida Lake in 2015 was 5.2×10^{12} individuals.

Measuring *Daphnia* performance on spring and summer phytoplankton. Juvenile growth rate, JGR, the specific rate of mass increase of individual *Daphnia*, has been shown to be a reliable measure of performance highly correlated with per capita rate of population increase, *r* (day^{-1}), under controlled laboratory conditions of varying food concentration[24]. It has been used as an effective bioassay of performance in a variety of food environments[10,12,17-19]. We used a four-day measure which in a study with another *Daphnia* species was found to be well correlated with *r* ($r^2 = 0.88$)[24]. The eight *Daphnia* isofemale lines (seven clones with one duplicated) were cultured clonally at 20°C for at least 10 generations, fed on good food (chlorophyte alga, *Scenedesmus obliquus*) before the start of the experiment. Clonal performances were determined using two different food conditions: phytoplankton typical of Oneida Lake either in spring or late summer.

We measured JGR over a four-day period starting with neonates ($\leq$ 24 h since hatching)[34]. Neonates were placed in triplicate 250-mL flow-through chambers maintained in a water bath at 20 ± 0.5°C with dim incandescent illumination. Each chamber was initiated with 8-12 neonates of the same isofemale line and was continuously supplied the test phytoplankton food suspension

using a peristaltic pump at a rate of 750 mL/day. The food concentration used, 1 mg C/L, assured that food density was not limiting to *Daphnia* growth and that only food quality was a factor. The two diets were created using laboratory-cultured phytoplankton meant to simulate spring and fall phytoplankton community compositions commonly found in Oneida Lake. The spring diet consisted of three algal taxa each comprising 1/3 of the food mixture by carbon content: the unicellular centric diatom *Cyclotella meneghiniana*, the green alga *Scenedesmus obliquus*, and the cryptophyte *Cryptomonas ozolini* (see Supplementary Table 4 for culture information). The late-summer diet consisted of a 50:50 mixture of the cyanobacterium *Microcystis aeruginosa* known to produce the toxin microcystin and the green alga *Scenedesmus obliquus*. Although cyanobacteria made >50% of Oneida Lake phytoplankton biovolume on some dates in 2015 (Fig. 1c), our late-summer 50:50 diet was chosen because only two diets were possible in our experimental set up, and that mixture was sufficient to distinguish effects of diet among the clones we used. The two phytoplankton food mixtures were prepared daily in 0.45 µm filtered Oneida Lake water, and maintained in suspension in aluminum-covered 10 L carboys on stir-plates.

The experimental setup consisted of 48 flow-through chambers allowing all eight isofemale lines to be tested at the same time (2 food treatments×8 lines×3 replicates). The flow-through experimental set-up was similar to established methods[18,24], but the chambers were made of a clear Plexiglas rather than glass. Each chamber was a tube 4.5 cm diameter, 30 cm long with a 75 µm mesh in the bottom to retain the *Daphnia* but to permit phytoplankton suspension to pass through. All chambers were suspended in a large water bath containing 0.45 µm filtered Oneida Lake water. The test food suspension was dripped into chambers continuously, and flowed out continuously at a rate of three replacement volumes per day, providing the *Daphnia* with a steady food supply.

Initial *Daphnia* dry weights were determined for 10–12 neonates per clonal lineage. At the end of the four-day experiment, we collected, dried, and weighed all remaining animals from each chamber; any animals that died during the experiment were excluded because they had stopped feeding before the experiment was complete. All animals were placed in aluminum tins, dried at 60°C for >24 h and weighted on a microbalance (Sartorius model SE2). Juvenile growth rate (day^{-1}) was calculated as: $JGR = [\ln W_t - \ln W_0]/t$, where W_0 and W_t are the initial and final weights per individual and t is the duration of the experiment (actual start and end times for

each chamber were recorded). Because individuals within a chamber were pooled for weighing, "chamber" is the unit of replication ($N = 3$).

Quantifying the magnitude of *Daphnia* evolution and its effect on consumer dynamics. We estimated how the JGR of each clone would have changed seasonally as a function of the proportion of the phytoplankton comprised of cyanobacteria (Fig. 1c) by fitting a $\log_e$-transformed reaction norm through JGR when fed either 0% cyanobacteria or 50% cyanobacteria (Supplementary Fig. 2). We use an exponential fit because a linear projection leads to nonsensical negative JGR values for some clones at high % cyanobacteria. The relationship in Supplementary Fig. 2 for each clone was used to infer JGR at other cyanobacterial proportions of the phytoplankton throughout the season (Fig. 4). Note that other plausible functions must all continue to decline as cyanobacteria fraction increases above 50%, or at the very least simply level off. Inspection of Supplementary Fig. 2 shows that any alternative monotonically decreasing function will give the same qualitative result in terms of the relative positions of the projected clonal JGRs. The only exception would be non-resistant clone 169 which would lie above only resistant clone 2 if JGRs did remained constant beyond 50% cyanobacteria, and even this would not substantively affect our analysis. For each date, the *Daphnia* population's mean JGR was estimated as the sum of the inferred JGR for each clone multiplied by its frequency, which gave us a picture of how JGR evolved as clonal frequencies changed over the season. We compared this evolving population JGR with the projected population JGR if evolution had not occurred and clonal frequencies had remained unchanged over the course of the season. For this non-evolving population, we used the average frequencies of the seven clones on the four sampling dates before the advent of the clear water phase (Fig. 1a); i.e., before the first major transition in phytoplankton quantity and quality (Fig. 1c). Since all clones had to be present at the start of the season (because all clones in the lake are established by hatching from diapausing eggs earlier in spring[21]), the frequencies of those from among the seven we studied that were not detected during the first four sampling dates were taken to be half the limit of detection ($0.5 \times 1/40 = 1/80$). The effect of evolution on *Daphnia* population performance over the course of the season was taken as the fractional increase in the clone-frequency-weighted JGR, EVOJGR, relative to the no-evolution projected JGR, NOEVOJGR, i.e., (EVOJGR–NOEVOJGR)/NOEVOJGR) (see Fig. 4c).

Finally, we projected the effect of evolution on the ecological process of seasonal change in consumer (*Daphnia*) population size by calculating a cost of not-evolving. For this, we calculated a "no-evo" mean JGR for each date using the same "baseline" we used above to construct Fig. 4c (i.e., constant clone frequencies set at the average of the frequencies on the first four sampling dates leading up to the start of the clear water phase). We then used these daily "no-evo" mean JGR values in the regression model for *r* to predict how much this substitution would change the value of *r*. Specifically, our calculated regression model for *r* is

$$\hat{r} = a_0 + a_1 \times (\text{chlA}) + a_2 \times (\text{mean JGR}) \qquad (1)$$

so the predicted effect on *Daphnia r* of holding clone frequencies at their "no-evo" baseline values is

$$\Delta r(t) = a_2 \times \left[\text{no-evo mean JGR}(t) - \text{mean JGR}(t)\right]. \qquad (2)$$

We projected what this change in *r* would have done to *Daphnia* population dynamics as follows. Let $N(t)$ be the observed *Daphnia* population as a function of time. The last of the four baseline clone-frequency samples was taken on day 151 (i.e., 31 May), so we project a "no-evo" *Daphnia* abundance $N^*(t)$ forward from that date as

$$N^*(t) = N(t) exp\left(\int_{151}^{t} \Delta r(t)\, dt\right). \qquad (3)$$

The result is plotted in Fig. 5. Note that we use the regression model only to estimate how much $r(t)$ would *change*, if mean JGR on day *t* were the result of baseline clone frequencies rather than the estimated actual clone frequencies for day *t*. We do not use the regression model to project $r(t)$ values based on food quantity and no-evo mean JGR because there are many additional factors (e.g., temperature, predators, pathogens, etc.) in the Oneida Lake environment that can affect the *Daphnia* population's $r(t)$.

Statistical analyses. Statistical analysis were carried out using R version 3.5.1[44]. Differences between clones and food treatment were assessed via ANOVA (Supplementary Table 1; Fig. 3a), followed by post-hoc tests for pairwise clone differences in their reaction norm slopes corrected

for multiple comparisons[47] using package **phia**, with the function `testInteractions()` (Supplementary Table 2).

The regression model exploring the effects of chlA and mean JGR on *Daphnia* population per capita growth rate (Table 1; Fig. 5) was a simple linear regression without random effects, fitted using the `lm()` function in R. Model assumptions were validated using standard regression diagnostics. A plot of residuals versus fitted values showed no signs of nonconstant variance or unmodeled nonlinear trends, and additions of interaction and quadratic terms were all non-significant ($p>0.1$ in the `summary` of the fitted models). Residuals were Gaussian (Gaussian quantile-quantile plot using function `qqPlot()` in the **car** package; $p = 0.67$ in Shapiro-Wilk normality test using function `shapiro.test()` and had no significant autocorrelations, using function `acf()`). The Akaike Information Criterion, R function `AIC()`[44] (Supplementary Table 3) was used to compare linear regression models estimating *Daphnia r* as a function of chlorophyll-a alone or chlorophyll-a and mean *Daphnia* population resistance to dietary cyanobacteria.

The temporal change in evolutionary gains was assessed using a linear regression correcting for autocorrelation using the `gls()` function from the **nlme** R package. The regression coefficient was calculated using the `rsquared()` function from the **piecewiseSEM** R package. The Akaike Information Criterion, R function `AIC()`[44] (Supplementary Table 3) was used to compare linear regression models estimating *Daphnia r* as a function of chlorophyll-a alone or chlorophyll-a and mean *Daphnia* population resistance to dietary cyanobacteria.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data and code availability

The data underlying each of the figures and statistical analyses, as well as R scripts for R-generated statistical analyses and figures, are freely available online at https://doi.org/10.7298/pgzv-2736.

[Note: this on-line information will be updated if/when the paper has been accepted.]

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Author contributions

B.E.M., S.P.E. and N.G.H. conceived the study. L.R.S., B.E.M. and N.G.H. designed the field sampling; E.F., L.R.S., and L.G.R. and his lab carried it out. L.R.S. and N.G.H. designed the lab experiment, and L.R.S. carried it out. P.S. and his lab did the microsatellite DNA sequencing. B.E.M. and L.R.S. analyzed the microsatellite data to calculate genotype frequencies. N.G.H., L.R.S., L.G., L.D.M and S.P.E. analyzed the experimental results. The first draft was written by L.R.S. and N.G.H. All authors contributed to revisions of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.7298/pgzv-2736.

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Table

Table 1| The effect of *Daphnia* evolution on projected *Daphnia* population dynamics. Regression model results for *Daphnia* population growth rate, *r*, as a function of food density and *Daphnia's* evolving adaptation to dietary cyanobacteria. *Daphnia* population rate of increase, *r* (day^{-1}), was calculated from smoothed densities on consecutive sampling dates. Food density is measured by total phytoplankton chlorophyll-a (ChlA). *Daphnia* population mean adaptation to dietary cyanobacteria is measured by the clone-frequency-weighted mean of estimated $\log_e$(JGR) (Fig. S2) at the fractional cyanobacteria abundance on each sampling date. Note that including an interaction term to the model was not significant (t = 1.73, p = 0.12).

Regression model: $r = \alpha_0 + \alpha_1 \times (\text{chlA}) + \alpha_2 \times (\text{mean } \log_e(\text{JGR}))$

Regression coefficients:		Estimate	Std. Error	t value	p-value
(Intercept)	α_0	-0.172	0.044	-3.898	0.0030
Chlorophyll-a	α_1	0.298	0.067	4.434	0.0013
Mean $\log_e$(JGR)	α_2	0.683	0.189	3.613	0.0048

Adjusted r^2 for full model = 0.71; for ChlA only = 0.38; for mean $\log_e$(JGR) only = 0.21

Figure 1

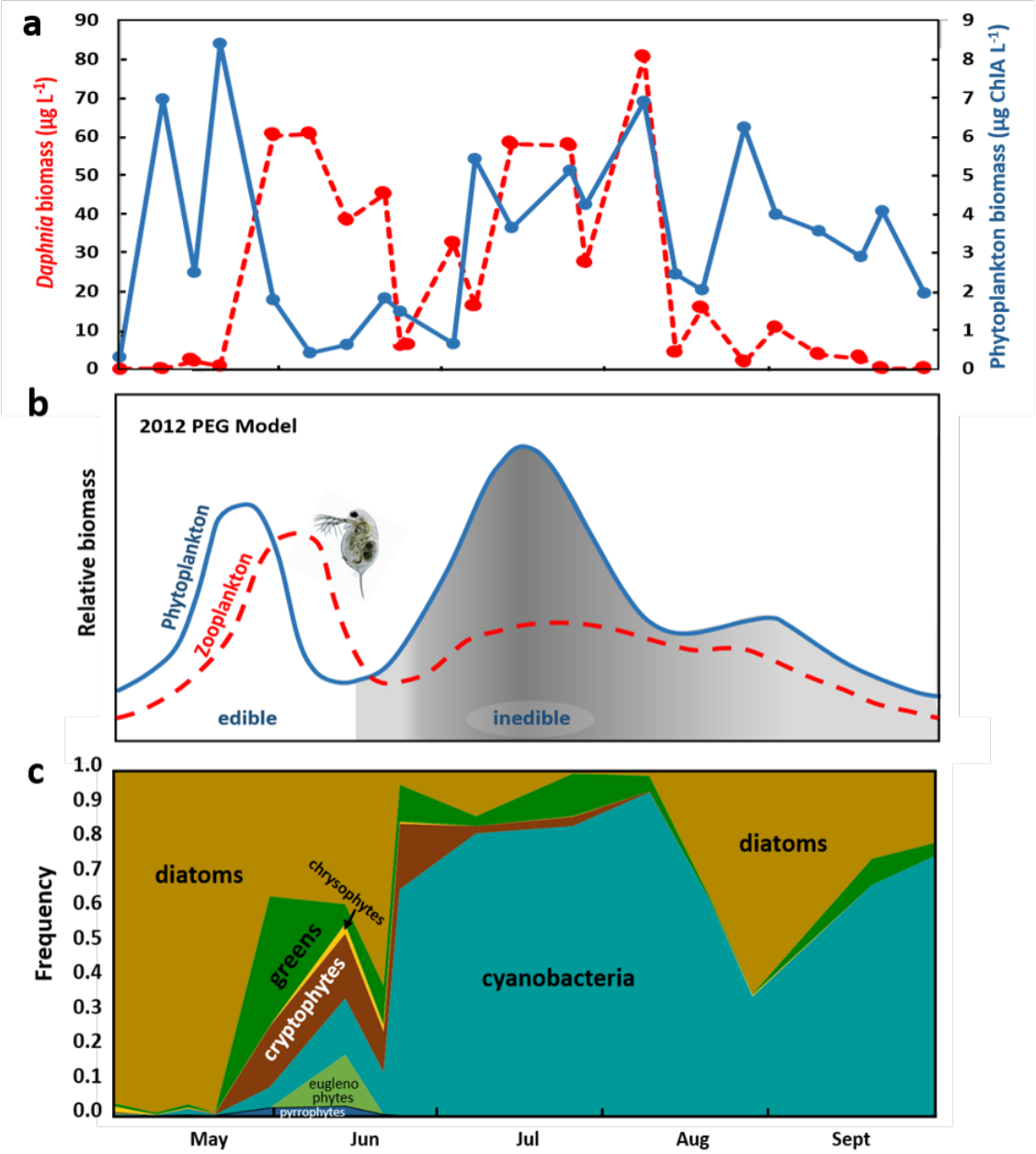

Fig. 1 | Seasonal dynamics of Oneida Lake plankton in 2015 compared with stereotypical PEG model. **a**, Biomass density of phytoplankton (blue; chlorophyll a, chlA) and the dominant zooplankton grazer, *Daphnia mendotae* (red) is similar to the Plankton Ecology Group (PEG) model in **b**, redrawn from Sommer *et al*.[23]. Phytoplankton phyla in **c** underwent succession from a spring bloom dominated by edible diatoms, followed by a clear water phase, caused by *Daphnia* grazing, dominated by diatoms and small flagellated green algae and cryptophytes, and then a summer bloom of relatively inedible cyanobacteria.

Figure 2

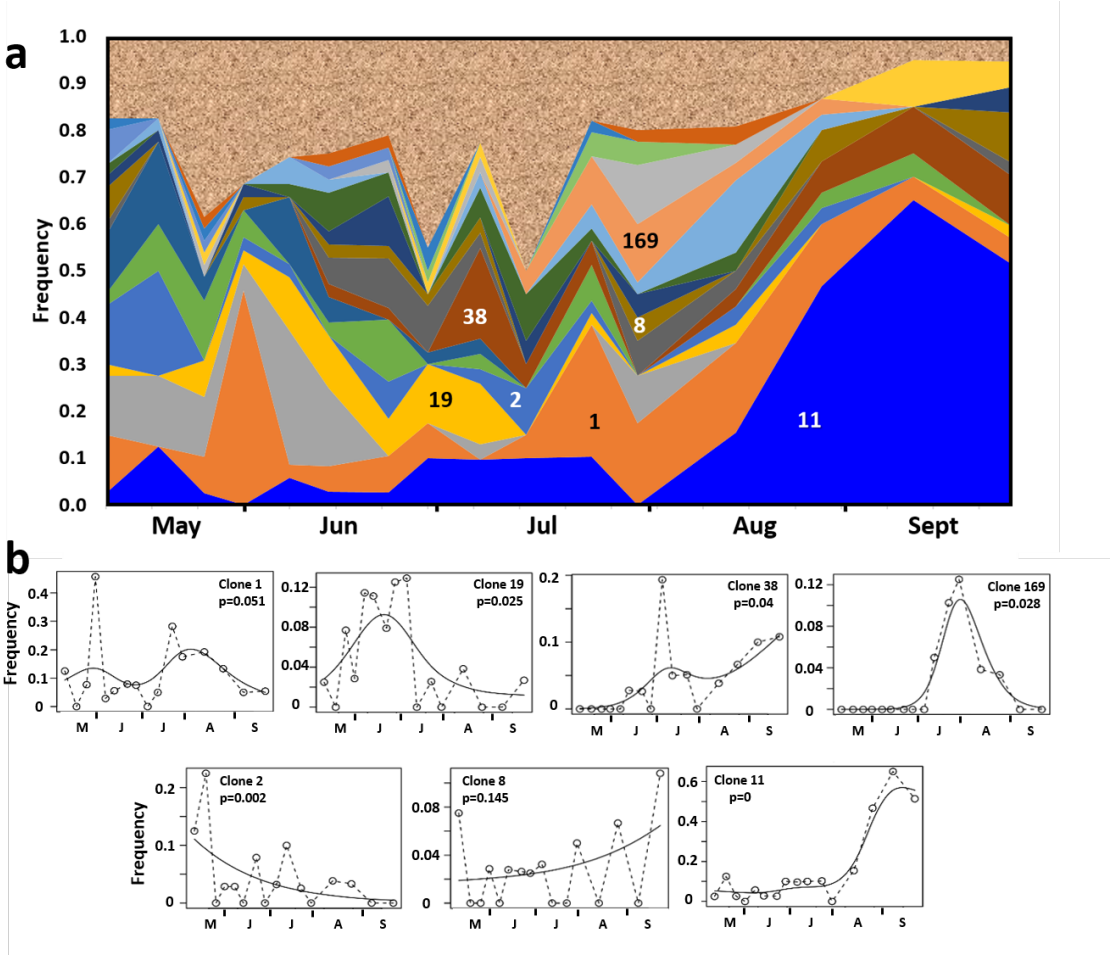

Fig. 2 | Frequencies of *Daphnia mendotae* clones in Oneida Lake 2015. **a**, Each color represents a different clone. Granular area at the top is the sum of clones only occurring in plankton samples as a single individual on a single date. **b**, Seasonal frequencies of the seven clones on which JGR was measured (numbered in **a**). Shown in **b** are the data and a fitted spline curve with the *p*-value testing against the null hypothesis of no temporal trend (**Methods**). Top row: clones 1, 19, 38 and 169 are non-resistant to cyanobacteria in juvenile growth rate experiments (Fig. 3a). Bottom row: clones 2, 8 and 11 are resistant to cyanobacteria.

Figure 3

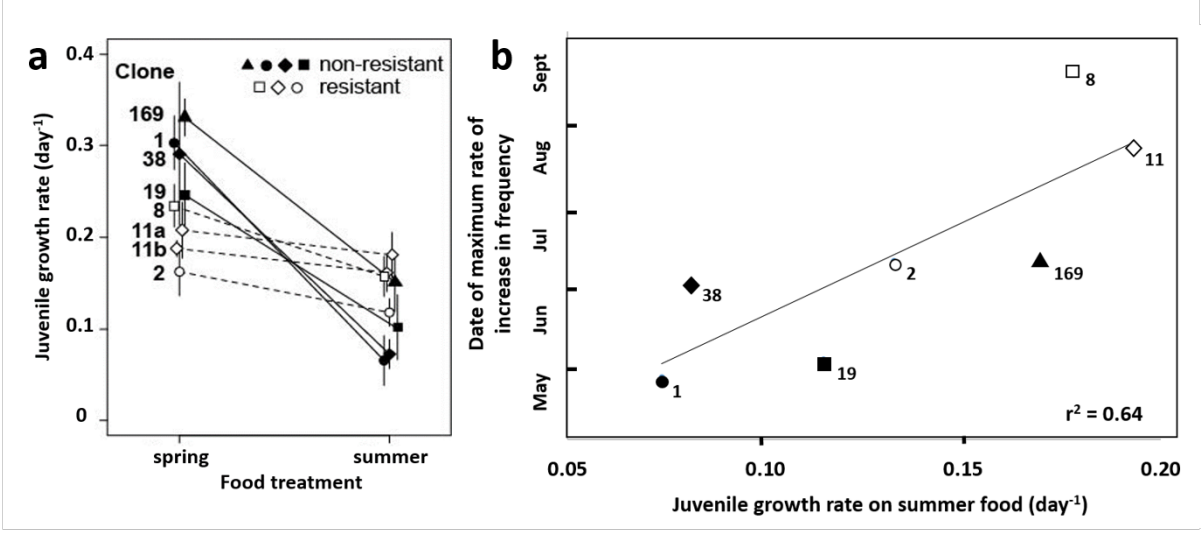

Fig. 3 | Performance of Oneida Lake *Daphnia mendotae* clones on spring and summer food. **a**, Juvenile growth rate, JGR, reaction norms of seven clones that peaked in frequency at different times during 2015, when fed phytoplankton typical of spring or summer. Clone 11 is represented by two independent clonal isolates. Resistant (unfilled symbols, dashed lines) and non-resistant (filled symbols, solid lines) clones are defined by their reaction norm slope (see text). Error bars: $\pm$1 sd. **b**, Relationship between date on which each clone had its maximum rate of increase in frequency and its JGR on cyanobacteria-rich summer diet ($r^2 = 0.64$, df = 5, $p = 0.034$).

Figure 4

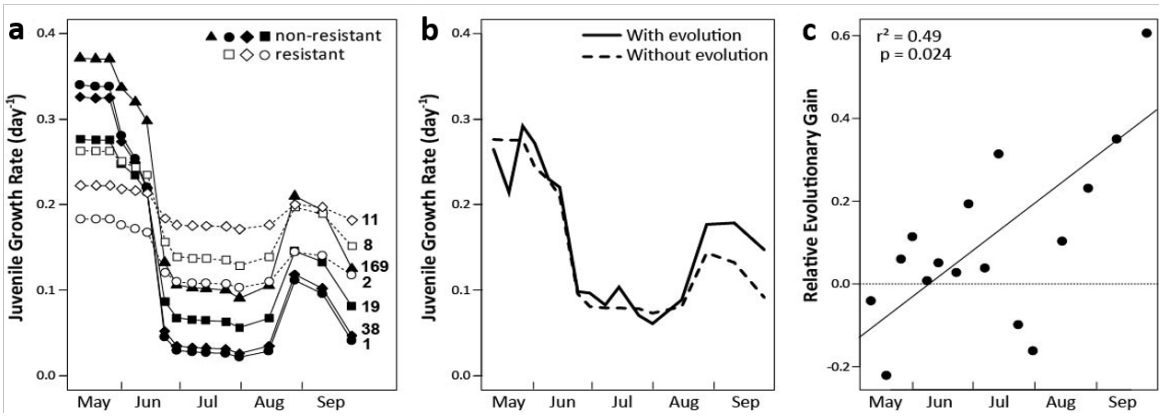

Fig. 4 | Evolution of *Daphnia* population mean juvenile growth rate based on clone-specific JGR values and seasonal phytoplankton composition. **a**, Projected clone-specific JGR for resistant and non-resistant clones based on Fig. 3a and Supplementary Fig. 2 combined with seasonal cyanobacteria frequency data (Fig. 1c). Clones favored by selection (JGR generally above the others) varies seasonally. **b**, Comparison of evolving mean JGR (solid line = JGR weighted by observed clone frequencies on each date) with projected non-evolving JGR (dashed line = JGR if clone frequencies remained constant at start-of-season values) (see Methods). **c**, Relative evolutionary gain (fractional increase in performance due to evolution = [solid-line in **b** – dashed-line in **b**]/[dashed-line in **b**]) increases over the season.

Figure 5

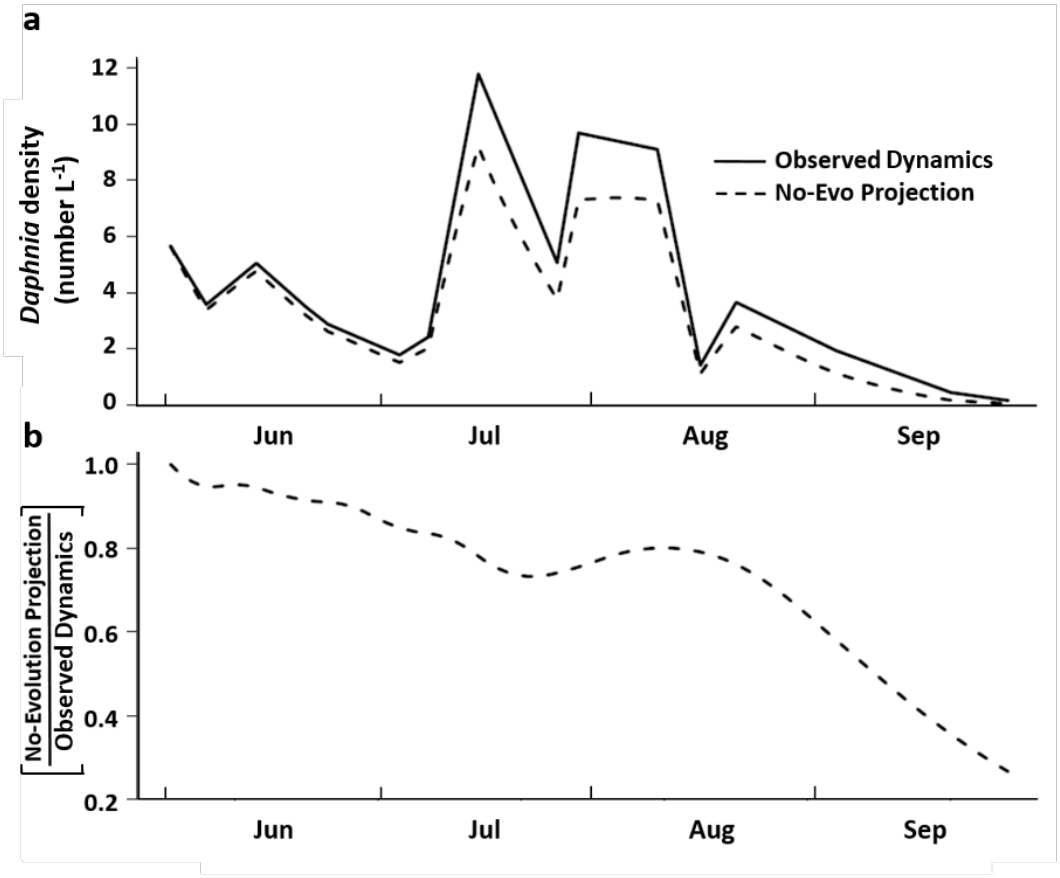

Fig. 5 | The projected effect on *Daphnia* population dynamics of its evolution in response to changing edibility of the phytoplankton food resource. **a**, The observed density of evolving *Daphnia* population in Oneida Lake between 7 June (first sample date after CWP) and 25 September (last sample date) is greater than the density projected if clonal frequencies had not evolved over this period (i.e., remained fixed at start-of-season frequencies). **b**, The projected effect of not evolving on *Daphnia* population size illustrated by the fractional decline in projected no-evolution *Daphnia* density relative to observed (i.e., with-evolution) *Daphnia* density.