CONSUMER-RESOURCE DYNAMICS IS AN ECO-EVOLUTIONARY PROCESS IN A NATURAL PLANKTON COMMUNITY

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ABSTRACT

While many laboratory and mesocosm studies have shown rapid evolution can occur on an ecologically relevant timescale leading to eco-evolutionary dynamics, these interactions are rarely documented in nature. This study is one of the first to demonstrate these processes in a natural lake. We used an important planktonic consumer, *Daphnia mendotae,* and the quality of its resource, phytoplankton, to demonstrate this eco-evolutionary process. We observed seasonal changes in phytoplankton species composition (an ecological process) drive changes in the frequency of consumer genotypes (evolution), which in turn has the potential to affect the consumer population's somatic growth rate (ecology). Genotypes predominant in spring, when edible phytoplankton dominated, grew well in the lab when fed spring algal taxa, but poorly on a diet containing relatively inedible cyanobacteria typical of summer. Conversely, genotypes that dominated in late summer, or showed no seasonal frequency pattern, were relatively resistant to dietary cyanobacteria.

BIOGRAPHICAL SKETCH

Lindsay Renee Schaffner was born in 1985 in Milwaukee, Wisconsin. At the age of 9, her family moved to New Berlin, WI where she lived until high school graduation from New Berlin West High School. Lindsay then attended the University of Wisconsin-Madison where she received a B.S. in Wildlife Ecology with a certificate (minor) in Environmental Studies. She became interested in aquatic ecology while working summers during her undergraduate studies at the Center for Limnology - Trout Lake Station in northern Wisconsin. After receiving her B.S., she worked several seasonal jobs from fish hatcheries in Alaska to herptile surveys in Wyoming. While in the process of applying for master's programs, Lindsay learned about an opportunity to work with Dr. Nelson Hairston, Jr. at Cornell University. She began as his lab manager in the spring of 2011. In addition to working with Dr. Hairston, Lindsay also worked as a technician for Dr. David Lodge and as an administrator of an IGERT grant for Dr. Christine Goodale. With the support of Dr. Hairston, Lindsay enrolled in Cornell University's Employee Degree Program through the Department of Ecology and Evolution to pursue a master's degree in 2014. In her free time, Lindsay enjoys hiking, gardening, crafting, and baking.

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Finally, thank you to Dr. Steve Ellner for acting as my committee member, adding thoughtful insight to the research, and being supportive throughout the entire process. Overall, thanks must be given to Dr. Nelson Hairston, Jr. for being a wonderful scientist, mentor, boss, advisor, and friend through the entire process.

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INTRODUCTION

With the recognition that organisms in nature frequently evolve quite rapidly (e.g., Endler 1986; Hendry and Kinnison 1999; Messer et al. 2016) has come the realization that when evolving traits are important in determining interaction strengths among species, ecological dynamics can be altered while they are occurring (e.g., Thompson 1998; Yoshida et al. 2003; Hairston et al. 2005; Carroll et al. 2007; Agrawal et al. 2013; Pelletier et al 2017). Although these eco-evolutionary dynamics increasingly appear to be common in nature (see reviews by Schoener 2011; Rudman et al. 2017; Hendry 2017), an intriguing question that remains is whether rapid contemporary evolution alters community processes in a way that is important for ecosystem functioning. There are excellent examples of genotypes within a single species having distinct and important effects on ecosystem processes such as nutrient cycling and consumer respiration (Whitham et al. 2003; Bassar et al. 2010; Rudman et al. 2015), community primary production (Cook-Patton et al. 2011; Chislock et al. 2013), and consumer abundance (Agrawal et al. 2013), among others in a field known broadly as community and ecosystem genetics (Agrawal 2003; Whitham et al. 2006). Yet, examples of effects on temporal dynamics in nature are still largely missing from this potentially powerful intersection of ecology and adaptive evolution: since evolution of ecologically important traits can be fast enough to occur on the time scale of ecological dynamics, do the evolving phenotypes alter short-term temporal changes in community structure or ecosystem functioning? Such temporal eco-evolutionary dynamics have been documented in laboratory microcosms (Yoshida et al. 2003; Hiltunen et al 2014; Turcotte et al. 2011a) and field mesocosms (Turcotte et al. 2011b; Pantel et al. 2015), but studies in unconfined natural systems are generally lacking. Further, an assessment of the ecological

importance of eco-evolutionary dynamics will be most convincing if an evolving organism plays a central ecological role in the functioning of its ecosystem.

A good candidate for exploring the occurrence and importance of eco-evolutionary dynamics in nature is the major freshwater planktonic herbivore, *Daphnia*. Members of this well-studied crustacean genus (de Bernardi and Peters 1987; Lampert 2011; Colbourne et al. 2011; Miner et al. 2012) are significant consumers of phytoplankton, capable of seasonally clearing the water column in many lakes (Sommer et al. 2012). In addition to its effects on primary producer abundance, *Daphnia* can affect phytoplankton community composition (Sarnelle 1993; Tessier and Woodruff 2002), and alter nutrient availability and stoichiometry via consumption and excretion (Sterner and Elser 2002). It also serves as a major food source for zooplanktivorous predators (e.g., Luecke et al. 1990; Rudstam et al. 1993), and hence plays a central role in the trophic cascade in lakes (e.g., Carpenter et al. 1987; Hambright et al. 2007).

One challenge that *Daphnia* faces in lakes with moderate to high nutrient enrichment, is the dominance of cyanobacteria in the phytoplankton in late summer and early autumn. These members of the phytoplankton are poor food for *Daphnia* because they lack or are extremely low in essential fatty acids (Müller-Navarra et al. 2000), often contain compounds toxic to *Daphnia* (Lampert 1981), and in many cases have complex colony shapes that are difficult for these grazers to handle. Because *Daphnia* is a generalist consumer with poor ability to discriminate among particles when feeding, dominance of the phytoplankton by cyanobacteria typically results in low consumer growth rate (Arnold 1971; Wilson et al. 2006; Martin-Creuzburg et al. 2008). As a result, there should be strong natural selection favoring *Daphnia* genotypes that are relatively insensitive to dietary cyanobacteria.

Consistent with expectation, Hairston et al. (1999, 2001) showed the evolution of this tolerance expressed as juvenile (somatic) growth rates of clonal lineages of *Daphnia* hatched from dormant eggs laid before and after a decade of eutrophication had resulted in summer cyanobacterial blooms in Lake Constance, Europe. Similarly, adaptive tolerance of *Daphnia* to dietary cyanobacteria has been demonstrated for individuals originating from lakes with high and low abundances of cyanobacteria (Sarnelle and Wilson 2005, Jiang et al. 2015). And finally, Chislock et al. (2013) showed that clones of *Daphnia*, known to be tolerant of dietary cyanobacteria, suppressed the growth of cyanobacteria in nutrient enriched mesocosms, whereas clones sensitive to cyanobacteria did not.

To explore the rate at which this adaptive evolution occurs, and how it affects the consumer-resource interaction in a natural system, we studied changes in genetic composition of *Daphnia mendotae* (hereafter, *Daphnia*) in Oneida Lake, New York State, over the course of a single season during which the taxonomic makeup of the phytoplankton underwent typical seasonal succession including a summer cyanobacterial bloom. We documented evolution as changes in clonal frequencies identified using microsatellite DNA genotyping, since these *Daphnia* only reproduce parthenogenetically in summer (Cáceres et al. 1998a). We then assayed the performance of seven representative clones with peak abundances at different times of year, by measuring their specific rate of mass increase as juveniles (juvenile growth rate, g_j) when fed either spring phytoplankton (diatoms, cryptophytes and chlorophytes) or summer phytoplankton (cyanobacteria and chlorophytes). From this we calculated how mean *Daphnia* growth rate changed seasonally, affected both by succession in phytoplankton quality as food, but also by evolution of *Daphnia* sensitivity to that changing diet, showing within-season eco-evolutionary consumer-resource dynamics.

METHODS

Study System

We studied *Daphnia* clonal evolution and its effect on planktonic consumer-resource dynamics in 2015 at Oneida Lake, New York State, 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), typically weekly from spring to autumn, for zooplankton starting in 1964, and for phytoplankton and nutrients starting in 1975 (reviewed by Rudstam et al. 2016). These data show that at the start of this record, total phosphorus (TP) concentrations exceeded 100 µgP/L and phytoplankton biomass (as chlorophyll a) was > 30 µg Chla/L in the 1960s, but both decreased steadily following watershed nutrient management starting in the 1970s so that by 2015, the year of our study, summer phosphorus was 20-30 µg TP/L and phytoplankton was $\leq$ 8 µg Chla/L (Cuhel and Aguilar 2016; Idrisi et al. 2016). 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, with cyanobacterial blooms taking place between July and October, including in 2015 (see below). Cyanobacteria typically dominate the phytoplankton in most years from late July through October (Idrisi et al. 2016), and the blooms that have occurred since 2000 have been dominated by cyanobacteria capable of producing toxins, including *Dolichospermum* (formerly *Anabaena*), *Aphanizomenon*, and *Microcystis* (Hotto et al. 2008).

The Oneida Lake zooplankton community is dominated by seasonally abundant *Daphnia* species (Cáceres et al. 2016), several calanoid and cyclopoid copepod species (Hairston and Van Brundt 1994; Hansen and Hairston 1998) and a diversity of rotifers (Hairston et al. 2000). 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 (Cáceres 1998b), 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 (Cáceres et al. 2016). For this study, we focused on *D. mendotae* because it dominated the *Daphnia* assemblage in the summer of our study, and because published microsatellite markers are available for closely related *D. galeata* (Brede et al. 2006) for clonal identification and tracking evolutionary changes in population genetic composition.

During 2015, sampling for plankton abundance was carried out weekly at a 12 m deep central lake site (CBFS "Shackelton Point Deep") as a part of the field station's long-term monitoring program (Rudstam et al. 2016). Zooplankton were collected by 64 µm mesh vertical plankton net tow, and phytoplankton by 10 m integrated tube sampler (methods given by Cuhel and Aguilar 2016; Rudstam et al. 2016). Zooplankton, including *D. mendotae*, were identified and counted by CBFS staff, and phytoplankton were identified, measured, and counted, and biovolumes were calculated by PhycoTech Inc.

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 CBFS "Shackelton Point Deep." 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 (Montero-Pau et al. 2008), and genotyped using seven microsatellite loci (swiD4, swiD5, swiD10, swiD14, swiD15, Dp512 and DaB10/14) described by Brede et al. (2006) for European *Daphnia*, closely related to our North American *D. mendotae*. Three additional loci of Brede et al. (2006) (SwiD2, SwiD12, and SwiD1) 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; Meirmans & Van Tienderen 2004) . 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 were highly variable 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 sampled and sequenced on at least one date; 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 were fitted with a spline generalized additive model (function gam() from the mgcv package in R, using method=REML and family=binomial) (R Core Team 2017). From among the 16 clones, we chose seven with different seasonal maximum frequencies on which to measure juvenile growth rate when fed either spring "good" food or summer "poor" food. For one clone (Clone 11), which came to dominate (frequency > 60%) the *Daphnia* population in late summer, we measured performance on two separately isolated isofemale lines to evaluate the consistency of the phenotypic response for a clone identified using microsatellite DNA.

Measuring Daphnia performance on spring and summer phytoplankton

The eight *Daphnia* isofemale lines (seven clones with one duplicated) were cultured clonally in 0.45 µm filtered Oneida Lake water at 20°C for at least 10 generations and 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. Performance for each clone was measured as the specific rate of mass increase (juvenile growth rate, g_j) as described below.

We measured g_j over a four-day period starting with neonates (< 24 hr since hatching), using the method of Lampert and Trubetskova (1996). Neonates of each clone 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 contained 8-12 neonates of the same isofemale line and were 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*. 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*. Phytoplankton culturing information is given in Table 1. The two phytoplankton food mixtures were prepared daily in 0.45µm filtered Oneida Lake water, and maintained in suspension in aluminum-covered 19 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 that described by Lampert and Trubetskova (1996), 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 tubes were suspended in a large water bath containing 0.45 µm filtered Oneida Lake water. Each tube had a culture volume of 250 mL, and the test food suspension was dripped into tubes continuously, and flowed out continuously at a rate of three replacement volumes per day, providing the *Daphnia* with a steady food supply.

Table 1. Phytoplankton culture sources and media used in juvenile growth rate experiment.

Phytoplankton taxon	Source	Culture method and medium*
Cyclotella meneghiniana	UTEX LB FD257† https://utex.org/products/utex-lb-fd-0257	batch; WC medium
Scenedesmus obliquus	UTEX 393 https://utex.org/products/utex-0393	chemostat; Bolds Basal medium
Cryptomonas ozolini	UTEX LB 2194 https://utex.org/products/utex-lb-2194	batch; WC medium
Microcystis aeruginosa	CPCC 300§ https://uwaterloo.ca/canadian-phycological-culture-centre/sites/ca.canadian-phycological-culture-centre/files/uploads/files/cpcc_list_of_cultures_nov_20_13.pdf	batch; BG-11 medium

*Recipes for media: Algal Culturing Techniques. 2005. ed. Anderson, R.A. Elsevier, Amsterdam.

†UTEX: Culture Collection of Algae at the University of Texas at Austin https://utex.org/

§Canadian Phycological Culture Centre, University of Waterloo https://uwaterloo.ca/canadian-phycological-culture-centre/

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. For the spring food treatment, the maximum number of animals that died per replicate was two with an average loss per replicate of

less than one animal. For the fall food treatment, clone 1 lost the most number of animals throughout the experiment with just under half dying before the end of the experiment. Overall, there was an average loss of two animals per replicate for the fall food treatment. All remaining 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: $g_j = [\ln W_t - \ln W_0]/t$, 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 or each tube were recorded). Because individuals within a chamber were pooled for weighing, "chamber" is the unit of replication (N = 3).

Statistical analyses were carried out using R version 3.5.1 (R Core Team 2017). For ANOVA, we used library 'phia', with the function testInteractions, followed by post-hoc tests for pairwise clone differences corrected for multiple comparisons using the method of Holm (1979). Seasonal patterns in clone frequencies were evaluated by fitting spines to clone frequencies.

RESULTS

Oneida Lake seasonal plankton dynamics

Seasonal phytoplankton-zooplankton dynamics in Oneida Lake in 2015 were consistent with the generalized pattern for eutrophic lakes described by Sommer et al. (1986, 2012) as the "Plankton Ecology Group" or "PEG model" (Figure 1). A spring diatom bloom, present when sampling started in May, was terminated in late-May by an increase in grazing *Daphnia* causing a spring (late-May to early-June) clear-water phase (CWP) dominated by diatoms and unicellular and small colonial algae, chlorophytes, chrysophytes, cryptophytes, and euglenophytes, comprising 85–100% of the phytoplankton biomass. The CWP was followed by a crash and then resurgence of the *Daphnia* population, accompanied by an increase in abundance of cyanobacteria (75–90%

of biomass) with filamentous forms dominating first (*Dolichospermum* then *Aphanizomenon*) followed by colonial *Microcystis*, and a late August return of diatoms.

The 124 unique *D. mendotae* clones we identified varied in frequency in Oneida Lake between May and September 2015 (Figure 2a), and the seven clones chosen for juvenile growth rate bioassay had distinct seasonal frequency patterns (Figure 2b).

Daphnia clonal performances on spring and summer phytoplankton

As measured by juvenile growth rate, all seven clones performed worse on the summer mixed diet of cyanobacteria and green algae than on the spring diet of cryptophytes, diatoms and greens (Figure 3a) with a highly significant food effect (Table 2). Clones differed in their overall performance (significant clone effect), and in their resistance to the cyanobacteria diet (significant food×clone effect) (Table 2, ANOVA all p values << 0.001).

In a pair-wise comparison of reaction norms, we found that Clones 2, 8 and 11, which all have relatively shallow slopes, did not differ significantly from each other (Table 3), so we designate them "resistant" clones (Figure 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. Finally, pair-wise comparisons between clones from the resistant and non-resistant categories show that they differ significantly, and we use these two groupings in order to simplify the discussion of our results. Clone 19 is intermediate but closer to the non-resistant clones, so we include it in that group.

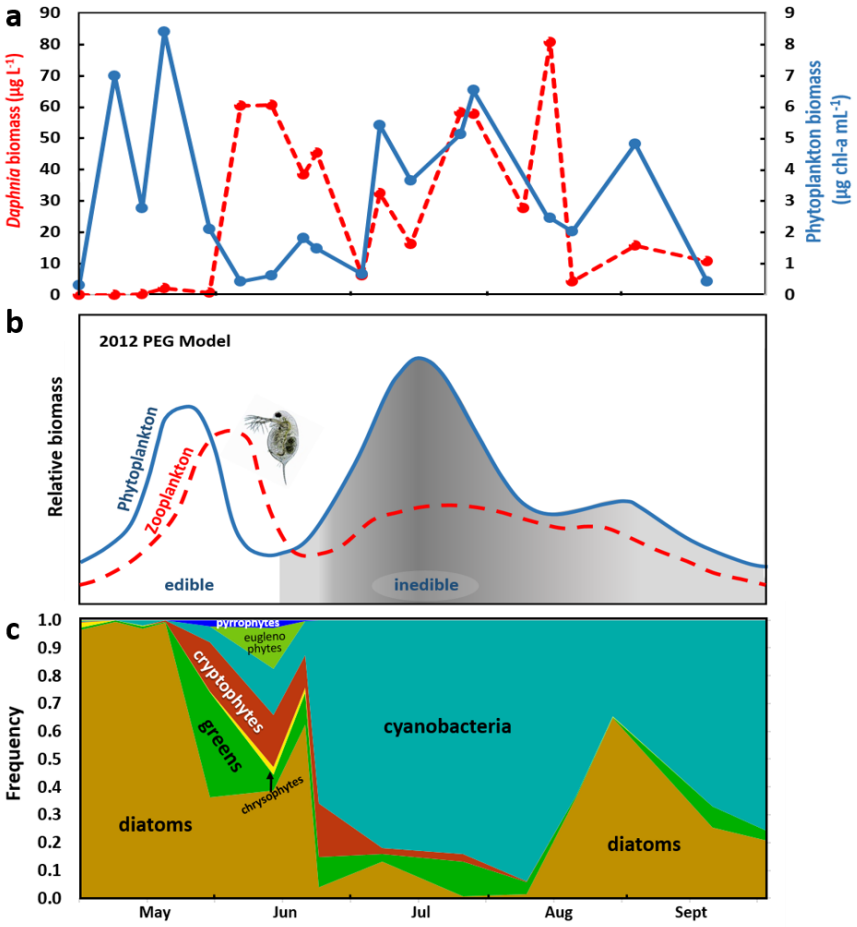

Figure 1. Oneida Lake 2015 phytoplankton and zooplankton dynamics compared with PEG model. (**a)** Seasonal abundances of phytoplankton (blue) and dominant zooplankton grazer, *Daphnia* (red). Note similarity with stereotypical pattern described by the plankton ecology group (PEG) model, (**b)** redrawn from Sommer et al. (2012). (**c)** Frequencies of major phytoplankton taxa with replacement of edible spring taxa by relatively inedible cyanobacteria and similarity with PEG model.

Clone 11 which dominated in late summer (Figure 2a), following a two month period of high concentrations of cyanobacteria in the lake (Figure 1c), had the highest performance of any of the clones on the summer diet, though like the other clones it was still negatively affected. At the same time, Clone 11 had a lower g_j than all but one of the other clones when feeding on the spring diet, suggesting that there is a cost to tolerance of dietary cyanobacteria. Furthermore, the two independent isolates of Clone 11 performed very similarly (not significant by ANOVA with post-hoc comparison, Table 3) indicating that the dietary-performance phenotype was consistent with microsatellite clonal identity. In addition to Clone 11, the other six clones likewise had seasonal patterns of frequency change consistent with their performance of summer (cyanobacteria) diet. There is a significant relationship between the date that each of the seven clones had its maximum rate of frequency increase and its growth rate on summer phytoplankton (Figure 3b; $r^2 = 0.64$, $p = 0.034$), so that clones that were more resistant to cyanobacteria generally increased in frequency later in the season as cyanobacteria became dominant, and vice versa.

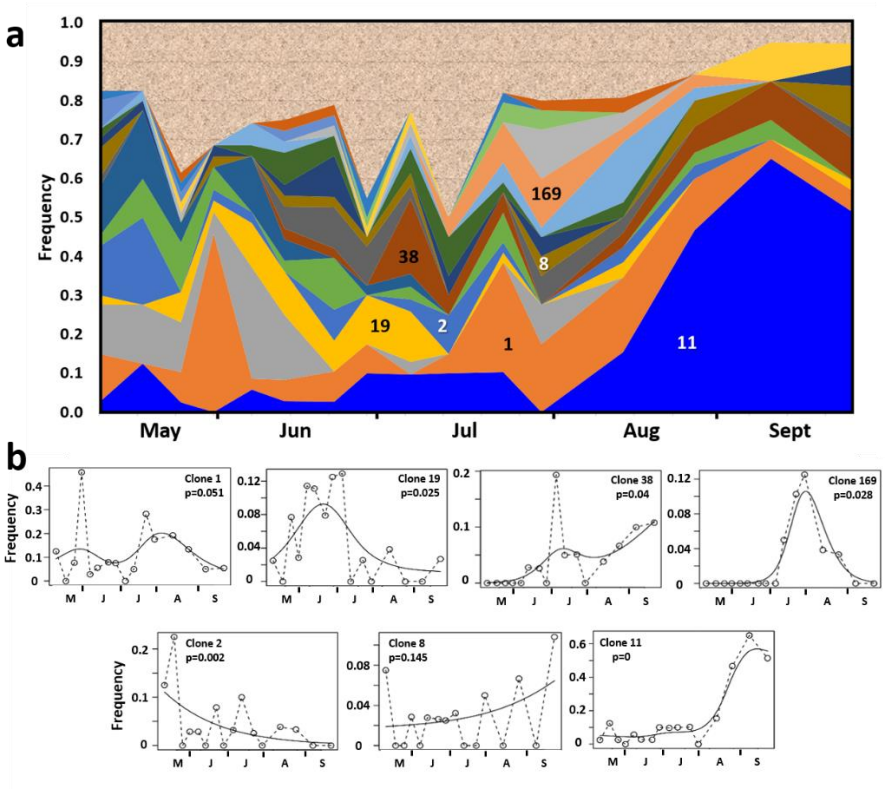

Figure 2. Frequencies of *Daphnia mendotae* clones in Oneida Lake 2015. (**a**) Each clone is represented by a different color. The granular area at the top is the sum of clones that only occurred as a single individual on a single date. (**b**) Frequencies of the seven clones on which g_j was measured (also numbered in panel **a**). Shown are the data, the fitted spline curve, and the p-value for the fit obtained by using the anova.gam() function in R (Wood 2017). Top row, and black numbers in **a**, are clones designated "non-resistant" to cyanobacteria; bottom row, and white numbers in **a**, are clones designated "resistant" to cyanobacteria, as described in text.

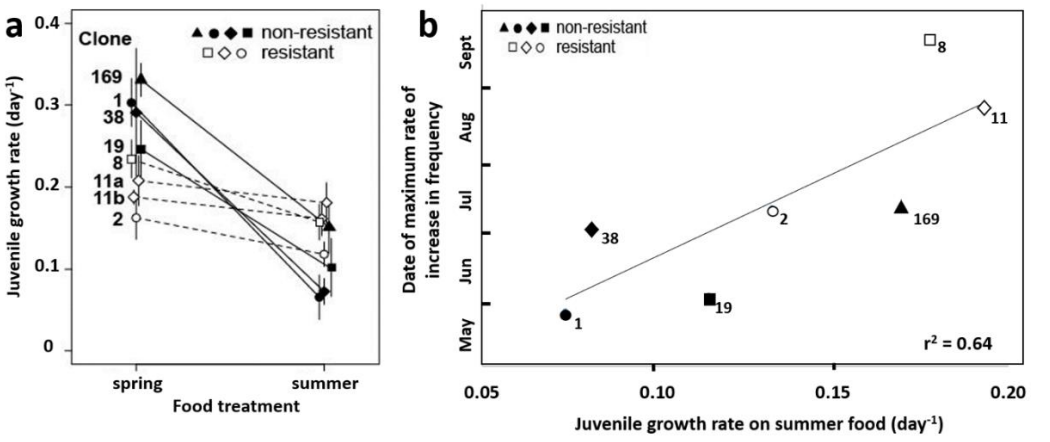

Figure 3. Sensitivity of Oneida Lake *Daphnia mendotae* to spring and summer food.

(**a**) Juvenile growth rate, g_j, of clones fed either phytoplankton typical of spring or summer. Each line is a reaction norm for one of seven clones that peaked in frequency at different times during 2015. Clone 11 represented by two independent clonal isolates. Resistant (unfilled symbols and dashed line) and non-resistant (filled symbols and solid line) clones are defined by slope of their reaction norms (see text). (**b**) Relationship between date on which each clone had it maximum rate of increase in frequency and its g_j on summer cyanobacteria-rich diet ($r^2 = 0.64$, df = 5, p = 0.034). Clones numbered; unfilled symbols represent resistant clones; filled symbols represent non-resistant clones (see text).

Table 2. ANOVA results of the juvenile growth rate experiment**.** Data analyzed are for seven clones of *Daphnia mendotae* that dominated at different times between May and September 2015 in Oneida Lake when fed phytoplankton typical of either spring or summer.

	df	Sum Sq	Mean Sq	F value	p value
Food treatment	1	0.2164	0.2164	174.11	1.73×10^{-14}
Clone	7	0.0422	0.0060	4.86	8.11×10^{-4}
Food×Clone	7	0.1002	0.0143	11.51	3.31×10^{-7}
Residuals	32	0.0398	0.0012		

Table 3. Pairwise comparisons of the reaction norms for all eight clonal lineages. Post-hoc pair-wise comparison tests following from ANOVA in Table 2 with p-values corrected for multiple comparisons (Holm 1979); ns indicates not significant at $\alpha = 0.05$.

		Resistant				**Non-resistant**			
	Clones	**2**	**8**	**11a**	**11b**	**1**	**19**	**38**	**169**
Resistant	**2**	-	ns	ns	ns	< 0.001	ns	< 0.001	0.013
	8	ns	-	ns	ns	0.002	ns	0.008	ns
	11a	ns	ns	-	ns	< 0.001	0.046	< 0.001	0.004
	11b	ns	ns	ns	-	< 0.001	0.046	< 0.001	0.004
Non- resistant	**1**	*redundant information*				-	ns	ns	ns
	19					ns	-	ns	ns
	38					ns	ns	-	ns
	169					ns	ns	ns	-

DISCUSSION

The *Daphnia mendotae* population in Oneida Lake evolved within a single season in response to changes in its phytoplankton food environment. Because the population reproduces exclusively by parthenogenesis during this period (Cáceres 1998), the evolution observed occurred via changes in clonal frequencies. Whereas most of the 124 clones identified by genotyping were rare – often only represented in our samples by a single individual on a single date – 16 reached at least 10% of the population at some point in the season, with three clones having greatest frequency in spring, two dominating in late-summer, two reaching their greatest proportion in the middle of the season, and nine showing no detectable pattern of seasonality (Supplemental Information 1). The seven clones that we chose for analysis of fitness response to food environment differed significantly in their resistance to late-summer dietary cyanobacteria, so that the three clones that were least negatively affected (shallowest reaction norm), had their maximum increases in frequency later in the season, whereas the four clones most negatively affected (steepest reaction norm) increased in frequency in spring or early summer. Provided that these seven clones are representative of the population, the *Daphnia* evolution was due to natural selection imposed by change in food quality.

The strength of this result is ecologically significant given that there are many other environmental factors that change seasonally in Oneida Lake which likely impose selective pressure on *Daphnia* including interspecific competition (Caceres 1998a), zooplanktivorous fish (Mills and Forney), predatory invertebrates (Caceres et al. 2016), pathogens (Hewson et al. 2013), and even water temperature (Peters 1987). Since all traits are genetically linked in a

clonal population, our result suggests that food quality is of major importance compared with these other factors in exerting selection on the population.

We assume here that the differences among clones in performance on different food types are genetic since the clones have distinct microsatellite genotypes. It is worth noting, however, that Macke et al. (2017) have shown that the ability of *Daphnia* to adjust to changes in food quality can also be affected by the activity of gut microbiota in response to dietary cyanobacteria. If cultured lineages of *Daphnia* differ in their gut microbiota, this would have the potential to provide an additional route by which they might differ in performance in relation to diet, so that both intrinsic genetic variation and extrinsic gut microbiota might be important. In our study iso-female clonal lineages, isolated from Oneida Lake, were cultured for ten or more generations in the laboratory, all in 0.45 µm filtered lake water before being assayed for juvenile grow rate. The microbiota in the Macke et al. (2017) study were transmitted externally from mother to neonates, so for this to have played a role in our findings, transmission from mother to offspring would have to have been very efficient, and for it to explain the changes in clonal succession we observed in the lake population, transmission would have to be specific to each clone even in a large well-mixed lake. If it were that efficient, it would then effectively be a heritable trait (in the sense of a high parent-offspring phenotype regression), though not one located in the *Daphnia* genome.

By the end of the season, the clonal diversity of the *D. mendotae* population had declined markedly. Clonal diversity, calculated using Simpson's Index, (1-D) corrected for sampling without replacement, $\mathrm{D} = \sum_{i=1}^{R}[n_i\ (n_i - 1)/(N(N-1)]$ (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), was high, varying between 0.88 and 0.98 from May to mid-August, and then dropped rapidly to between

0.58 and 0.77 in late-August and September (Figure 4) driven primarily by the increasing dominance of Clone 11 which reached over 60% of the population on the second to last date of the season (Figure 2a). While 124 clones were identified over the course of the summer, on the last date sampled (25 September) 8 clones comprised 95% of the population, a result consistent with general simulation of *Daphnia* clonal erosion under natural selection (Vanoverbeke and De Meester 2010). In addition, at the end of the season, the seven clones we analyzed for g_j made up 81% of the genotyped population, and the three clones we identified as "resistant" made up 62% of that population. It is unclear if any of the 124 clones originally identified actually went extinct by then end of the season since 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 (based *D. mendotae*/L and the volume of Oneida Lake). This population size approximation is based on the product of mean *Daphnia* density and total lake volume, where volume was approximated as an ellipse-based cone: $V=1/3\pi rRh$ where r is the half minor axis (0.5×8,000m), R is the half major axis (0.5×34,000m) and h is maximum lake depth of 16.8 m: $V = 1.2\times10^{7}$ m^{-3}. Average *D. mendotae* density in Oneida May-Sept 2015 was 3,700 m^{-3} (Cornell Biological Field Station data archive), so mean total *D. mendotae* population size in Oneida Lake in 2015 = 4×10^{12} individuals.

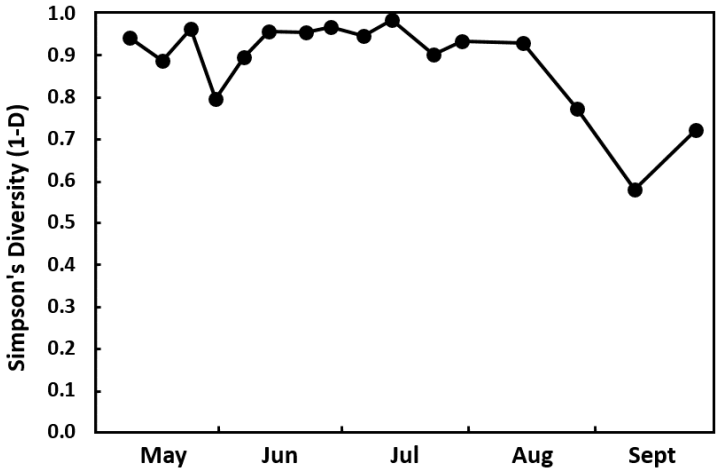

Figure 4. Clonal diversity of *D. mendotae* in Oneida Lake, 2015. Diversity was calculated using Simpson's Index (1-D).

To evaluate further the action of natural selection on each of the seven clones, we estimated how a clone's juvenile growth rate would have changed over the course of the season as cyanobacteria frequency varied (Figure 1c). We know the g_j of each clone on food that either had 0% cyanobacteria (spring food) or 50% cyanobacteria (summer food), and using this information we projected g_j at other frequencies of the poor food observed in the field assuming a monotonic relationship (given by the reaction norm in Figure 3a). We used $\log_{10}$-transformed g_j so that the extrapolation at very high cyanobacteria frequencies did not project nonsensical negative juvenile growth rates (Figure 5).

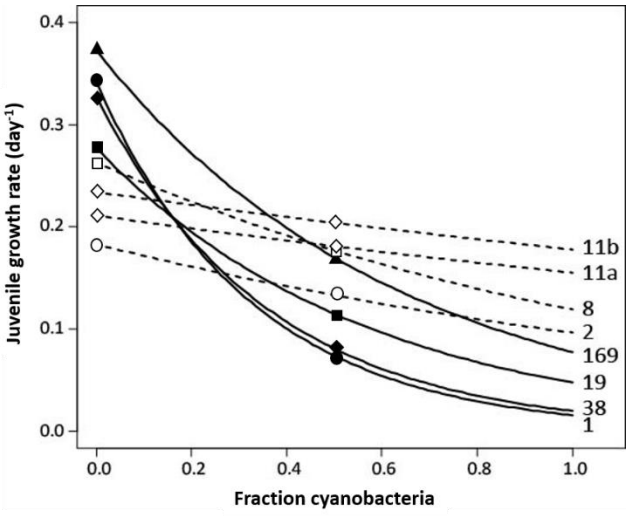

Figure 5. Projected g_j for each *D. mendotae* clone as a function of faction of cyanobacteria in diet. Data points are measured g_j from Figure 3a; lines are exponential fits.

The results, with the resistant and non-resistant clones plotted separately (Figure 6), show how g_j changes relative to the mean for all seven clones. The effect of the onset of the cyanobacterial bloom on *Daphnia* growth rate is seen by the distinct drop in g_j for all clones in late June as cyanobacteria increases. The resistant clones have g_j above the mean, showing that they would have had a selective advantage, while the non-resistant clones were selected against, having g_j below the mean. The reverse is true during the spring diatom bloom and flagellate-dominated CWP with non-resistant clones having g_j above the mean and resistant clones with g_j below the mean. This latter pattern again suggests a tradeoff between relative fitness on cyanobacteria-rich poor food and that on spring good food, though we do not know of a mechanism that might underlie this tradeoff. In late summer when diatoms returned to being a substantial portion of the phytoplankton (Figure 1c), the resistant and non-resistant clones show a

mixed pattern in relation to the mean phenotype suggesting that selection by food quality is relaxed at this point.

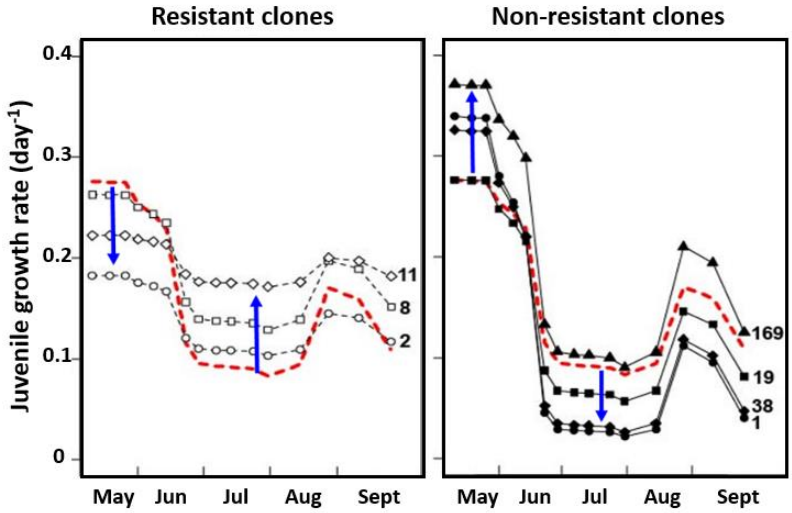

Figure 6. Projected seasonal changes in juvenile growth rates based on g_j values for resistant clones and non-resistant clones. Projected g_j based on values in Figure 3a and seasonal changes in frequency of cyanobacteria in Oneida Lake May-September 2015 shown in Figure 1c. Red dashed lines are the mean projected g_j for all seven clones (same line in both panels), and blue arrows show direction of selection with downward arrows showing selection against clones with projected g_j values falling below the mean, and upward arrows showing selection favoring clones with projected g_j values lying above the mean.

A central question in the study of rapid contemporary evolution is how important adaptive phenotypic changes of ecologically important taxa are for understanding ecosystem structure and function when they occur on the time scale of ecological dynamics (Schoener 2011; Ellner et al 2011; Hendry 2017). *Daphnia*, as a critical species in the functioning of many lake

ecosystems (Lampert 2011; Miner et al. 2012; Sommer et al. 2012) including in Oneida Lake (Mills and Forney 1988; Cáceres et al. 2016), provides an opportunity to assess this question both for this particular system, and for many lakes in which *Daphnia* affects phytoplankton dynamics. Our question then becomes: did the evolution of *Daphnia* clonal phenotypes in response to the changing phytoplankton composition feedback on the zooplankton-phytoplankton consumer-resource dynamics of Oneida Lake?

Others have found that genetically based phenotypic differences in *Daphnia* do affect community and ecosystem-level processes in mesocosms (Chislock et al. 2013, Pantel et al. 2015), so the potential exists for a lake-wide effect. The Geber method, and its extensions and related approaches, for partitioning the effects of evolutionary and ecological change on ecological dynamics (Hairston et al. 2005, Pelletier et al. 2007, Ellner et al. 2011, Govaert et al. 2016) is currently being explored as a way of providing insight by weighing the dependence of seasonally changing *Daphnia* growth rate on changing phytoplankton composition (ecology), changing *Daphnia* performance genotype (clone effect: evolution), food (plasticity) and clone×food interaction (evolution of plasticity). While we do not yet have a direct answer to this question, we point out that if only a single *Daphnia* genotype of a particular phenotype had occurred in Oneida Lake in 2015 instead of the diversity of genotypes actually present, the resulting phytoplankton-zooplankton dynamics would surely have looked very different – and would differ depending on whether the single clone had been a resistant clone that grew poorly on spring bloom algae but relatively well on summer cyanobacteria, or a non-resistant clone what grew well in the spring bloom and poorly in the summer bloom.

The "Plankton Ecology Group" or PEG model of freshwater plankton dynamics treats *Daphnia* as a single entity, but nevertheless has been successful in depicting general seasonal

changes in phytoplankton and zooplankton abundances, and in phytoplankton taxonomic composition. The question our research raises is whether such seasonal patterns will be even better understood if evolution of the consumer (and likely the consumed) is considered as well. For example, is the substantial increase in *Daphnia* abundance we observed in Oneida Lake at the start of the cyanobacterial bloom in July explained at least in part by the evolution of the consumer population toward more resistant clonal phenotypes?

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