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The effect of toxic Microcystis aeruginosa on four different populations of Daphnia

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Abstract

Cyanobacteria reduce the fitness of many *Daphnia* species, and blooms in eutrophic lakes may place strong selective pressure upon these primary consumers. This study examines the ability of daphnids to resist the deleterious effects of toxic *Microcystis* and determine if this resistance is related to the trophic conditions of their native lakes. Three populations of *Daphnia pulex/pulicaria* were examined; *D. pulicaria* from eutrophic Klamath Lake in Oregon, *D. pulex* from eutrophic Old Durham Reservoir in New Hampshire, and *D. pulicaria* from oligotrophic Russell Pond in New Hampshire. *D. carinata* from meso-oligotrophic Lake Rotoaira in New Zealand was used as a known cyanobacteria-sensitive species. Ten-day old 5^{th}-6^{th} instar animals were exposed to a mixture of *Microcystis aeruginosa* and *Chlorella vulgaris* (25% and 100% *M. aeruginosa*). Body length, lipid index, reproductive index and clearance rate were assessed for each population after 120 hours of treatment. A feeding bioassay response quantifying the energetic (feeding rate) cost of post abdominal rejections was also determined for a gradient of *M. aeruginosa* concentrations from 0% to 100%. The four populations of *Daphnia* exhibited different rates of decline in overall fitness when exposed to *Microcystis.* Populations exposed to *Microcystis* exhibited reduced thoracic beat rate, lower lipid and reproductive indexes, and higher cost of post abdominal rejections in comparison to daphnids in the control *Chlorella*. Length was not a sensitive indicator of fitness level. *D. pulex* from eutrophic Klamath Lake had a mean clearance rate in 100% *Microcystis* that was three to four times higher than *D. pulicaria* from oligotrophic Russell Pond. In general, daphnids from oligotrophic lakes exhibited a more drastic decline in fitness than daphnids from eutrophic lakes. This suggests that taxonomically related populations of *Daphnia* have evolved a suite of adaptations to *Microcystis* depending upon their history of exposure.

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Introduction

Cyanobacteria blooms occur when excess amounts of nitrogen and phosphorous are incorporated into a freshwater ecosystem (Chorus 2000). These blooms are often toxic and have been responsible for human and animal death (Chorus 2000).

The hepatotoxin called microcystin was first isolated from the cyanobacteria *Microcystis* (Chorus 2000). The presence of *Microcystis* in a lake ecosystem may affect populations of filter-feeders particularly the zooplankton grazing community. Specific physiological and behavioral responses of the zooplankton have been investigated. Rohrlack (1999) found that *Daphnia galeata* exposed to toxic *Microcystis aeruginosa* tended to die faster than organisms that were starved. Feeding rate and thoracic appendage beat rate are often sensitive indicators of fitness. Short-term exposure of *D. carinata* to toxins from *Aphanizomenon flos-aquae* resulted in 30-50% depression of thoracic beat rate and elevation of post-abdominal rejections (Haney *et al*. 1995).

Zooplankton also may change their swimming behavior in the presence of *Microcystis*. *D. carinata* exhibits a vertical migration pattern in which it will actively avoid strata that contain the cyanobacteria (Kinder 1995). Zooplankton can also accumulate the toxin, acting as a potential vector for transfer of the toxin to higher trophic levels in the food web (Rohrlack *et al*. 1999, Thostrup *et al.* 1999).

Acknowledgements

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Daphnia pulex
Old Durham Reservoir
Durham, NH USA

Daphnia pulicaria
Klamath Lake
Oregon, USA

Daphnia carinata
Lake Rotoaria
New Zealand

Daphnia pulicaria
Russell Pond
Woodstock, NH USA

Photo gallery of *Daphnia* species/populations. Organisms shown here post-120-h exposure to 100% *Microcystis.*

Daphnia magna fed *M. aeruginosa* had lower weight, decreased survivorship, and decreased neonate production (Trubetskova and Haney, 2000). Neonates born to mothers exposed to the toxin were smaller and lighter. It was found that responses in growth and reproduction were similar to starvation. Similarly, Demott (1999) found that five different *Daphnia* species exposed to *M. aeruginosa* all had inhibited feeding, depressed growth rates, and lower egg production. These responses varied among the different species, indicating that different *Daphnia* species vary in their sensitivity to toxic cyanobacteria.

The varying fitness levels *Daphnia* species exhibit in the presence of toxic cyanobacteria suggest the evolution of adaptive responses. Evidence for local adaptation of neighboring *Daphnia* populations has frequently been reported. Two populations of *D. galeata* in separate ponds separated by only 5 m of land were found to be genetically different in life history traits. Allozyme electrophoresis revealed that these two geographically close populations were different in genetic and allelic structure (Declerck 2001).

These authors also suggested that local adaptations of *Daphnia* to environmental conditions other than predation occur readily and can be extremely rapid. Hairston, Lampert, *et al* (1999) proposed that when phytoplankton assemblages change in lakes, zooplankton are subject to strong selective pressures. It was demonstrated that by hatching dormant eggs found in the lake sediments, resistance of *Daphnia galeata* increased during a decade of eutrophication in Lake Constance.

Our experiment was based on the hypothesis that *Microcystis* in the phytoplankton of eutrophic lakes exert selective pressure on the zooplankton grazing community. We predicted that genetically similar *Daphnia* populations from more eutrophic lakes would have higher fitness levels than populations from oligotrophic lakes when exposed to the toxic cyanobacteria *Microcystis aeruginosa*.

Table 1. Location and characteristics of the native lakes of four *Daphnnia* populations

Species	Lake	Location	Trophic Status
D. pulex	Old Durham Reservoir	Durham, NH, USA	Eutrophic
D. pulicaria	Klamath Lake	Oregon, USA	Eutrophic
D. carinata	Lake Rotoaira	New Zealand	Meso-oligotrophic
D. pulicaria	Russell Pond	Woodstock, NH, USA	Oligotrophic

Materials and Methods

Lab Cultures - *Daphnia* populations were collected from lakes of different trophic status (Table 1). The *Daphnia* were identified using keys written by Pennak, Hebert, and Brooks (see references).

Daphnia were cultured in unfiltered, aerated well water from AFAIR Lab at the Univ. of New Hampshire and fed *C. vulgaris* maintained in a goldfish tank. *Daphnia* were fed every other day and food was not limiting. The daphnids were cultured at 20˚C under continuous light conditions (0.3 μM s^{-1} m^{-2}).

Microcystis aeruginosa was cultured in GTk medium (Table 2). Unicellular microcystin-producing *M. aeruginosa* was purchased from UTEX, the Culture Collection of Algae at the University of Texas, USA. *Chlorella vulgaris* was cultured with ASM-1 media (Gorham *et al* 1964). Both species were grown in 0.5-liter aerated, air-filtered culture tubes at 20˚C under continuous light conditions (15.0 μM s^{-1} m^{-2}). *Chlorella* and *Microcystis* concentrations were determined by hemacytometer counts of at least 50 cells and appropriate dilutions were prepared thereafter.

To test the response of lipid index, reproductive index, and clearance rate ten day-old cohorts of *Daphnia* were exposed to 100% *C. vulgaris* and 100% and 25% *M. aeruginosa* for 120 hours. *Daphnia* cohorts were prepared by pouring cultures through a 505-μm screen allowing the smallest juveniles to pass through the sieve Juveniles were allowed to mature for 10 more days fed on a culture of mixed green algae, mainly *Ankistrodesmus*. The 120-h experiment began at this adolescent stage to facilitate quantification of life history characteristics as the daphnids reached sexual maturity.

Food suspensions were prepared based upon cell dry weight (Table 3). Dry weights of *Chlorella* and *Microcystis* were 16.9+/- 0.1 pg $cell^{-1}$ and 7.5+/- 0.2 pg $cell^{-1}$, respectively. Carbon content was estimated as 50% of dry weight (Trubetskova and Haney 2000).

Measuring fitness - Daphnid lipid Index, reproductive index, and body length (mm) were collected from three replicates in the control (*Chlorella*) and treatment (*Microcystis*) animals. Each replicate consisted of five daphnids per 100 ml of suspension in a 150-ml jar. Water used to prepare the suspensions were obtained from the AFAIR Lab well in Durham, NH and filtered through Whatman GFC glass microfibre 4.7-cm filters.

After 100 ml of the suspensions were added to the jars, animals were systematically pipetted

Table 2. GTk Growth Medium for freshwater species. To make 1L of medium, start with 970 ml (DI) deionized water, add solutions G1, T2, through K3 in the order listed. S= Stock concentration, F=Final concentration.

MACRONUTRIENTS (Stock Solution) Use 10 ml/L			
G1	KNO_3	12.5g S= 247 mM	F= 2.5 mM
	$MgSO_4 \bullet 7H_2O$	3.75g S= 30 mM	F= 0.3 mM
	$CaCl_2 \bullet 2H_2O$	1.30g S= 17 mM	F= 0.17 mM
	In 500 ml of DI water		
T2	Na_2HPO_4	2.60g S=40 mM	F=0.4 mM
	MW= 142 In 500ml DI water		
MICRONUTRIENTS (Stock Solution) Use 1.0 ml/L			
K3	EDTA, Na_3	2.15g/40ml S=150 mM	F= 150 μM
	Titrate to approx. range pH 6-7		
K4	$FeSO_4 \bullet 7H_2O$	224mg/40ml S=20mM	F= 20.0 μM
	+1 drop 10% H_2SO_4		
K5	$ZnSO_4 \bullet 7H_2O$	23mg	F= 2.0 μM
	MoO_3	10mg	F= 1.0 μM
	$CuSO_4 \bullet 5H_2O$	6mg	F= 0.6 μM
	$CoCl_2 \bullet 6H_2O$	2mg	F= 0.2 μM
	$MnCl_2 \bullet 4H_2O$	120 mg	F= 14.0 μM
	In 40ml +2 drops 10% H_2SO_4		

*** All stock solutions may be autoclaved ***with the exception of K4***

Table 3: *Chlorella* and *Microcystis* concentrations for life history and thoracic beat experiments.

		Dry Weight	Carbon
Suspension	Cells ml^{-1}	(μg ml^{-1})	(μg ml^{-1})
100% *Chlorella*	1.75×10^5	3.00	~1.50
100% *Microcystis*	4.00×10^5	3.00	~1.50
25% *Microcystis*			
Mic. Content	1.00×10^5	0.75	~0.375
Chl. Content	1.31×10^5	2.25	~1.130
Thoracic Beat Trt.			
100% *Microcystis*	5.30×10^4	4.00	~2.00
100% *Chlorella*	2.40×10^4	4.00	~2.00

into the jars to reduce bias. Each daphnid was pipetted individually into different 100-ml jars until each of the 30 jars had five daphnids. Since the larger, slower daphnids were easier to capture and transfer, pipetting one animal at a time into different jars distributed similar body sizes among the replicates. Black caps were overturned on top of open 100-ml jars to prevent entrapment of the animals in the surface tension. The bottoms of the jars were stirred twice daily to prevent settling of the cells (Fig. 1).

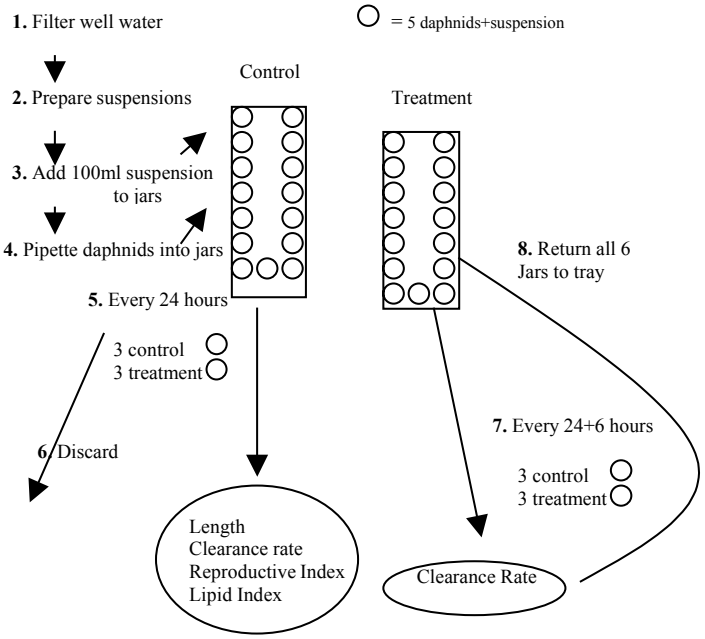

Fig. 1. Experimental design to measure fitness: Lipid Index, Reproductive Index, Clearance Rate, and Length.

Lipid index was estimated as follows: individual animals with 0-10 lipid droplets had an index of 1, 11-50 an index of 2, 51-100 an index of 3, >100 lipids an index of 4. Reproductive index was based upon the "reproductive unit (ru)," one unit being either one egg or one neonate. Eggs were counted in the carapace of the daphnid and neonates were counted freely swimming in the suspension. Length was determined using the MetaMorph™ digital imaging software and recorded as body length from head to base of tail spine.

Feeding rate was measured twice daily by hemacytometer counts at 6 hours and 18 hours. Number of cells eaten in a specific time period was calculated as the difference between the initial and final cell counts. Clearance rate was calculated as ml of culture cleared per individual daphnid per hour according to:

$$CR\ (ml\ ind^{-1}\ h^{-1}) = \frac{\ln(C_0) - \ln(C_t)}{t_h} * \frac{Expt.\ Vol.\ (ml)}{n}$$

C_o= Initial cell concentration (cells ml^{-1})
C_t= Final cell concentration (cells ml^{-1})
t = Length of feeding time (h)
n = # daphnia per experimental volume

Thoracic beat (TB) rate and post abdominal rejections (PARs) were measured to determine the relative cost of the post-abdominal rejections. Every time a daphnid performs a post-abdominal rejection the thoracic beat rate slows; there is a "lull" in thoracic beat rate. The cost associated with post-abdominal rejection is the depressed thoracic beat rate as a result of this cleaning mechanism. During the PAR the organisms "kick" to remove unwanted material from their feeding chambers. The cost was calculated to be the average total thoracic beats per minute minus the average thoracic beats per minute excluding lulls in thoracic beating due to post abdominal rejections.

Daphnids were pipetted individually into a petri dish and the animal was affixed to the bottom of the petri dish with petroleum jelly (Haney *et al* 1995). Animals were oriented to view the dorsal carapace laterally which was secured with the 0.2-0.4-mm wide line of petroleum jelly extruded from a syringe needle. Each daphnid was prepared in a separate petri dish and considered in independent replicate. Suspensions were administered after a one-hour post-refrigeration acclimation period to eliminate the effects of temperature variations on thoracic appendage beating. Control suspension (100% *Chlorella*) was pipetted onto the animal (10 ml) and a 10-minute acclimation period ensued. Thoracic beating activity was viewed through a dissecting microscope at 2.25X and recorded on a VHS video tape recorder for 90 seconds. After recording, the control suspension was pipetted out of the petri dish and replaced with a 3% *Microcystis* suspension (10 ml). Acclimation time to the 3% suspension was 10 minutes and

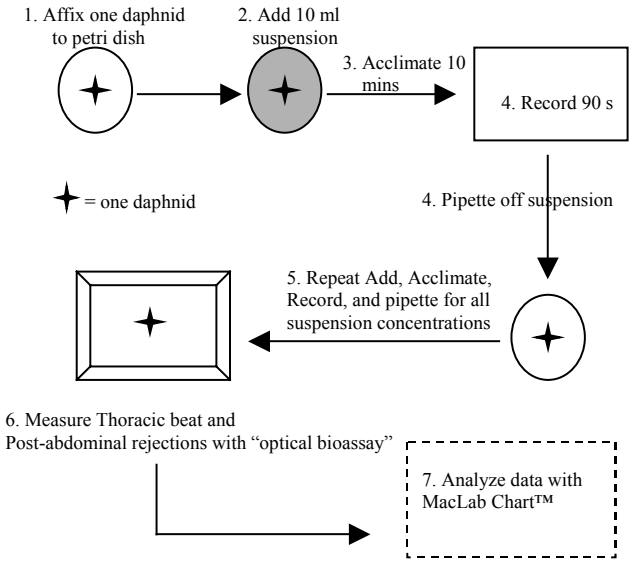

Fig.2: Experimental design to measure thoracic beats and post abdominal rejections.

thoracic beating activity was recorded for 90 seconds. This procedure was repeated for 6%, 12%, 25%, 50%, and 100% *Microcystis* suspensions. All suspensions were stirred thoroughly before and during the procedure to prevent settling. This procedure was repeated for each daphnid (N=5).

Five daphnids were tested to represent each of the four populations.

A MacLab™ (Analog Digital Instruments) A/D converter was used to measure TB rates from the videotapes.

Tapes were played on a television monitor. A diffuse, low intensity (~10 μM s^{-1}) fiber-optic photosensor was pressed to the television screen to record TB rate. TB rate was viewed and recorded on a Macintosh computer using the MacLab Chart™ software (Fig. 2, 3a, 3b).

Results

Length was not a useful indication of fitness at 100%, 25% *Microcystis, or* 100% *Chlorella* (Fig. 4). In the control *Chlorella D. pulex* from Old Durham Reservoir (ODR) had the largest growth (+0.54 mm, se=0.27) and *D. pulicaria* from Russell Pond (RP) had the least change in body length (+0.15mm, se=0.11). In the 25% *Microcystis* there was much variation in all populations with no conspicuous pattern. In 100% *Microcystis D. pulicaria* RP exhibited no change in body length and *D. pulex ODR* exhibited the greatest change (+0.32mm, se=0.16.)

All populations of *Daphnia* had decreases in lipid index after 120 h in 25% and 100% *Microcystis* (Fig. 5). In the control *Chlorella* all species showed little change in lipid content. In 25% *Mi-crocystis* the meso-oligotrophic species *D. carinata* exhibited the highest decline in fitness as measured by lipid loss (-2.20, se=0.491). *D. pulex* from Old Durham Reservoir (ODR) had the smallest decrease in lipids in 25% *Microcystis* (-0.930, se=0.382). However, in 100% *Microcystis D. pulicaria* from Klamath lake (KLAM) had the least change in lipid index

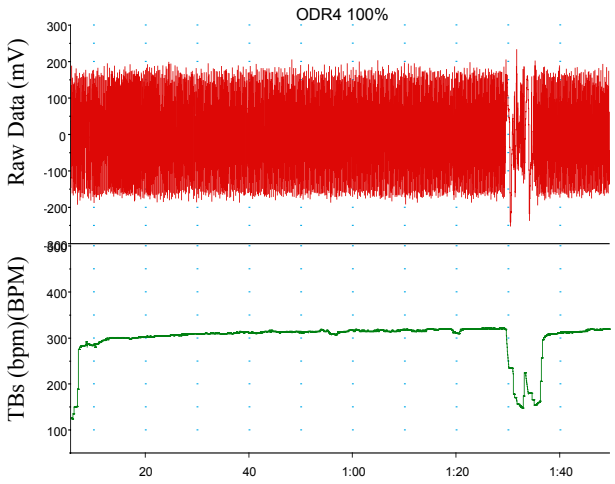

Fig. 3a: Thoracic beat rate of *D. pulex* ODR in 100% *Microcystis*. X-axis represents time in seconds and minutes.

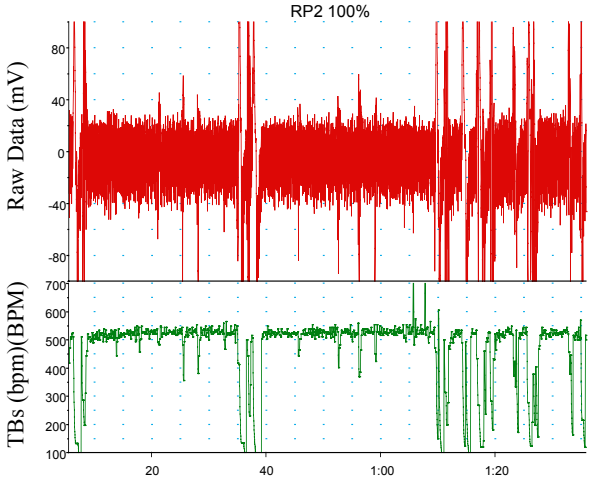

Fig. 3b: Thoracic beat rate of *D. pulicaria* RP in 100% *Microcystis*. X-axis represents time in seconds and minute

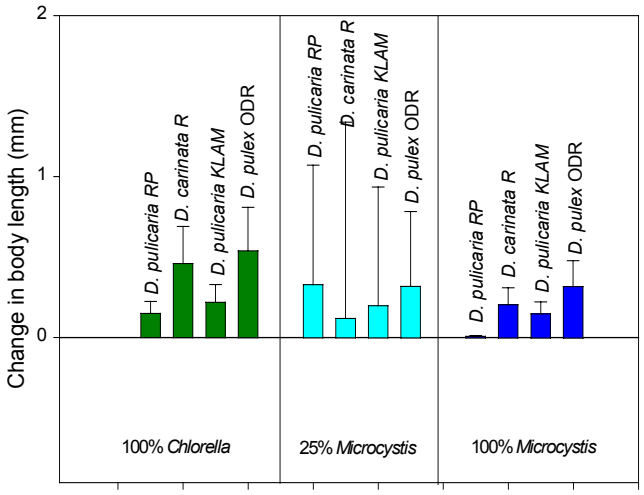

Fig. 4. Change in body length of four *Daphnia* populations after 120h in 100% *Chlorella*, 25% *Microcystis,* and 100% *Microcystis*. Total food concentration 0.15 µg C ml^{-1}

(-0.750, se=0.382).

In 25% *Microcystis* both eutrophic species, *D. pulex* ODR and *D. pulicaria* KLAM, had significantly higher ($p<0.05$) reproductive indexes (+6.26 ru, se=3.14 and +5.43 ru, se=2.72, respectively) than meso- and oligotrophic species *D. carinata* Rotoaira and *D. pulicaria* from Russell Pond (RP) (0 ru, and –0.0830 ru, se=0.0416, respectively) (Fig. 6). In 100% *Microcystis* all species exhibited severely reduced reproductive output. Despite this, populations

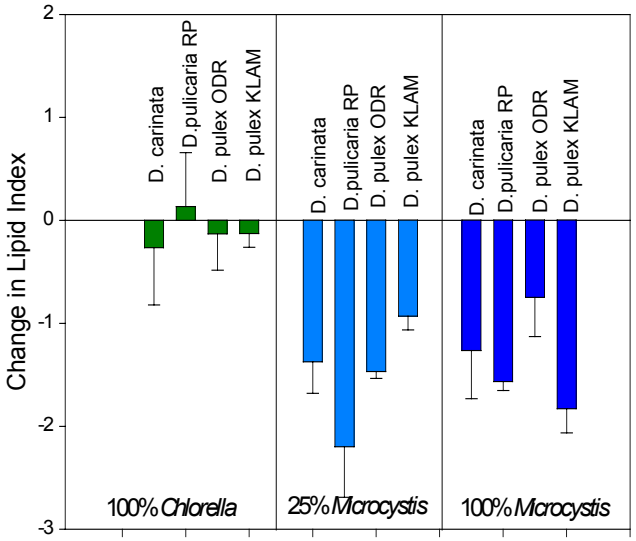

Fig. 5. Change in lipid index after 120 hours of exposure to 100% *Chlorella*, 25% *Microcystis*, and 100% *Microcystis*.

from eutrophic lakes had higher reproductive indices than oligotrophic populations.

Thoracic beat rates and post-abdominal rejections seen in figures 3a and 3b illustrate differences in *Daphnia* TBs and PARs. The eutrophic lake populations (such as *D. pulex* ODR) had a steadier thoracic beat rate and fewer PARS than the oligotrophic *D. pulicaria* RP.

In 100% *Microcystis* the mean rate of ingestion of toxic *Microcystis* cells by *D. pulicaria* KLAM (1.25 ml h^{-1}) was 3.68 times higher than that of *D. pulicaria* RP (0.34 ml h^{-1}) (Fig. 7). All species had similar rates of post-abdominal rejections in 100% *Microcystis* (2.5-4.2 rejections min^{-1}) (Fig. 8). However, oligotrophic species *D. pulicaria* RP and meso-oligotrophic *D. carinata* incurred a higher thoracic beat rate "cost" due to post-abdominal rejections (-35.1 thoracic beats min^{-1}, se=10.2 and –30.9 thoracic beats min^{-1}, se=5.38, respectively) approximately three times that of the eutrophic populations (*D. pulex* ODR –6.94 thoracic beats min^{-1}, se=2.17 and *D. pulicaria* KLAM –10.12 thoracic beats min^{-1}, se=2.96).

In 25% *Microcystis* concentrations, populations had different rates of post-abdominal rejections (1.07-8.99 rejections min^{-1}) (Fig. 8). Meso-oligotrophic population *D. carinata* R and oligotrophic population *D. pulicaria* RP exhibited the highest thoracic beat cost of post-abdominal rejection (-41.0 thoracic beats min^{-1}, se=7.52 and –22.7 thoracic beats min^{-1}, se=6.16, respectively). Eutrophic populations *D. pulex* ODR and *D. pulicaria* KLAM had low thoracic beat costs of post-abdominal rejection (-17.3 thoracic beats min^{-1}, se=2.92 and -7.6 thoracic beats min^{-1} , se=2.90).

There was a range of responses by the different *Daphnia* populations after 5 days of exposure to toxic *Microcystis.* The relative fitness ranks of the four *Daphnia* populations were calculated (Tables 4 & 5). In 25% *Microcystis D. pulicaria* KLAM and *D. pulex* ODR had the highest fitness (2.75 and 3.5, respectively). In 100% *Microcystis D. pulicaria* KLAM (3.4) and *D. pulex* ODR (2.8) again had the highest fitness.

D carinata had the highest lipid loss, low reproductive index, and incurred a high cost of post-abdominal rejections. Similarly, *D. pulicaria*

Table 4: Relative fitness rank of four *Daphnia* species after 120-h exposure to 25% *Microcystis*. Ranking as follows: 1=Least Fit, 4=Most Fit.

	D. carinata NZ	*D. pulicaria* RP	*D. pulicaria* KLAM	*D. pulex* ODR
Length	1	4	2	3
Lipid Index	1	3	2	4
Reproductive Index	2	1	3	4
Clearance Rate	N/A	N/A	N/A	N/A
TB Cost of PAR	1	2	4	3
Overall Average Fitness	1.25	2.5	2.75	3.5

RP had a low reproductive index, low lipid index, and a high cost of post-abdominal rejection. *D. pulex* ODR had the highest lipid content, high reproductive index, and the lowest cost of post-abdominal rejections.

D. pulicaria KLAM had a high reproductive index, high clearance rate, and low cost of post-abdominal rejection.

Discussion

Isolated populations of genetically similar *Daphnia* species responded differently to toxic *Microcystis aeruginosa* under controlled laboratory conditions. *Daphnia* populations varied in their degree and mechanism of response to feeding on toxic cyanobacteria. In general, populations from eutrophic Klamath Lake and Old Durham reservoir had higher fitness when fed *Microcystis* than populations from oligotrophic Lake Rotoaira and Russell Pond (Tables 4 & 5). The results of this study suggest that under eutrophic lake conditions *Daphnia* populations have evolved mechanisms that allow them to coexist with toxic cyanobacteria.

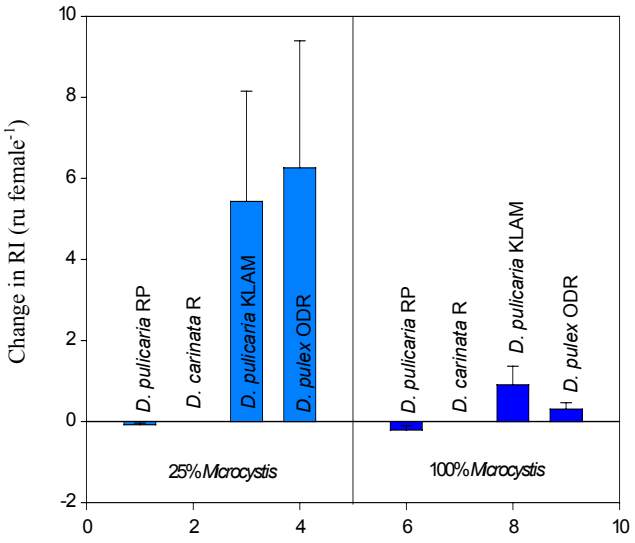

Fig. 6: Change in reproductive index (RI) after a 120-h exposure to 25% and 100% *Microcystis*. Measured in Reproductive Units (ru).

Despite this general trend, the mechanism of resistance varied among and within the different populations when exposed to 25% or 100% *Microcystis*. Populations that exhibited high levels of fitness in one concentration of *Microcystis* often did not perform as well in another *Microcystis* concentration. For example, the *D. pulex* ODR population had the highest lipid index fitness rank (4) in 25% *Microcystis*, but in 100% *Microcystis* had a fitness rank for lipids of 1 (Tables 4 & 5).

The fitness inconsistencies that occurred between 25% and 100% *Microcystis* treatments indicate that pure *Microcystis* may exceed the natural response of the *Daphnia*. The populations from eutrophic lakes had the highest overall fitness in both 25% and 100%

Microcystis, but they did not exhibit consistently high ratings in all categories (Tables 4 & 5). For example, in 100% *Microcystis D. pulicaria* KLAM had the highest overall fitness (3.4) but had fitness ranks of 2 in length and 3 in thoracic beat cost of post-abdominal rejection.

In general, the observed pattern between history of exposure through lake trophic level and resistance is not readily seen at 100% *Microcystis* concentrations. It is more likely at the concentrations used in this study ($4x10^5$ cells ml^{-1}) 25% *Microcystis* is within the range of natural

Table 5. Relative fitness rank of four *Daphnia* species after 120-h of exposure to 100% *Microcystis*. Ranking as follows: 1=Least Fit, 4=Most Fit.

	D. carinata NZ	*D. pulicaria* RP	*D. pulicaria* KLAM	*D. pulex* ODR
Length	3	1	2	4
Lipid Index	2	3	4	1
Reproductive Index	2	1	4	3
Clearance Rate	3	1	4	2
TB Cost of PAR	2	1	3	4
Overall Average Fitness	2.4	1.4	3.4	2.8

lake conditions, and is therefore more useful in testing the responses of *Daphnia* to cyanobacteria.

The response of different Daphnia population to M. aeruginosa indicates that *Daphnia* species have evolved different mechanisms to coexist with toxic cyanobacteria. *D. carinata* is a species known to be sensitive to toxic *Microcystis* despite the fact that it is from a meso-oligotrophic lake. In nature, this population is likely exposed to periodic, infrequent cyanobacteria blooms.

In our experiments, fitness of *D. carinata* declined sharply upon exposure to toxic *Microcystis*. However, this species could coexist with the toxic cyanobacteria due to its behavioral adaptations that allow it to evade *Microcystis* through vertical migration (Kinder 1995). Thus, it appears *D. carinata* has not developed extensive physiological adaptations, suggesting a trade-off between behavioral and physiological adaptations. Indeed, *D. carinata* maintained very high clearance rates in the presence of 100% *Microcystis*, which, in the absence of a spatial refuge exacerbated the effects of the toxic *Microcystis*.

D. pulex from the highly eutrophic Old Durham Reservoir appear to have physiological adaptations to toxic cyanobacteria. This species had a low cost of post-abdominal rejections indicating that they have become efficient at clearing toxic *Microcystis* from their filtering chambers with minimal reduction in thoracic beat rate. Similarly, Klamath Lake *Daphnia* may also have developed physiological mechanisms to persist in extremely eutrophic conditions with prolonged periods of cyanobacteria blooms. However, clearance rate of *D. pulicaria* KLAM remained high in 100% M*icrocystis*, suggesting that this population has a mec-hanism of "detoxifying" ingested microcystins.

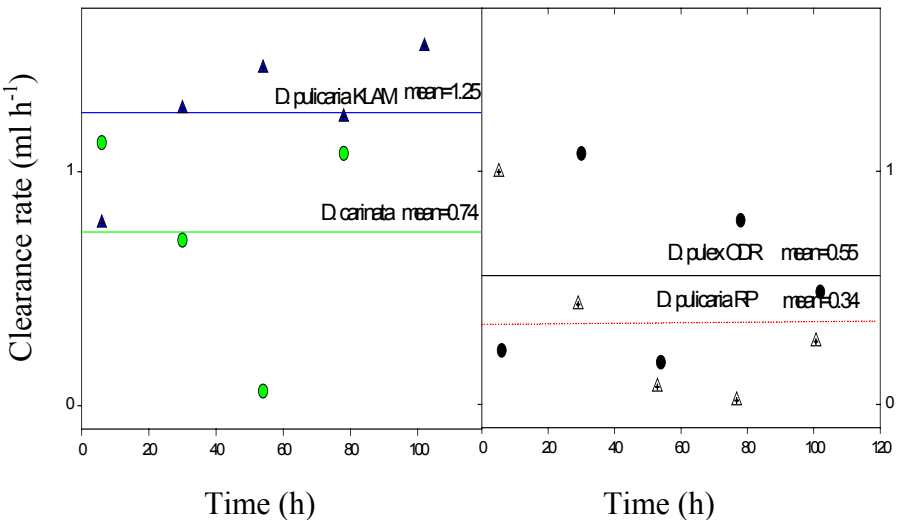

Fig. 7: Change in clearance rates after a 120-h exposure to 100% *Microcystis*. Mean clearance rates of four *Daphnia* populations represented.

There is little information on daphnid populations from ultraoligotrophic lakes, especially regarding mechanisms to coexist with *Microcystis*. *D. pulicaria* from oligotrophic Russell Pond had poor fitness when exposed to the toxin, presumably because it has historically not had been exposed to selective pressures to develop these defenses. It is likely that as a result, *D. pulicaria* RP may

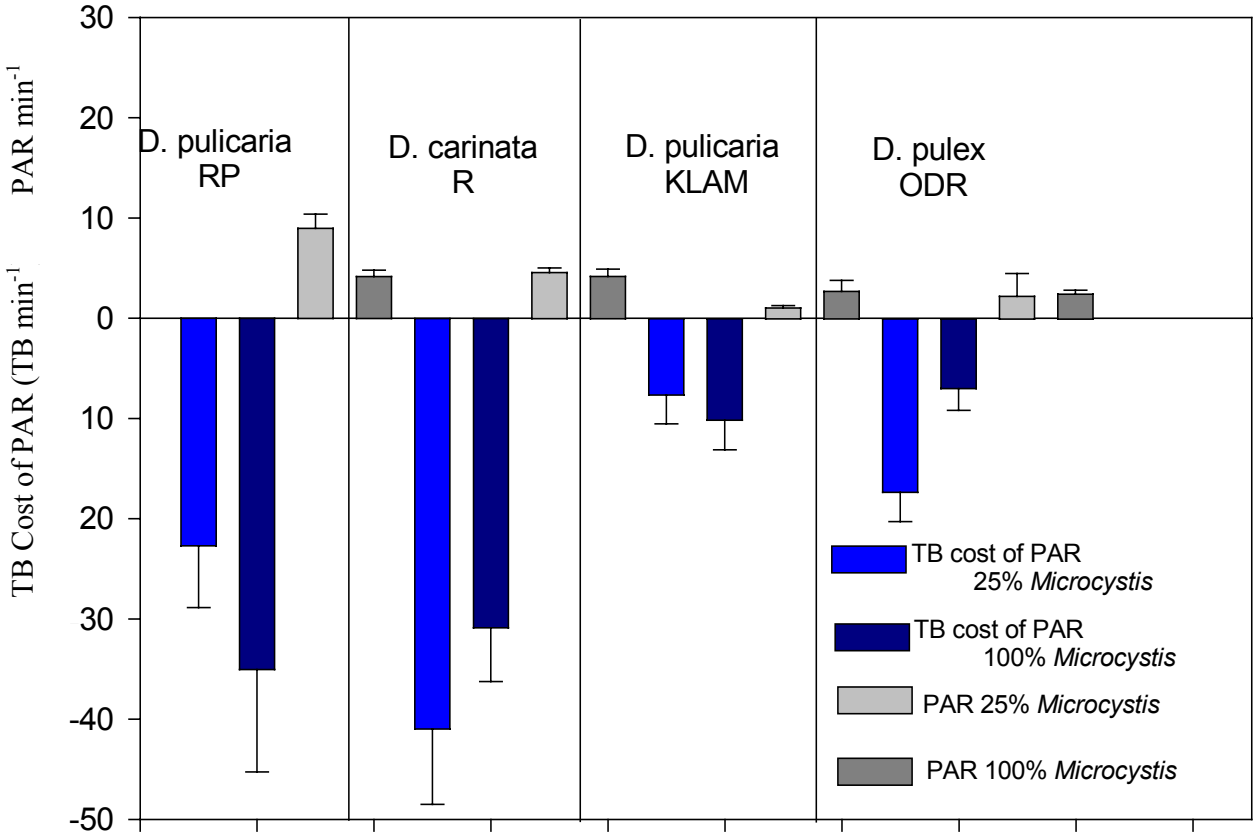

Fig. 8. Average rates of post-abdominal rejection (PAR) and estimated thoracic beat (TB) cost of PAR for four *Daphnia* populations exposed to 25% and 100% *Microcystis* ($4x10^5$ cells ml^{-1}).

be able to allocate more energy to other adaptations to exist in extremely oligotrophic environments. Interestingly, *D. pulicaria* RP exhibits an unusually fast and irregular swimming pattern that may be associated with avoidance of predators, presumable because of their higher visibility in the clear water. More studies need to be conducted with *Daphnia* populations to determine their specific adaptations to oligotrophic environments.

The effect of toxic cyanobacteria on the zooplankton community may promote instability in the aquatic food web. Toxic *Microcystis* may have lethal effects upon *Daphnia*, especially if blooms occur infrequently such as in mesotrophic systems. Elimination of *Daphnia* by cyanobacteria blooms would have dramatic effects on the aquatic food web because they are important primary consumers in lake ecosystems. Additionally, toxic microcystin can accumulate in *Daphnia* (Thostrup 1999). This indicates that zooplankton may be vectors of bioaccumulation in the food web. The concentration of toxic *Microcystis* varies among lakes, and, based upon our results, it is likely that the concentration of toxin in the *Daphnia* should depend upon the species and population history of the *Daphnia*. In the present study we have no means to distinguish between the effects of microcystin contained in the *Microcystis* cells and other chemicals produced by this cyanobacterium.

Future work is needed to further explore the physiological and behavioral mechanisms that allow *Daphnia* to coexist with toxic cyanobacteria, testing different measures of fitness, and a wider variety of *Daphnia* species. Genetic investigations may also help pinpoint the rate of divergence among closely related *Daphnia* species.

We hypothesized that toxic cyanobacteria exert selective pressures upon the zooplankton grazer community. Our work suggests that toxic cyanobacteria promote differentiation between and within closely related *Daphnia* species.

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