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Nadejda Mirochnitchenko
nmiroch@bgsu.edu

Kevin E. McCluney
Bowling Green State University, kmclun@bgsu.edu

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The Influence of Beached Harmful Algal Blooms On Terrestrial Arthropods on the Shore of Lake Erie

Mirochnitchenko, N. A. & McCluney K. E.

Abstract:

Marine primary inputs, such as sea wrack and algae, offer a great niche for insects and other animals to exploit. The existence of a similar niche on the coast of lakes has received less attention. To complicate matters, many freshwater systems are seeing increases in proliferation of toxic and non-toxic cyanobacteria blooms. This study examined patterns in lake shore terrestrial arthropod abundance, diversity, and community composition across gradients of beached algae, with varying toxicity. We detected water microcystin effects on arthropod richness in survey three, beached material effects on Shannon's Diversity in survey two, and water microcystin effects on the community structure on survey three. Our results suggest the beached aquatic inputs have diversifying effect and microcystin has a positive indirect relationship with certain orders of arthropods such as flies and spiders. We recommend further study into the mechanisms surrounding shore arthropod resource utilization and predator release from toxic blooms.

Keywords: arthropod, toxic cyanobacteria, harmful algal bloom, spiders, flies, beetles, lake shore, microcystin, beached material

Introduction:

Island biogeography teaches that species richness is a balance between colonization and extinction on islands and that smaller islands support less biomass and species richness (McArthur & Wilson 2015, Simberloff 1974, Kohn & Walsh 1994, Toft & Schoener 1983, Brown 1978, Brown, 1971, Brown 1978) However, Polis and Hurd found the opposite trend on the Baja California islands; these islands had a significant negative correlation between spider density and island size, which suggests that smaller islands were more productive than larger islands. They explained this unusual biodiversity trend as being due to the higher perimeter to area ratio of small islands, allowing for relatively more marine material, such as sea wrack, to be washed ashore, compared to the larger islands. More than 99% of collected primary consumers were detritivores, thus the spiders and higher predators were supported by arthropods eating primarily allochthonous marine primary material on these dry and otherwise unproductive islands (Polis & Hurd 1995, Polis & Hurd 1996). Marine inputs have been shown to enhance other taxa: soil invertebrates, arthropods, reptiles, birds, and carnivores (Polis & Hurd 1996). Similar primary subsidy effects have been observed in rodent populations in the Gulf of California (Stapp & Polis 2003). Such a discovery shows that marine and terrestrial systems are linked and can promote biodiversity.

Islands do not have as many resources typically as the mainland. Marine inputs offer a great niche for insects and other animals to exploit. The existence of a similar niche on the coast of lakes has received less attention. To complicate matters, many freshwater systems are seeing increases in proliferation of toxic and non-toxic cyanobacteria blooms (Hallegraeff 1993, Sukenik *et al.* 2015), commonly referred to as harmful algal blooms (HAB). These HABs might increase fluxes of primary material that could be utilized by terrestrial arthropods, but the potential toxicity increases the risk associated with utilizing such allochthonous material. The goal of this study is to examine how beached aquatic material and its toxicity might influence lake shore arthropod food webs.

The system in this study is Lake Erie, which suffers annually from blooms of toxic cyanobacteria that negatively impact human health, local economies and wildlife. Most noticeably,

the previous summer's winds pushed the blooms to the water intake of the largest Western Basin City on the Lake Erie shore: Toledo Ohio. The high density of microcystis in this HAB, which secretes the hepatotoxin microcystin, overloaded the Toledo filtration system: contaminating the drinking water for half-million residents (Henry 2014, Wines 2014).

The National Oceanic Atmospheric Administration predicted that the 2015 HAB would be second worst since 2002, but due to unusually high nutrient loads early in the summer, the bloom exceeded expectations and became the worst bloom in recorded history. The bloom expanded to cover much of the central basin, but away from shore, unlike the primarily western basin blooms in previous years. Two cold fronts and strong winds hastened the blooms termination in late September (NOAA Nov. 2015).

These blooms, although named for their toxicity, can be non-toxic. Lake Erie's blooms are composed of *Microcystis spp.* and *Planktothrix spp.* which can produce microcystin, a hepatotoxin, but have varying toxicity depending on the strain and environmental factors (Ouellette *et al.* 2006, Rinta-Kanto & Wilhelm 2006). Increased temperatures, nitrogen and phosphorus levels yielded more toxic *Microcystis* compared to nontoxic strains (Davis *et al.* 2009, Vezie *et al.* 2002). Thus, global warming and high nutrient loads could cause more toxic HABs. Understanding the impacts of mixed toxic and nontoxic primary inputs on both freshwater and adjacent terrestrial systems, could aid management to curb biodiversity loss due to HABs.

Here we examine patterns in lake shore terrestrial arthropod abundance, diversity, and community composition across gradients of beached algae, with varying toxicity.

Methods:

We took field observations and samples on the southern shore of Lake Erie during three sampling periods: once in late July, once in early September (peak HAB extent), and once in late September.

Study Site. The study sites comprised 13 unarmored beaches spanning 140 km from Toledo (41.71° N, 83.46° W) to Lorain (41.46° N, 82.20° W) in Ohio. Nine of the 13 sites are on the shore of the western basin of Lake Erie, which historically has had blooms that exceed the World Health Organization's provisional microcystin concentrations (Kanto *et al.* 2005). The other four sites are along the western portion of the Central Basin, which has had lower harmful algal blooms (Kanto *et al.* 2005). The sites were visited over a period of three days in a randomized order.

Environmental characteristics, such as tree cover, impervious surface, and lawn cover, were calculated by estimating each cover value over a 100m *100m grid using Google Earth images of each of the sites. The side of the grid was line up with the coast with the site at the midpoint of that side.

At each site, we flagged three ten meter transects, starting at the shoreline, perpendicular to the shore, and five meters apart from each other. We hand collected, with the assistance of aspirators, all living arthropods encountered on one walk of this transect and stored the specimens in glass vials. Collection proceeded for the entire transect unless a physical barrier severely limited safety or access. Insects were identified using dichotomous keys to order. The primary keys were compiled in Borror and DeLong's Introduction to the Study of Insects. Average arthropod abundance, richness, and Shannon's diversity were calculated for each site after the arthropods were identified to order.

Covariate data, such as temperature and humidity, were collected at each site. On each transect, quarter meter quadrats were randomly tossed every two meters on the transect. Total

beached material was calculated as the average of the percent cover of aquatic inputs, such as detritus, algae, and cyanobacteria, in the quadrats within two meters of shore. Vegetation cover was calculated as the average percent cover of living terrestrial plants, such as grass and reeds, in the quadrat at the last two meters of the transect. Canopy cover was measured using a concave spherical densiometer facing the shore at every two meters of the transect, however due to almost universally low canopy cover within a few meters of the shore, only the average of the values associated with the last two meters of the transects were used. As mentioned above, we complemented this approach with identification of tree cover in a 100m² grid using satellite imagery.

Filtered water was also collected at each site and stored in glass vials in a freezer. Water was scooped at arm's length from shore and was filtered using 45 µm cellulose filters to reduce particulate matter in the water. Samples were frozen until the all samples were collected and ready for analysis. Water microcystin levels were measured by enzyme-linked immunosorbent assay (ELISA) with an Abraxis Microcystins-ADDA ELISA kit (product number 520011) (Loftin *et al.* 2008). Results were reported as parts per billion (ppb).

Because much of the HAB was not beached during the sampling times, we used chlorophyll A as an approximation for the potential amount of photosynthetic material to be beached at that time. Chlorophyll A water samples were also collected at arm's length from the shore during the third survey, kept out of the light, filtered using 60 mL of sample through FF 24mm filters within two weeks of collection, and sent on ice to Louisiana University for analysis using the Environmental Protection Agency's method 445 with Turner Designs TD-700 Spectrofluorometry (Lu *et al.* 2014, Parson 1984). Results were reported in micrograms per liter (ug/L).

Data Analysis. Environmental co-variates, such as canopy cover, that were correlated with the response variable, were included as nuisance variables in our generalized linear models (glm) in R v.0.99.467. Principal component analysis in R, using the vegan package, allowed us to distill several of the correlated covariates (impervious surface over 100m², tree cover over 100m², and lawn cover over 100m²) into fewer variables (PC1 and PC2). A second principal component analysis distilled impervious surface over 100m² and tree cover over 100m² into fewer variables (PC2A1 and PC2A2) for when lawn cover was not strongly associated with the response variable. We used 0.1 as our level of significance, since we were primarily interested in detecting any potential patterns of correlation within the data for future manipulative hypothesis testing. Survey one was not analyzed for this analysis due to the lack of HAB.

To test whether arthropod community composition was related to algae and its toxicity, we used nonparametric permutational multivariate ANOVA (adonis) in vegan package of R (4999 permutations). To identify specific taxa that were affected, similarity percentage (simper) analysis was conducted. To display community differences and associations of arthropods with environmental and hypothesis variables, non-metric multidimensional scaling plots (metaMDS) with envfit vector plotting function in the vegan package of R were utilized. These community structure analyses all used Bray- Curtis distances and followed procedures similar to those in McCluney & Sabo (2012).

Results:

Abundance. Site abundance ranged from 1 to 88 arthropods. The average abundance was 19.67 arthropods with a standard deviation of 18.43. Over the three surveys Coleoptera (beetles), Araneae (spiders), Hymenoptera (mostly ants), and Diptera (flies) were the most abundant arthropods on the coast line, respectively. 50% of the beetles collected were of two species: the

spotted cucumber beetles (*Diabrotica undecimpunctata*), which were 39.3% of the beetles, and pink spotted beetle (*Coleomegilla maculata*), which were 10.8% of the beetles. These beetles were primarily found on or nearby beached sea wrack.

Richness: The number of orders at each site varied between 1 to 9 orders. The average richness was 4.38 orders with a standard deviation of 1.88.

Microcystin Effects. We did not detect any significant environmental effects in arthropod abundance, richness, Shannon's diversity, or community structure for either survey two or three (Table 1). We detected water microcystin effects on arthropod richness in survey 3 ($\chi^2 = 3.07$, $df=1,10$, $p=0.06$) at the 0.1 alpha level (Table 1). Beached material had significant effects at the 0.1 alpha level on Shannon's Diversity in survey two ($F=4.53$, $df=1,9$, $p=0.07$) (Table 1.) We also detected water microcystin effects at the 0.1 alpha level on the community structure of survey three ($df=1$, $R^2=0.15$, $p=0.06$) using a nonparametric permutational multivariate ANOVA (adonis) (Table 1.).

Response Variable	Survey	Test	Distribution	Environmental factor	X ²	F	Degrees of freedom	P	R ²	Direction of relationship
Total Arthropod Abundance	2	Chisq	Quasipoisson	Canopy Cover	4.10		1,11	0.39		+
	2	Chisq	Quasipoisson	Temperature	2.48		1,10	0.51		-
	2	Chisq	Quasipoisson	Water Microcystin	1.50		1,9	0.60		-
	2	Chisq	Quasipoisson	Beached Material	1.22		1,8	0.64		-
	2	Chisq	Quasipoisson	Microcystin*Beached Material	0.61		1,7	0.74		
Total Arthropod Abundance	3	Chisq	Quasipoisson	PC1	51.74		1,11	0.20		-
	3	Chisq	Quasipoisson	Water Microcystin	79.44		1,10	0.11		+
	3	Chisq	Quasipoisson	Chlorophyll A	16.33		1,9	0.47		-
	3	Chisq	Quasipoisson	Microcystin*Chlorophyll A	4.16		1,8	0.72		
Arthropod Richness	2	Chisq	Poisson	PC2A1	0.14		1,11	0.71		-
	2	Chisq	Poisson	Water Microcystin	0.13		1,10	0.72		+
	2	Chisq	Poisson	Beached Material	1.27		1,9	0.26		+
	2	Chisq	Poisson	Microcystin*Beached Material	0.08		1,8	0.78		
Arthropod Richness	3	Chisq	Quasipoisson	PC1	1.73		1,11	0.16		-
	3	Chisq	Quasipoisson	Water Microcystin	3.07		1,10	0.06		+
	3	Chisq	Quasipoisson	Chlorophyll A	1.15		1,9	0.26		-
	3	Chisq	Quasipoisson	Microcystin*Chlorophyll A	0.25		1,8	0.60		
Shannon's Diversity	2	F	Normal	PC2A1		0.42	1,11	0.54		-
	2	F	Normal	Water Microcystin		0.75	1,10	0.41		+
	2	F	Normal	Beached Material		4.53	1,9	0.07		+
	2	F	Normal	Microcystin*Beached Material		0.04	1,8	0.84		
Shannon's Diversity	3	F	Normal	PC1		0.83	1,11	0.39		-
	3	F	Normal	Water Microcystin		0.00	1,10	0.97		+
	3	F	Normal	Chlorophyll A		0.15	1,9	0.71		-
	3	F	Normal	Microcystin*Chlorophyll A		0.05	1,8	0.82		
Community Structure	2	Adonis		PC2			1	0.57	0.07	
	2	Adonis		Water Microcystin			1	0.53	0.07	
	2	Adonis		Beached Material			1	0.46	0.08	
	2	Adonis		Microcystin*Beached Material			1	0.19	0.11	
Community Structure	3	Adonis		PC2			1	0.16	0.12	
	3	Adonis		Water Microcystin			1	0.06	0.15	
	3	Adonis		Chlorophyll A			1	0.58	0.06	
	3	Adonis		Microcystin*Chlorophyll A			1	0.78	0.04	

Table 1. This table shows the results of the statistical tests performed for arthropod abundance, richness, Shannon's diversity, and community structure for survey two and three. The table shows the statistical test used, the data distribution, the environmental factors involved in

each test, the χ^2 values, F values, degrees of freedom (df), p values, and R^2 values associated with each test. The last column show the direction of relationship between the response variable and that environmental variable. PC1 and PC2 refer to the first and second axis respectively of the principal component analysis that involved impervious surface, lawn cover, and tree cover. PC2A1 refers to the first axis of the principal component analysis that involved impervious surface and tree cover.

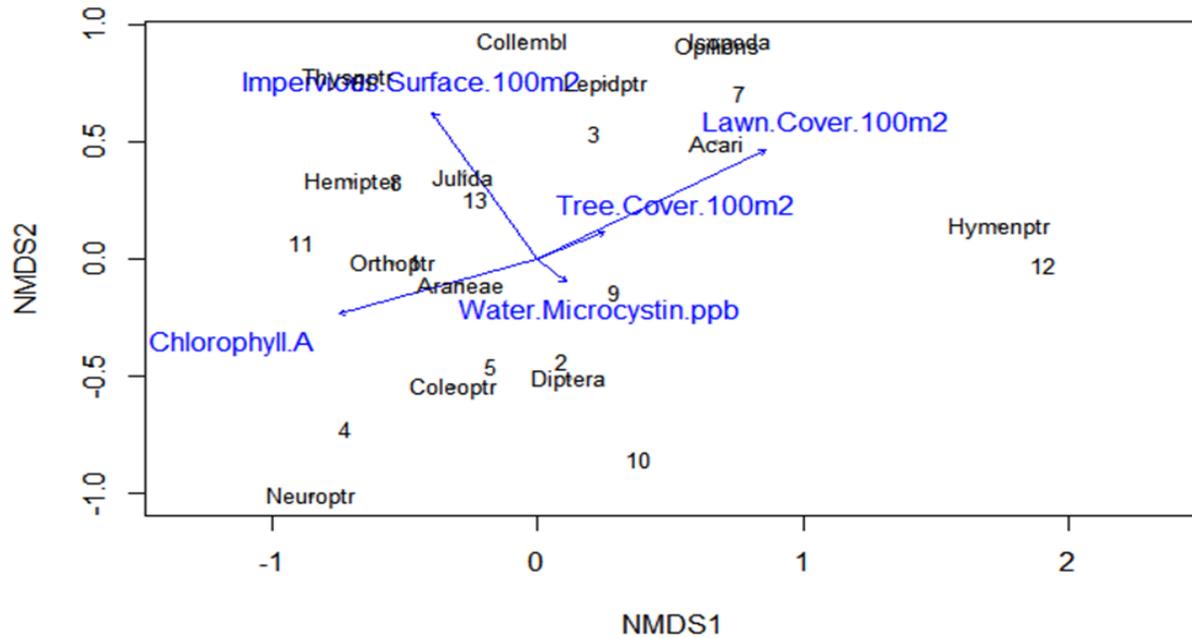


Figure 1: This non-metric multidimensional scaling plots shows the association between each environmental factor and order of arthropod community composition. The rays show the direction and importance of the factor's variation along the first two axes. Black text refers to the orders of arthropods in the analysis. See Table 1 for R^2 and p values for each factor. This plot shows impervious surface, lawn cover, and tree cover separately but these factors were included in a principal component analysis of which only the second axis, PC2, was used in the statistical calculations. The numbers refer to the site composition.

A post-hoc similarity percentage (simpler) analysis showed that these effects were most strongly associated with Coleoptera (contribution=0.333), Diptera (contribution=0.222), Araneae (contribution=0.111), Hemiptera (contribution=0.111), and Hymenoptera (contribution =0.111). These taxa graphed against water microcystin levels and all five displayed positive relationships with microcystin levels. Araneae had the closest fit to the positive line ($R^2=0.55$), followed by Diptera ($R^2=0.30$) and Coleoptera ($R^2=0.14$) (Fig.2, Table 2).

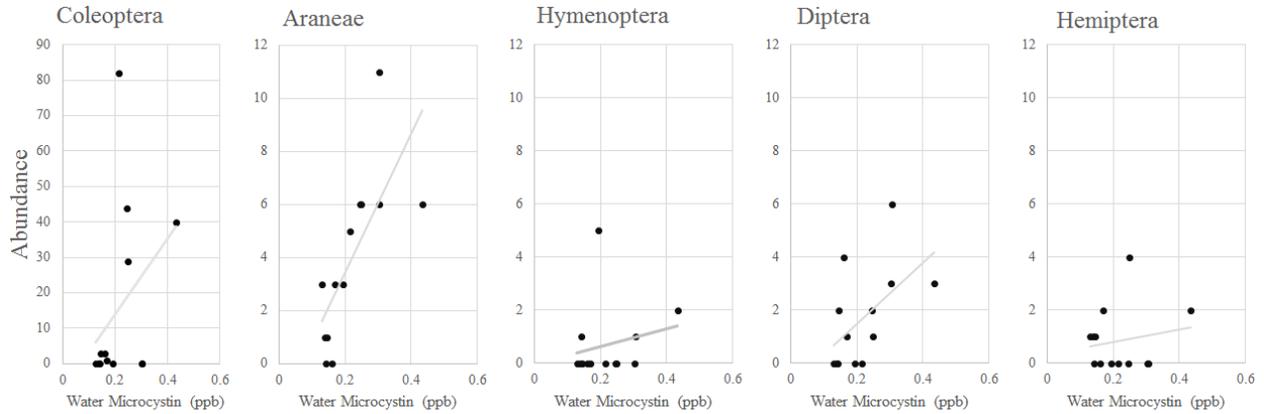


Figure 2. This figure shows the abundance of the arthropod orders that contributed most to the variation associated with the nonparametric permutational multivariate ANOVA results, which were determined by similarity percentage analysis, in relationship to the water microcystin (ppb) levels during survey three. All of these orders display positive relationships with microcystin. See table 2 for the equations of these linear fits and their associated R^2 value.

Order	Direction of Relationship	Equation	R2
Coleoptera	+	$107.18x-7.79$	0.14
Araneae	+	$26.15x-1.76$	0.55
Hymenoptera	+	$3.36x-0.04$	0.04
Diptera	+	$11.57x-0.82$	0.30
Hemiptera	+	$2.31x+0.34$	0.03

Table 2. This table shows the direction of relationship, equation, and closeness of fit (R^2) for the arthropod orders that contributed most to the variation associated with the nonparametric permutational multivariate ANOVA results, which were determined by similarity percentage analysis, during survey three. All of these orders' abundances display positive relationships with microcystin (ppb) levels in the water. See Figure 2 for distribution trends.

Discussion:

Our initial hypothesis was that beached primary material could provide a niche for arthropod that live on or adjacent to relatively unproductive beaches. We observed one significant relationship between beached material and Shannon's diversity, which was a positive relationship (Table 1). This result may indicate a diversifying influence that beached material has on the lakeshore arthropods, supporting our hypothesis. In the field, we observed beetles crawling over beached sea wrack and a harvestman dragging a piece of sea wrack away from shore. We thought that these arthropods could be using the beached material as food or shelter. We did not observe such behavior associated with beached cyanobacteria. The cyanobacteria is variably toxic, which could have led to avoidance, and may not have provided the structure that some arthropods may be searching for. Alternatively, not as much cyanobacteria was beached at our sites as sea wrack or filamentous algae, thus the frequency of each observation may be due to chance relative to occurrence of the beached material on shore. Although, our results suggest that beached material has diversifying effects, however the mechanism of such diversification cannot be teased out from this study.

We expected arthropod abundance and richness to be negatively correlated with microcystin water levels. Microcystin effects are not well understood in terrestrial invertebrates but the toxins are constantly seen to increase mortality of insects and macroinvertebrates that are fed toxic cyanobacteria (Delaney & Wilkins 1995, Laurén-Määttä *et al.* 1995, White *et al.* 2005). However, we detected significant microcystin effects on the arthropod richness of survey two and community structure in survey three (Table 1). When microcystin level was plotted against arthropod richness from survey two, a positive relationship emerged (Table 1). Further, the taxa that were most affected by the microcystin water concentration all independently showed a positive relationship when plotted against water microcystin level (Fig. 2, Table 2). Beetles had the most positive correlation but the R^2 value was low ($R^2=0.14$). Since most of these beetles were found on sea wrack, maybe microcystin and other cyanobacteria chemicals were attracting was attracting these beetles to the sea wrack that was laced with these chemicals. Our results suggest that microcystin may be associated with indirect positive impacts on the abundance of some of the most abundant arthropods on utilizing the coast, contradictory to our hypothesis.

An alternative hypothesis could be that microcystin level was a better indicator of allochthonous inputs that arthropods utilize than our more direct methods of measuring allochthonous inputs, i.e., quadrat beached primary material values and chlorophyll A water content. At sub-lethal microcystin levels, various arthropods can still utilize the aquatic primary inputs. The spotted cucumber beetle, the red spotted beetle, a couple harvestmen were observed interacting with aquatic sea wrack material, which could support this hypothesis but more research is needed to understand arthropod food selection in these ecotones.

Spiders and flies had the highest R^2 values, associated with a positive relationship with water microcystin level, out of the five most impacted taxa. This result might suggest that the higher water microcystin levels could be killing freshwater organisms such even big fish such as catfish (Zimba *et al.* 2001, Phillips *et al.* 1985), which were observed at some of the sites. We propose that the dead carcasses attracted flies leading to spiders having more prey on the shore (Fig 3). Thus, microcystin might have a stronger indirect positive impact on beach arthropod food webs by increasing food arriving to relatively unproductive shores than the toxic direct effects that we hypothesize. Further studies could explore this new link between toxic marine and shore environments.

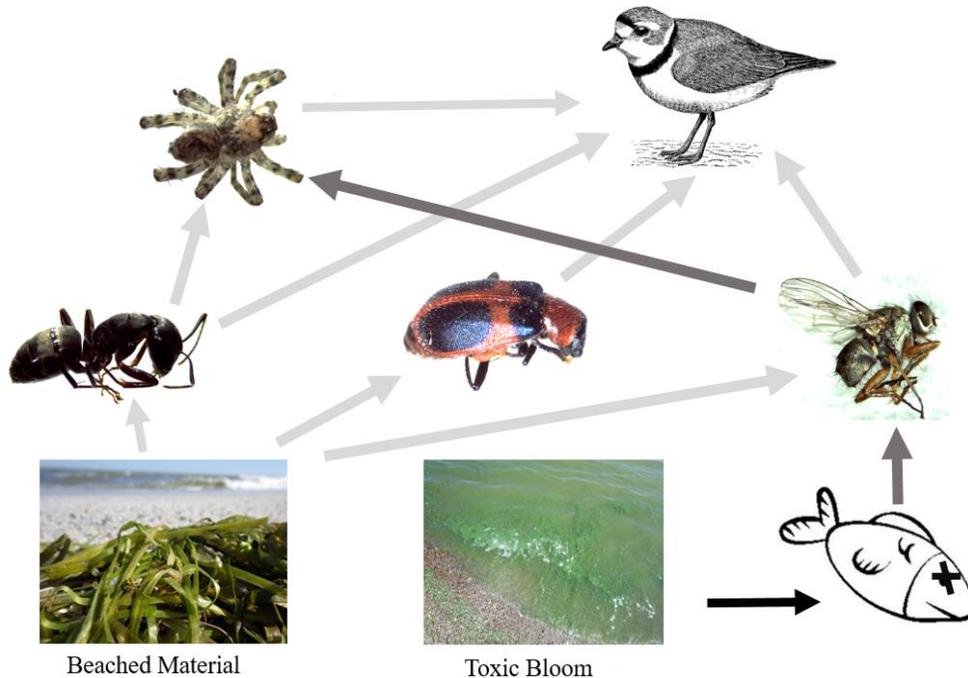


Figure 3. This proposed food web shows the strength of interaction among beached material, the toxic bloom, freshwater organisms, flies, beetles, ants, spiders, and shore birds. The darkest arrow from the toxic bloom to the freshwater organisms, represented here as a dead fish, indicated that the proposed strongest interaction in this web. The medium arrows indicate the next strongest interaction of flies being attracted by the beached dead organisms and spiders being attracted to the shore by the prospect of catching flies feasting on the carcasses of the dead fish. The light arrows represent weak interactions we suspect are happening, especially the interaction between beetles and beached material because we observed so many beetles utilizing beached sea wrack. Shore birds, such as plovers, were also observed searching for food, typically insects, along the beached sea wrack.

The increased numbers of some taxa could pose a threat to higher trophic levels if microcystin bioaccumulates in the tissues of organisms ingesting the toxin. If arthropods were consuming toxic material, whether the material was laced with microcystin from burst cyanobacteria, from the cyanobacteria itself or from organisms that died from their toxin exposure, the arthropods could be a vector for further toxin impacts on the predators of these lakeshore detritivores. Migratory shore birds might be the most at risk from such bioaccumulation because large concentrations of birds stopover especially at the shore of the Great Lakes basin to refuel for their flight over the vast lakes (Bonter *et al.* 2009). Although bioaccumulation is a risk especially for higher trophic, research is inconclusive whether microcystin accumulates in insect tissues (Laurén-Määttä 1995, Kotak *et al.* 1996, Chen *et al.* 2009), although more research points to the affirmative. If various taxa increase in association with microcystin and are consuming the toxic material, insectivore conservation groups, such as birds, may need to watch for this potential threat.

We examined bottom-up explanations of our results, however top-bottom effect could also influence the lakeshore arthropod communities. We observed that there were more arthropods of specific taxa, such as spiders, flies, beetles, true bugs, and ants, on the shore when water toxicity increased. In marine HAB, seabirds have been observed to reduce feeding activity and many birds

die (Shumway *et al.* 2003, Kvitek & Bretz 2005). Freshwater predators such as fish, turtles, ducks, and water birds have also seen increased mortality associated with toxic cyanobacteria (Chen *et al.* 2009). The mortality of predators may release the lower trophic levels from some predation, which could explain the increases various arthropod taxa on the shore of more toxic areas. Our study does not indicate that total abundance increased in more toxic areas (Table 1), which does not support this hypothesis. To further support this hypothesis, studies would need to observe increased predator mortality in more toxic areas and concurrently increases in arthropod abundances.

The patterns we observed during this study indicate that various arthropods are utilizing the beaches by Lake Erie during a HAB, the worst HAB in history (NOAA Nov. 2015). These patterns are most likely not as intense as other years because this year had unusual wind patterns that blew most of the bloom away from the southern shore of Lake Erie. If observations were to occur during a different HAB year, and with more sites, we predict that stronger relationships will be observed.

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