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Biogeography and co-occurrence of 16 planktonic species of *Keratella* Bory de St. Vincent, 1822 (Rotifera, Ploima, Brachionidae) in lakes and reservoirs of the United States

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Abstract

We report current distributions of 16 species of *Keratella* Bory de St. Vincent, 1822 within lakes and reservoirs of the U.S. Specimens were identified from 988 lakes and reservoirs during spring and summer 2012 as part of the U.S. Environmental Protection Agency's National Lakes Assessment (NLA). We performed a co-occurrence analysis to determine correlations between species-pair occurrences and a niche-centroid analysis to predict optimal water quality conditions for each species. While a high degree of overlap in geographic range was observed among the species, distribution maps showed that *K. quadrata* was largely confined to more northern latitudes and *K. americana* was confined primarily to the eastern U.S. Other common species were either ubiquitously or sparsely distributed across ecoregions, suggesting that their distribution may be more related to inter-species competition or local water quality parameters. This study expands the limited pool of knowledge on rotifer biogeography within the U.S.

Key words: ecoregion, lentic waters, National Lakes Assessment, Rotifera, zooplankton

Introduction

Species of the genus *Keratella* Bory de St Vincent, 1822 are among the most commonly encountered rotifers, often making up a substantial portion of the zooplankton community in aquatic systems worldwide (Ahlstrom 1943). Some species of *Keratella* are ubiquitous throughout many regions of the globe, e.g., *Keratella cochlearis* (Gosse, 1951), while others exhibit endemism only in certain regions: e.g., the Nearctic species *Keratella earlinae* Ahlstrom, 1943 (Segers & De Smet, 2008). Most studies have focused on a single lake or a few lakes within a small region; only a few have examined rotifer biogeography on a broad scale (e.g., Dumont 1983, Segers & De Smet 2008). Compared to Europe there have been few surveys that have examined the distribution of rotifers in the U.S. The last major review of *Keratella* species that included distributions within the country was performed in the early 1940's (Ahlstrom 1943). Given the degree of urban development, landscape alteration and climatic changes that have occurred since that time, there is a need to re-examine rotifer distributions in the U.S. and provide updated information for taxonomists working at the local or regional level. Rotifers as a group are sensitive to water quality and may be used as indicators of environmental conditions (Sládeček 1983, Saksena 1987, Duggan *et al*. 2001). Because there is a high likelihood of encountering species of *Keratella* within the zooplankton community of any lake, we chose to focus our attention on that genus. Our aim was to provide detailed geographic information regarding species of *Keratella*, which is requisite to augment information needed to meet water quality challenges.

Keratella are characterized by a hard lorica and are thus resistant to distortion in preserved samples, lending to relative ease of identification by polygonal facet patterns on the lorica surface. This characteristic, along with being commonly encountered, makes *Keratella* an ideal model for examining the question of whether some rotifer species are subject to habitat constraints across an expansive and variable landscape. We hypothesized that *Keratella*

species display distinct biogeographical distributions within the U.S. Biogeographical information from this study, along with other species data collected during the NLA, can be used to inform rotifer researchers about shifts in species distributions, both in comparison to past studies and as a baseline for changes that may occur in the future.

Methods

National Lakes Assessment study sites. Data from the U.S. Environmental Protection Agency's 2012 National Lakes Assessment were used in this study. The NLA is a broad-scale survey of multiple limnological parameters that occurs every five years in lakes and reservoirs (hereinafter, lakes) of the conterminous U.S., a useful public resource for both scientists and citizens interested in water quality metrics (Pollard *et al.* 2018). Both artificial and natural lakes greater than 1 ha in size (excluding the Great Lakes) were selected randomly from the USGS/EPA National Hydrography Dataset (NHDPlus) (Simley & Carswell 2009) and were sampled either once or twice in spring and summer (May–September) 2012. Lakes were selected without bias using probability-based selections and constituted a statistically valid representation of lakes in similar regions (USEPA 2012a). A total of 1,038 lakes were sampled during the 2012 NLA. Samples collected from Wisconsin were excluded from this study due to inconsistency in laboratory personnel performing taxonomic determinations, leaving a total of 988 lakes analyzed.

Sample collection. All samples were collected from an index site in each lake, which was considered an open water area up to 50 m deep, or at the mid-point in reservoirs. Secchi depth (SD) was recorded using a standard 20 cm diameter Secchi disk, painted with quadrants, lowered to disappearance then raised to reappearance on the shady side of the boat. An integrated tube sampler designed by the Minnesota Water Control Agency (USEPA 2012a, section 5.3) was used to collect whole water grab samples from the euphotic zone. This zone was defined as either 2x SD (when the SD was > 2 m) or within the top 2 m of the water column (when the SD was ≤ 2 m). Water was transferred from the sampler into a rinsed 4 L Cubitainer® and this process was repeated until the Cubitainer was filled. Subsamples were then taken from the Cubitainer for nutrients, turbidity, and chlorophyll. A 250 ml subsample for nutrient analysis was acidified and shipped overnight to processing labs. For chlorophyll, a 2 L subsample was taken and at least 100 ml of sample water was filtered through a glass fiber filter (GF/F) immediately following collection. Filters were flash-frozen in the field and subsequently shipped on ice to processing labs, where they were analyzed within 30 days. Turbidity subsamples were not filtered or preserved; they were processed within 72 hrs of collection. Vertical temperature profiles were measured at the index site using a multiparameter water quality meter equipped with a temperature probe; however, in this study only mean water temperatures from the upper 5 m of the water column were evaluated.

Microzooplankton samples were collected using a fine (50 μm) mesh Wisconsin-style net. Although many rotifers are smaller than 50 μm in size, mesh < 64 μm has been shown to capture rotifers effectively (Mack *et al.* 2012). Each sample consisted of a 5 m vertical tow, and in situations where the water body was < 5 m deep, multiple tows were taken to obtain a cumulative tow of 5 m. Samples were preserved with 70% ethanol upon collection and then shipped to BSA Environmental Services, Inc. (Beachwood, OH) for taxonomic identification. Additional details regarding sample collection are provided in USEPA (2012a).

Laboratory analyses. All chemical analyses used the following EPA protocols (USEPA 2012b). Total nitrogen (mg L^{-1}) and total phosphorus (μg L^{-1}) were determined using automated colorimetric analysis following persulfate digestion (EPA method 353.2). Turbidity (NTU) was determined nephelometrically (EPA method 180.1). Chlorophyll-a extraction was performed using 90% acetone; the concentration (μg L^{-1}) was measured fluorometrically (EPA method 445.0, EPA method 446.0).

A consistent problem with taxonomic studies is the inability to verify identifications made by different researchers using diverse references (Koste & Shiel 1989, Segers 1998). During the 2012 NLA a small team of taxonomists in a single laboratory identified the species, using a common set of references and nomenclature. All zooplankton samples used in this study were analyzed at BSA Environmental Services, Inc. (Beachwood, OH). Additionally, the NLA provided quality assurance and quality control of taxonomic identifications both internally and externally for a subset of samples to verify identifications and validate initial species designations (USEPA 2012b). A trained taxonomist identified the taxa found in each sample to lowest possible taxonomic level; these were enumerated and measured to calculate biomass estimates (see below). Prior to microscopic analysis, concentrated sample volume was measured and each sample was homogenized using a magnetic spinner at low speed. A subsample was taken

using a wide-bore volumetric pipette and transferred into an Utermöhl chamber (20 mm diameter). Appropriate aliquots containing at least 400 organisms (including rotifers, copepod nauplii, and cladocerans < 20 µm in length) were examined at 100X magnification or higher using inverted microscopes (Wilovert). For biomass estimates, 20 individuals were measured for dominant taxa (> 40 individuals per aliquot). Ten measurements were taken for taxa encountered less than 40 times in an aliquot, and five measurements were taken for taxa encountered less than 20 times in an aliquot. Biomass estimates were based on established relationships between body length and dry weight (McCauley 1984). Abundance (organisms per liter) was calculated for individual taxa using the following equation:

Taxon abundance = [(concentrated sample volume ÷ volume counted) ÷ tow volume] × abundance in sample

Biomass (µg dry weight per liter, McCauley 1984) was computed for the appropriate number of individuals for each sample and the arithmetic mean biomass was multiplied by the taxon abundance to produce a taxon biomass for each sample. Additional details regarding laboratory analyses are provided in USEPA (2012b).

Nomenclature. A total of 21 individual taxa within the genus *Keratella* were identified in the 2012 NLA, including individuals that could not be identified to species. Those specimens were designated as *Keratella* spp. (found in n = 18 samples) were excluded from analyses. Taxonomic sources used for the identification of *Keratella* included Ruttner-Kolisko (1974), Koste (1978), and Stemberger (1979), as well as online resources including the Rotifer World Catalog (rotifera.hausdernatur.at) and the Image-Based Key to Zooplankton of North America (cfb. unh.edu/cfbkey/html/rotifers.htm). Following protocol specified by the NLA, species names were validated using the Integrated Taxonomic Information System (www.itis.gov). Since that time, a consensus on acceptable rotifer nomenclature (Segers *et al.* 2012) was released, which is somewhat incongruous with nomenclatural determinations made for the NLA. All data used in this study are available for public download (https://www.epa.gov/national-aquatic-resource-surveys). However, in the context of this study, nomenclatural determinations follow those outlined in Segers *et al.* (2012). Specific changes were made as follows:

Specimens originally described as *Keratella cochlearis tecta* are treated in this study as *Keratella tecta* (Gosse, 1851), an independent species instead of a junior subjective synonym of *K. cochlearis* (Gosse, 1851). Two junior subjective synonyms of *K. cochlearis* were identified in this study (*Keratella cochlearis faluta* and *Keratella cochlearis hispida*), which were agglomerated into *K. cochlearis* for all analyses in this study. *Keratella quadrata testudo* was determined by Segers *et al.* (2012) to be synonymous with *Keratella testudo* (Ehrenberg, 1832). As such, specimens identified as *K. quadrata testudo* were agglomerated with *K. testudo* for all analyses in this study. One specimen was identified as *Keratella quadrata* f. *tropica*, which was determined to be synonymous with *Keratella tropica aspina* Kutikova, 1970. In this study, it is treated as *K. tropica aspina*. *Keratella quadrata dispersa* Carlin 1943 is treated as an independent species from *Keratella quadrata*. The species designation *Keratella serrulata* (Ehrenberg, 1838) includes all forms of *K. serrulata* identified in this data set, including *Keratella serrulata curvicornis*. All other species designations made during the NLA remain valid. Under the nomenclatural scheme employed in this study, a total of 16 species of *Keratella* are analyzed.

Mapping. Using the latitude and longitude for sampling sites and total sample biomass for each species, the data were plotted onto a map using Arc GIS® software. For the purposes of the NLA and other components of the EPA's National Aquatic Resource Surveys (NARS), the 85 Level III ecoregions of the conterminous U.S. (Omernik 1987) were agglomerated into 9 broader ecoregions (Herlihy *et al.* 2008). These were assessed on the basis of uniformity in reference-site quality and naturally occurring variation in stream macroinvertebrate assemblages. Hereinafter, the term ecoregion will refer to the nine, agglomerated ecoregions outlined by Herlihy *et al.* (2008).

Niche Centroids. Relationships between environmental variables and individual *Keratella* species biomass were quantified using niche centroid analysis (terBraak & Verdonschot 1995). In this analysis, the weighted mean biomass for species and environmental variables were used to determine the optimum value at which maximal biomass was observed. For example, the niche centroid optima for biomass were calculated as follows.

$$u_k = \sum_{i=1}^{n} \frac{y_{ik}}{y_{+k}} x_i$$

where:

y_{ik} = the biomass of species k in the ith sample,
y_{+k} = the summed biomass of species k in all samples, and
x_i = the environmental variable in the ith sample.

Correlation Analysis. To determine whether there were significant correlations among taxon occurrence in lakes, a co-occurrence analysis was performed using the package *cooccur* in R (version 1.1.456). This package applies a probabilistic model of species co-occurrence (Veech 2013) to a set of taxa distributed among a set of survey or sampling sites (Griffith *et. al*. 2016). In this study, data from 16 species of *Keratella* in 1,168 samples were employed to develop the model.

Results

Species distributions. Biogeographical distributions of 16 species (Figure 1) were examined across nine distinct ecoregions (Figure 2). Distribution patterns are described below.

Keratella americana Carlin, 1943 (Figure 1a)
Keratella americana was found in 233 lakes and in all U.S. ecoregions, except for the Northern Plains. However, it was found in only one lake in the Western Mountains and sparsely in the Xeric and Upper Midwest ecoregions. Highest mean biomass values for *K. americana* were observed in the Coastal Plains (2.20 µg d.w. L^{-1}), Southern Plains (2.32 µg d.w. L^{-1}) and Temperate Plains (2.43 µg d.w. L^{-1}) (Table 1). *Keratella americana* was observed in lakes at a broad range of elevations, from 0.3 to 2, 968 m (Figure 3), however most observations occurred at elevations below 500 m.

Keratella cochlearis (Gosse, 1851) (Figure 1b)
Keratella cochlearis constituted the most abundant species of *Keratella* in the survey, observed in 885 lakes and in all nine U.S. ecoregions and contributing a mean of about 6.5% to total rotifer biomass (Table 1). Although *K. cochlearis* was ubiquitously distributed across all ecoregions with multiple observances in all nine ecoregions, highest mean biomass was observed in the Northern Plains (1.84 µg d.w. L^{-1}). *Keratella cochlearis* occurred at a broad range of elevations, from 0.01 to 3,595 m (Figure 3), however was primarily observed in lakes at elevations below 1,000 m.

Keratella crassa Ahlstrom, 1943 (Figure 1c)
Keratella crassa was observed in 375 lakes during the 2012 NLA. Highest mean biomass for *K. crassa* was observed in the Northern Appalachians ecoregion (0.84 µg d.w. L^{-1}) (Table 1). Although observed in all nine U.S. ecoregions, *K. crassa* was found in only two lakes in the Northern Plains and was found sparsely in the Southern Plains, Western Mountains and Xeric ecoregions. *Keratella crassa* was observed at a wide range of elevations, from 0.01 to 3,307 m, however, was more common at lower elevations (below 500 m, Figure 3) with a mean elevation of occurrence of 341 m.

Keratella earlinae Ahlstrom, 1943 (Figure 1d)
Keratella earlinae was observed in all nine U.S. ecoregions, however occurred more frequently at higher latitudes. Being found in 169 lakes, *K. earlinae* can be considered common. Highest mean biomass for *K. earlinae* was observed in the Temperate Plains (1.87 µg d.w. L^{-1}) (Table 1). *Keratella earlinae* was observed at elevations ranging from 0.01 to 3,307 m, with the majority of observations occurring in lakes at elevations below 1,000 m (Figure 3).

Keratella mixta (Oparina-Charitonova, 1924) (Figure 1e)
Keratella mixta was observed in only three lakes in the U.S. during the 2012 NLA, including two lakes in the Western Mountains ecoregion and one lake in the Coastal Plains ecoregion. *Keratella mixta* was observed at elevations ranging from 43 to 1,083 m (Figure 3).

Keratella testudo (Ehrenberg, 1832) (Figure 1f)
Keratella testudo was geographically isolated to only eight lakes, within the Western Mountains and Xeric ecoregions. Lakes in the Western Mountains region saw relatively high biomass for *K. testudo* (32.62 µg d.w. L^{-1}), while Xeric lakes saw lower biomass for this species (1.18 µg d.w. L^{-1}) (Table 1). Lakes in which *K. testudo* were recorded were between 678 and 2,594 m, however the majority of biomass was observed in lakes between 1,000 and 2,500 m (Figure 3).

Keratella hiemalis Carlin, 1943 (Figure 1g)
Keratella hiemalis was observed in four of the nine U.S. ecoregions, most frequently (four lakes) in the Western Mountains region. *Keratella hiemalis* was observed in one lake in the Southern Appalachians ecoregion, one

lake in the Temperate Plains ecoregion, and one lake in the Xeric ecoregion. Elevation of occurrence for *K. hiemalis* ranged from 303 to 3,435 m, with the majority of observations occuring between 1,000 to 3,000 m (Figure 3). Generally, *K. hiemalis* was observed at higher elevations than other species of *Keratella*.

Keratella quadrata (Müller, 1786) (Figure 1h)

Keratella quadrata was observed primarily in lakes located west of the Mississippi River, however was observed at least once in all ecoregions except for the Southern Appalachians, in a total of 163 lakes. Highest mean biomasses for *K. quadrata* were observed in the Upper Midwest (5.05 µg d.w. L^{-1}), Temperate Plains (3.98 µg d.w. L^{-1}) and Northern Plains (3.21 µg d.w. L^{-1}) (Table 1). Mean biomass of *K. quadrata* was relatively low in the Northern Appalachians (0.02 µg d.w. L^{-1}) and Coastal Plains (0.01 µg d.w. L^{-1}). *Keratella quadrata* was observed at elevations between 11 and 3,299 m (Figure 3).

Keratella quadrata dispersa Carlin 1943 (Figure 1i)

Keratella quadrata dispersa was found in 10 lakes across three U.S. ecoregions. One observation occurred in the Southern Appalachians, while all other observations occurred in the Western Mountains (mean biomass 1.76 µg d.w. L^{-1}) and Xeric (mean biomass 0.50 µg d.w. L^{-1}) ecoregions. *Keratella quadrata dispersa* occurred in lakes at elevations between 152 and 3,074 m, with the majority of biomass occurring at elevations between 1,000 and 3,000 m (Figure 3)

Keratella serrulata (Ehrenberg, 1838) (Figure 1j)

Keratella serrulata was found in 12 lakes across three U.S. ecoregions. Three observations were located in the Coastal Plains (mean biomass 0.02 µg d.w. L^{-1}), two observations were located in the Northern Appalachians (mean biomass 0.04 µg d.w. L^{-1}) and eight observations were located in the Western Mountains (mean biomass 0.34 µg d.w. L^{-1}) (Table 1). Minimum elevation of occurrence for *K. serrulata* was 5 m, while maximum elevation of occurrence was 2,970 m (Figure 3).

Keratella taurocephala Myers, 1938 (Figure 1k)

Keratella taurocephala was observed in 71 lakes in five U.S. ecoregions, including the Coastal Plains, Northern Appalachians, Southern Appalachians, Upper Midwest and Western Mountains. Occurrence was most frequent in the Northern Appalachians; however mean biomass was highest for *K. taurocephala* in the Western Mountains (1.84 µg d.w. L^{-1}) (Table 1). *Keratella taurocephala* tended to occur at lower elevations, ranging from 7 to 1,621 m, with a mean elevation of occurrence of 285 m (Figure 3).

Keratella tecta (Gosse, 1851) (Figure 1l)

Keratella tecta was observed in 232 lakes, across all nine U.S. ecoregions. Highest mean biomass for *K. tecta* was observed in the Western Mountains (0.94 µg d.w. L^{-1}), with second highest mean biomass seen in the Northern Plains (0.47 µg d.w. L^{-1}). *Keratella tecta* was observed at a wide range of elevations, from 0.1 to 2,754 m. The majority of observations for *K. tecta*, however, were confined to lakes at elevations below 1,000 m (Figure 3).

Keratella ticinensis (Callerio, 1920) (Figure 1m)

Keratella ticinensis was observed in only four lakes, all located within the Western Mountains ecoregion (Oregon and Washington). Biomass for *K. ticinensis* ranged from 0.01 to 2.09 µg d.w. L^{-1} (Table 1). All observations of *K. ticinensis* occurred in lakes at elevations below 1,000 m (Figure 3).

Keratella tropica (Apstein, 1907) (Figure 1n)

Keratella tropica was widely distributed across the conterminous U.S., occurring in seven ecoregions, however it was also relatively rare, occurring in only 26 lakes. Highest mean biomasses for *K. tropica* were observed in the Coastal Plains (4.39 µg d.w. L^{-1}) and Southern Plains (3.46 µg d.w. L^{-1}) (Table 1). *Keratella tropica* was observed at a range of elevations, from 0.01 to 3,595 m; however it occurred primarily in lakes at elevations below 1,000 m (Figure 3).

Keratella tropica aspina Kutikova, 1970

Keratella tropica aspina was observed at relatively low biovolume (0.20 µg d.w. L^{-1}) in only one lake in Utah, located within the Xeric ecoregion, at an elevation of 1,651 m.

Keratella valga (Ehrenberg, 1834) (Figure 1o)

Keratella valga was relatively rare, occurring in only seven lakes in the 2012 NLA. *Keratella valga* was observed in four ecoregions: the Southern Plains, Temperate Plains, Western Mountains and Xeric regions. Biomass of *K. valga* was generally low, ranging from 0.02 to 1.97 µg d.w. L^{-1} (Table 1). Elevation of occurrence for *K. valga* ranged from 3 to 1,797 m (Figure 3).
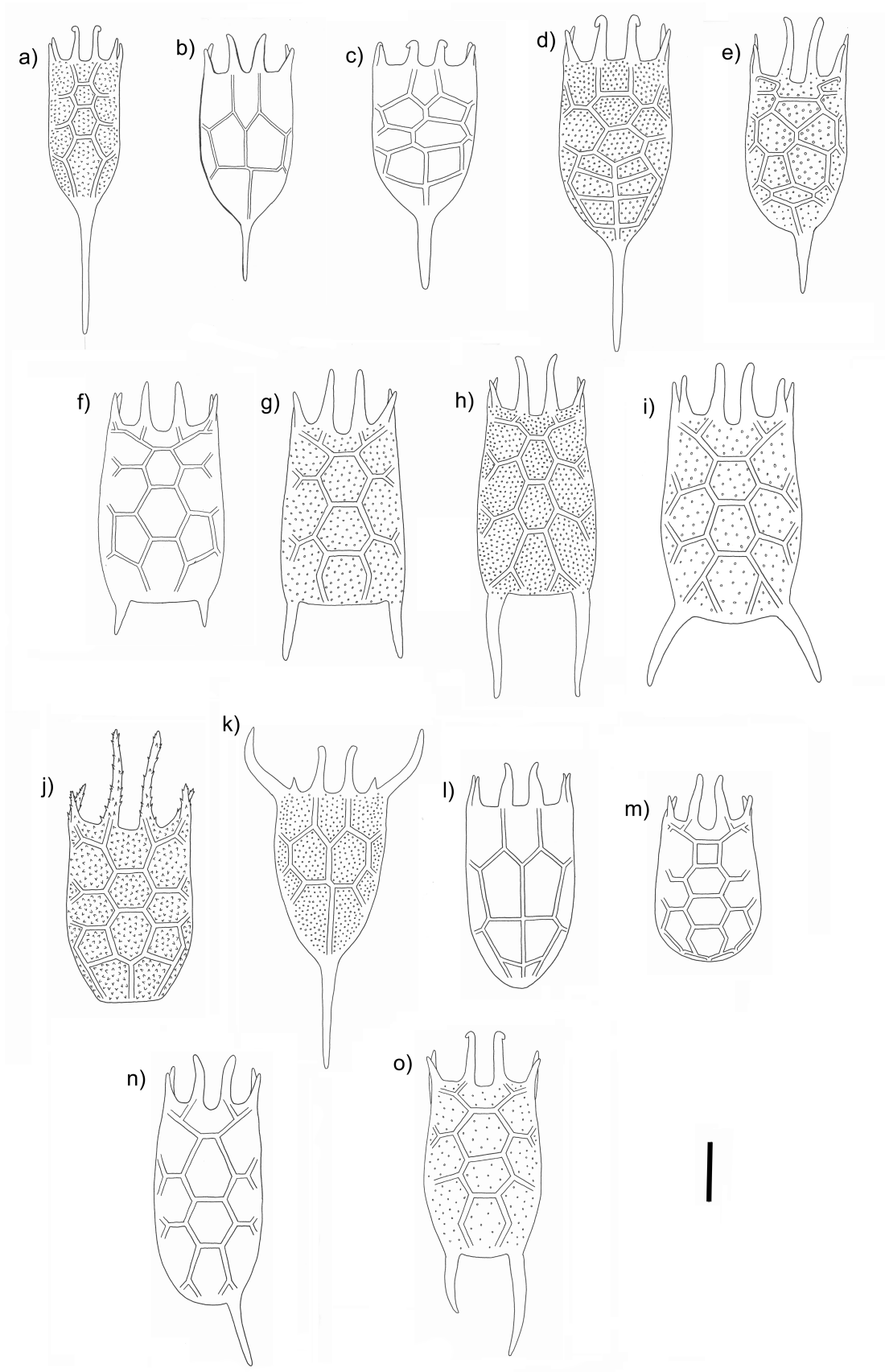
FIGURE 1. Dorsal views of the loricas of 15 species of *Keratella*. a) *Keratella americana*, b) *Keratella cochlearis*, c) *Keratella crassa*, d) *Keratella earlinae*, e) *Keratella mixta*, f) *Keratella testudo*, g) *Keratella hiemalis*, h) *Keratella quadrata*, i) *Keratella quadrata dispersa*, j) *Keratella serrulata*, k) *Keratella taurocephala*, l) *Keratella tecta*, m) *Keratella ticinensis*, n) *Keratella tropica*, o) *Keratella valga*. Scale bar represents 50 μm.

TABLE 1. Percentage of lakes in which each of the 16 species of *Keratella* was observed, along with mean percentage of total rotifer biomass (n=1,168 samples), and mean biomass (µg dry weight per liter) in each of the nine ecoregions (Abbreviations: CPL = Coastal Plains, NAP = Northern Appalachians, NPL = Northern Plains, SAP = Southern Appalachians, SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, WMT = Western Mountains, XER = Xeric).

| | occuring in % of lakes | mean % of total rotifer biomass | mean biomass (µg d.w. L⁻¹) | | | | | | | | |
			CPL	NAP	NPL	SAP	SPL	TPL	UMW	WMT	XER
Keratella cochlearis	90%	6.5%	1.35	0.80	1.84	0.63	0.78	1.52	1.12	0.74	0.28
Keratella crassa	38%	2.1%	0.68	0.84	0.64	0.71	0.81	0.18	0.77	0.62	0.48
Keratella americana	24%	2.4%	2.20	1.00		0.89	2.32	2.43	0.05	0.00	0.82
Keratella tecta	23%	0.2%	0.18	0.03	0.47	0.04	0.26	0.35	0.02	0.94	0.04
Keratella earlinae	17%	0.6%	0.12	0.44	0.09	0.14	0.70	1.87	0.30	0.83	0.09
Keratella quadrata	16%	2.3%	0.01	0.02	3.21		1.07	3.98	5.05	1.72	1.54
Keratella taurocephala	7%	0.6%	0.21	0.39		0.55			0.51	1.84	
Keratella tropica	3%	0.2%	4.39		0.31	0.02	3.46	0.98		0.19	0.31
Keratella serrulata	1%	<1%	0.02	0.04						0.33	
Keratella quadrata dispersa	<1%	<1%				0.01				1.76	0.50
Keratella testudo	<1%	<1%								32.62	1.18
Keratella valga	<1%	<1%					0.24	0.99		0.01	0.10
Keratella hiemalis	<1%	<1%				0.05		0.32		0.62	0.05
Keratella ticinensis	<1%	<1%									
Keratella mixta	<1%	<1%	1.528							0.005	
Keratella tropica aspina	<1%	<1%									0.2
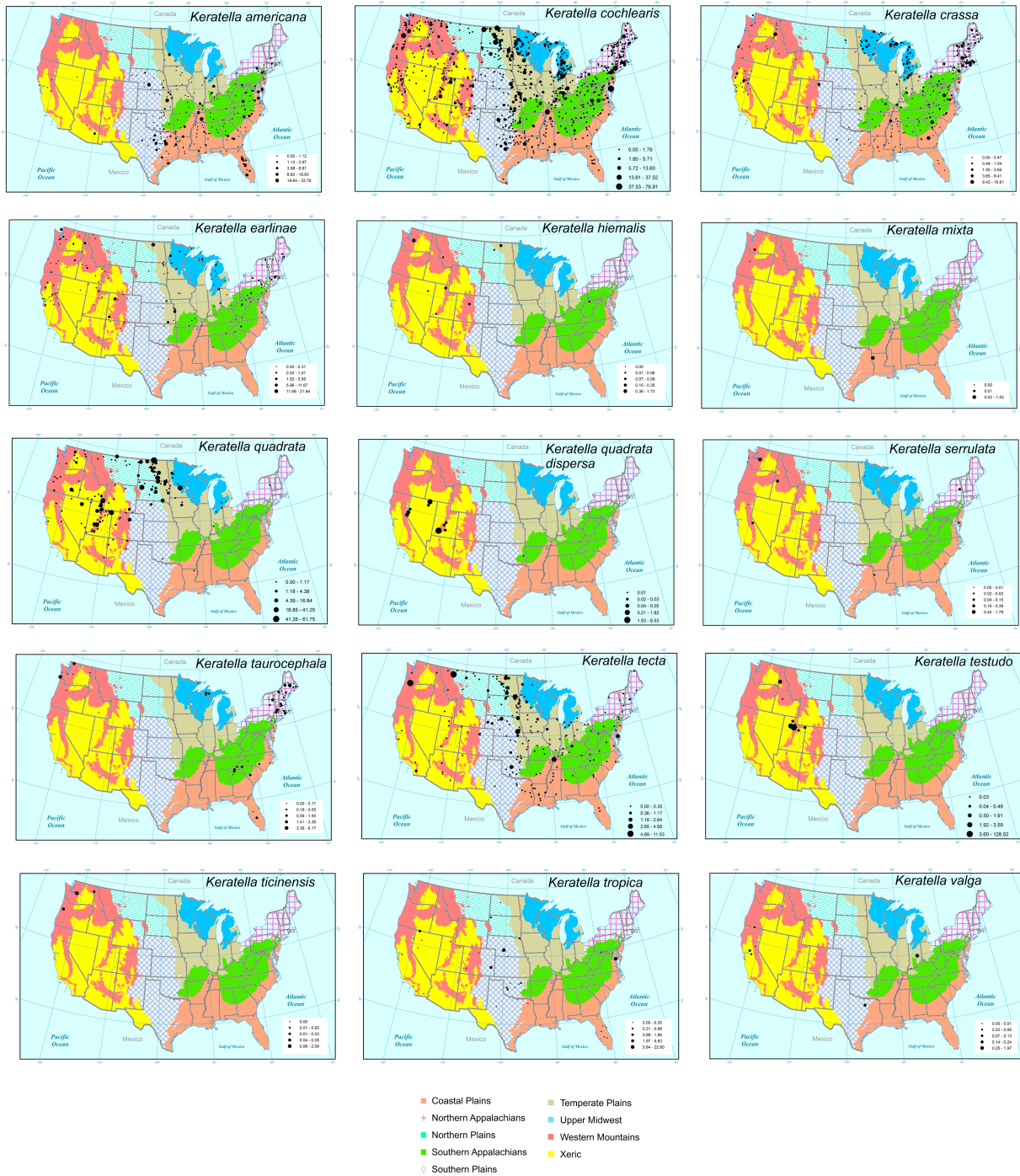
FIGURE 2. Distributions of 15 species of *Keratella* within nine agglomerated ecoregions of the continental U.S. Each circle represents one sample; circle size represents total biomass (µg dry weight L⁻¹) for that species in a sample. Biomass values for each species are set to individual scales.

Co-occurrence analysis. Out of 120 potential species pair combinations 61 pairs (50.83 %) were removed from the analysis because expected co-occurrence was < 1 and 59 pairs were analyzed. Out of the 59 pairs of *Keratella*, eight significant negative correlations and nine significant positive correlations were revealed (Figure 4). All other taxon pair combinations showed no significant correlations, indicating that instances of co-occurrence were random and not ecologically significant. Both *K. quadrata* and *K. tecta* were negatively correlated with *K. taurocephala* and *K. crassa*, despite the fact that all four of those species overlap to some degree in geographic range. *Keratella*

americana showed negative correlations with *K. quadrata* and *K. earlinae*, while *K. crassa* showed negative correlations with *K. tropica* and *K. quadrata dispersa*. *Keratella crassa* showed the highest number of positive correlations of co-occurrence, including with *K. earlinae*, *K. cochlearis*, *K. taurocephala* and *K. americana*. *Keratella crassa* overlaps in geographical range with all four of those species. *Keratella earlinae* showed significant positive correlations with *K. testudo* and *K. cochlearis*. *Keratella quadrata* showed significant positive correlations with *K. testudo* and *K. quadrata dispersa*. *Keratella cochlearis* was positively correlated with *K. tecta*.
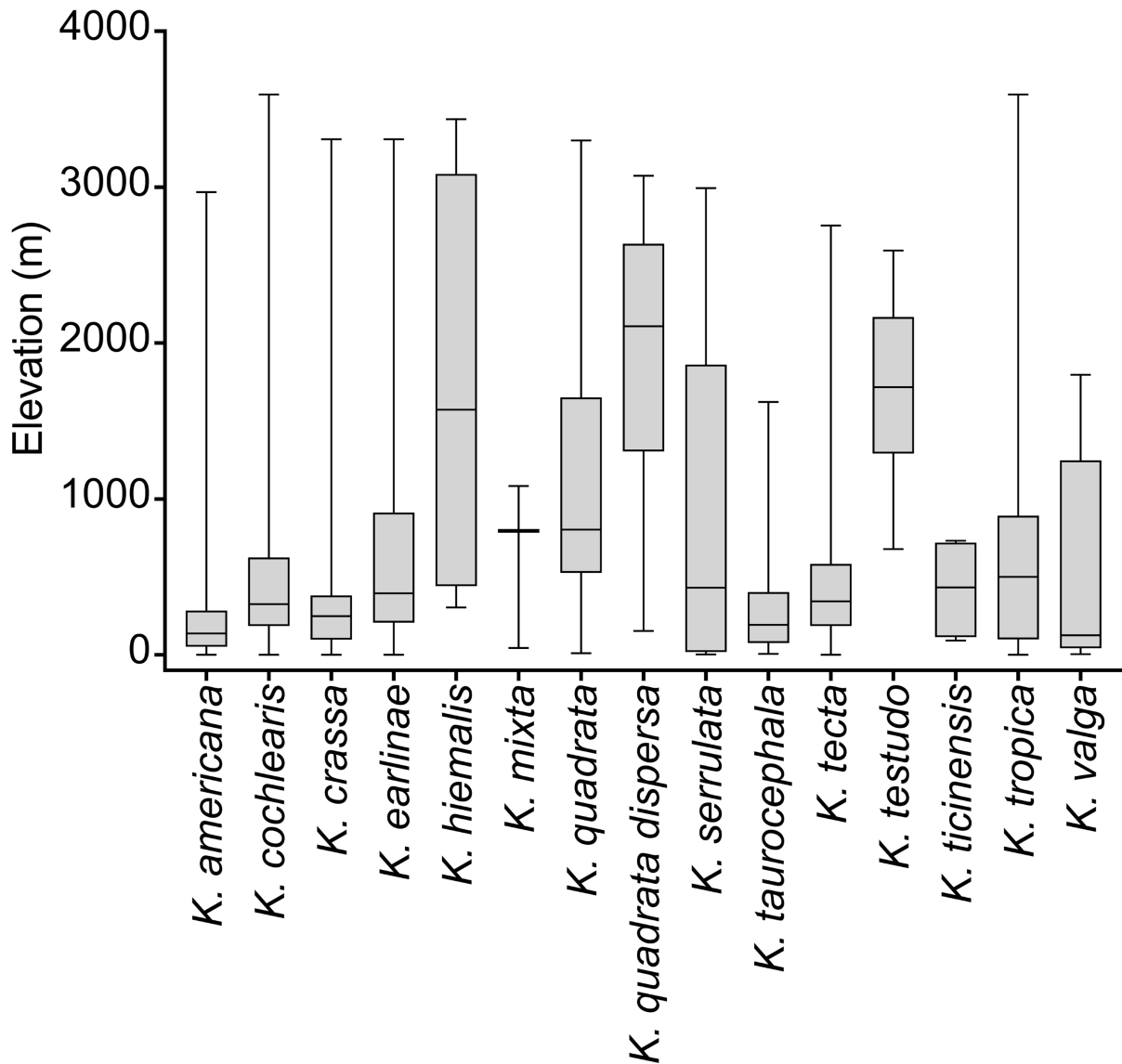

FIGURE 3. Box and whisker plot showing elevation range (m) by quartile for 15 species of *Keratella*.

Niche Centroids. Niche centroid optima for the 16 species of *Keratella* were determined for turbidity, total nitrogen, total phosphorus, water temperature, pH, and chlorophyll-a (Table 2). Both *K. americana* and *K. quadrata* displayed optima at relatively high turbidities (14.4 and 15.5 NTU, respectively), however temperature optima for the two species differed by more than 7° C (27.2 and 19.6° C, respectively). *Keratella tecta* and *K. tropica* also exhibited high turbidity optima (21.4 and 23.8 NTU, respectively), but showed similar temperature optima (22.3 and 25.2° C, respectively). *Keratella crassa*, *K. earlinae*, *K. hiemalis*, and *K. taurocephala* were predicted to exhibit highest biomass at relatively low turbidities (>6 NTU), indicating a preference for clearer water. Overall, *K. taurocephala* exhibited the most acidic optimum (5.9) of all 13 species, while *K. quadrata* exhibited the most alkaline (9.0) pH optimum. The highest optima for total nitrogen, total phosphorus, temperature, and chlorophyll-a were

exhibited by *K. mixta*, suggesting that this species has a tolerance for more eutrophic conditions. However, given the small number of lakes in which it was observed (n = 3), it is difficult to draw conclusions on the water quality preferences for this species. Similarly, niche centroid optima for *K. quadrata dispersa*, *K. tropica aspina*, *K. serrulata*, *K. testudo*, *K. ticinensis*, *K. tropica*, and *K. valga* are based on small sample sizes and may not represent the full scale of environmental preferences for these species.

TABLE 2. Locations of niche centroid optima between *Keratella* species and water quality variables.

|	Turbidity (NTU)	Total N (mg L⁻¹)	Total P (µg L⁻¹)	Temperature (0-5 m, °C)	pH	Chlorophyll-a (µg L⁻¹)
Keratella americana	14.4	1.5	136.4	27.2	7.3	45.1
Keratella cochlearis	8.3	1.5	131.2	23.0	8.0	35.9
Keratella tecta	21.4	3.0	229.9	22.3	8.5	85.6
Keratella crassa	2.8	0.6	38.7	23.2	7.2	18.2
Keratella earlinae	5.3	0.9	58.5	21.3	8.2	15.4
Keratella hiemalis	3.5	1.5	150.4	17.8	6.9	7.2
Keratella mixta	8.0	6.5	1047.6	29.0	6.2	248.4
Keratella quadrata	15.5	2.6	215.0	19.6	9.0	62.9
Keratella quadrata dispersa	2.8	1.1	126.3	18.0	8.6	12.3
Keratella tropica aspina	0.0	0.0	0.5	16.1	8.3	1.1
Keratella serrulata	2.4	1.4	156.2	18.3	6.5	8.0
Keratella taurocephala	1.1	0.3	18.9	21.0	5.7	6.8
Keratella testudo	0.8	0.4	29.0	16.1	8.3	1.1
Keratella ticinensis	2.5	1.5	178.1	18.4	6.4	5.4
Keratella tropica	23.8	2.9	245.7	25.2	8.6	145.6
Keratella valga	5.8	5.5	827.2	24.3	8.2	145.8
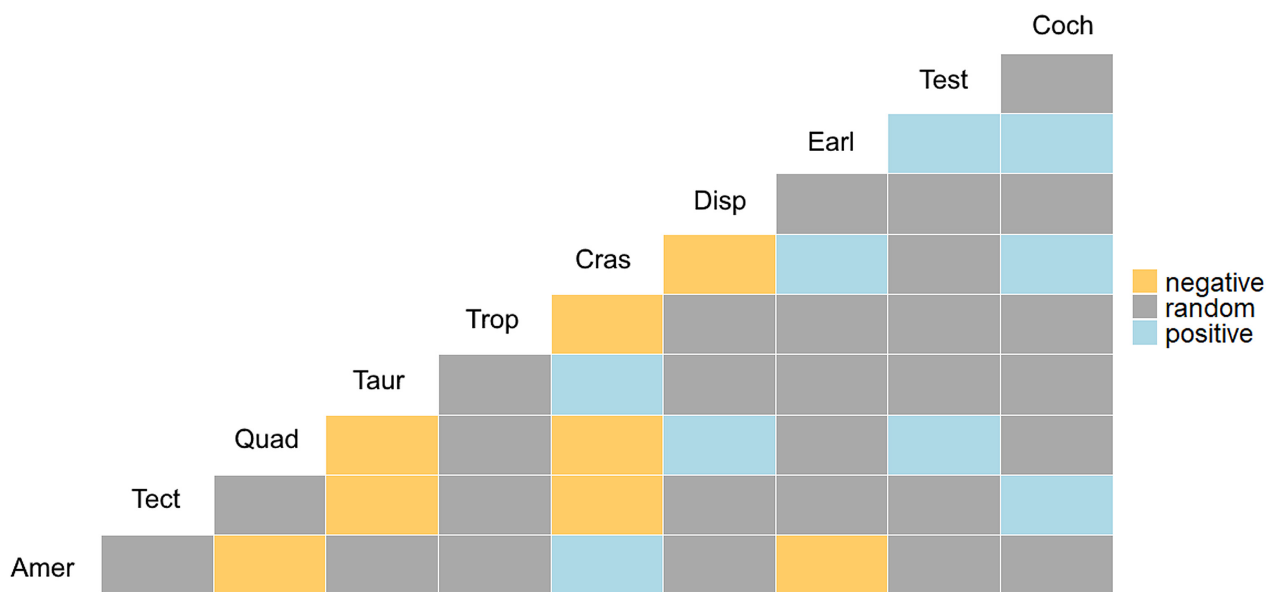
FIGURE 4. Co-occurrence matrix for ten *Keratella* species. All other taxon pair combinations showed no significant negative or positive co-occurrence correlations. (Abbreviations: Amer = *K. americana*, Tect = *K. tecta*, Quad = *K. quadrata*, Taur = *K. taurocephala*, Trop = *K. tropica*, Cras = *K. crassa,* Disp = *K. quadrata dispersa*, Earl = *K. earlinae*, Test = *K. testudo*, Coch = *K. cochlearis*)

Discussion

Rotifers are subject to passive dispersal (Rivas *et al*. 2018). As such, the argument has been made that rotifers make

poor subjects for biogeographical studies (Pejler 1995). However global-scale studies and reviews have determined that some species do appear to be endemic to certain regions (Dumont 1983, Segers & DeSmet 2008). Of the 16 species of *Keratella* found in this study, some are considered to be endemic to the Nearctic region (*K. crassa*, *K. earlinae*, *K. taurocephala*, and *K. americana*), while others have extended ranges in the Holarctic region (*K. hiemalis*, *K. mixta*, and *K. ticinensis*); still others appear to be truly cosmopolitan and have been found in lakes in all, or nearly all regions of the globe (*K. cochlearis*, *K. quadrata*, *K. tecta*, *K. tropica, K. valga*, and *K. serrulata*) (Segers & DeSmet 2008). Like Segers & DeSmet (2008), we observed few instances of cosmopolitan species over a broad scale, albeit our study was confined to a single country. *Keratella cochlearis*, *K. tecta* and *K. earlinae* were widely distributed within the U.S., indicating that these species may be eurytopic. For such taxa with apparent ubiquity, it is believed that distributions are determined primarily by local environmental factors and preferences for certain habitat conditions.

For *K. cochlearis*, the most common and widespread species of *Keratella*, several studies have shown that individual populations display cyclomorphic features adapted to changing environmental conditions, such as temperature (Green 2005) or presence of predatory species (Stemberger & Gilbert 1984). In this study, the idea that *K. cochlearis* can be successful in a diversity of habitats was supported by frequent observation of *K. cochlearis* across nine different ecoregions and in all continental states in the U.S. However, *K. cochlearis* also likely represents a cryptic species complex (Derry *et al.* 2003, Cieplinski *et al.* 2017), for which DNA sequencing is required to determine an unknown number of morphologically identical species. Rotifers as a group likely have a high incidence of cryptic speciation. High rates of passive transport combined with production of dormant propagules can lead to widespread distributions of organisms that adapt quickly to their local environment (i.e. high dispersal rates but restricted gene-flow between neighboring populations) (De Meester *et al.* 2002). Cryptic species occupying the same niche may also co-exist in the same lake, surviving via alternating phases of population reduction due to energy investment in sexual reproduction or rapid parthenogenic population growth (Montero-Pau & Serra 2011). The notion that morphologically similar species can constitute a cryptic species complex has been supported by the recent discovery of 15 cryptic species of *Brachionus plicatilis* Mueller, 1786 (Mills *et al.* 2016). For the *B. plicatilis* species complex, it was found that genetically distinct species exhibit slightly different environmental preferences and confined geographical ranges. This may also be the case with *K. cochlearis*, which could help to explain its apparent widespread distribution across a diversity of lakes from nine ecoregions. Given that cryptic speciation is a possibility for *K. cochlearis*, apparent environmental drivers of distribution can only be speculative.

Other *Keratella* species identified from the NLA showed clear biogeographical distributions. *Keratella americana* was found primarily in the eastern portion of the U.S. and was confined (with one exception) to latitudes below 45.3 °N. Although *K. americana* is thought to be endemic to the United States (Segers & DeSmet 2008), it has become established in southeast Asia (Segers 2001) and the River Murray, Australia (Wedderburn *et al.* 2017), indicating that it has the potential to invade and adapt to new environments outside of its native range. Niche centroid analysis showed that *K. americana* exhibited higher biomass at increased temperatures, which is consistent with the observation of this species primarily in lower latitudes. *Keratella quadrata* showed a near-opposite distribution pattern to *K. americana*, with observations primarily in the western portion of the U.S. and at higher latitudes. Although both *K. americana* and *K. quadrata* showed an affinity for high turbidity systems, their differing temperature optima may allow them to occupy a similar niche in different geographic regions. While no clear separation of these two species among ecoregions was observed, their differential distributions in the U.S. lend support to the idea that distinct biogeographical ranges exist for individual rotifer species.

Co-occurrence analysis indicated that differing biogeographical ranges might prevent co-occurrence of some species pairs (i.e., the negative co-occurrence correlation between *K. americana* and *K. quadrata*). In contrast, overlap in biogeographical range does not necessarily indicate significant co-occurrence. Species that occur within the same ecoregion showed significant negative co-occurrences, indicating that those species may have incongruent preferences for environmental conditions on a more localized scale. For example, *K. taurocephala* overlaps in range with *K. quadrata* in the Pacific Northwest region (Western Mountains ecoregion) of the U.S., but the two species exhibit opposing pH optima. Similarly, both *K. crassa* and *K. taurocephala* are negatively correlated with *K. tecta*, despite geographic overlap. In this case, it is likely that trophic status of the lake influences the prevalence of certain species over others, as *K. tecta* showed higher optima for eutrophic indicator variables including turbidity, total nitrogen, total phosphorus and chlorophyll-a.

Both *K. crassa* and *K. taurocephala* showed a preference for lakes with lower nutrients and chlorophyll and

greater water clarity: these parameters are considered indicative of more oligotrophic conditions. Both of these species were frequently observed in the northeastern region of the U.S. (Northern Appalachian ecoregion). The positive correlation of co-occurrence between *K. crassa* and *K. taurocephala* supports the perception that these species inhabit oligotrophic lakes. However, *K. taurocephala* may have an advantage over *K. crassa* in more acidic environments, as it has been shown to outcompete other *Keratella* species under increased acidity (Frost *et al*. 1998). In addition to the positive correlation with *K. taurocephala*, *K. crassa* also showed positive associations with *K. cochlearis*, *K. earlinae*, and *K. americana*, none of which displayed an obvious preference for water quality conditions. These patterns indicate that *K. crassa* may occupy a separate niche from other *Keratella* species, such as variation in grazing efficiency (Bogdan & Gilbert 1987) or timing in reproductive cycles (Magnien & Gilbert 1983). These characteristics would allow it to coexist with other species as long as water quality conditions are suitable. For rarer species (i.e., *K. hiemalis, K mixta, K. serrulata, K. testudo, K. quadrata dispersa, K. tropica aspina, K. tropica, K. ticinensis*, and *K. valga*), it is difficult to draw conclusions about the extent to which geography or water quality restricts distributional patterns, although observations from this survey suggest that both *K. ticinensis* and *K. testudo* appear to be confined to the mountainous western U.S.

Unexplored factors including interspecific competition among rotifers of different genera, trophic level interactions and land use characteristics may have significant influences on shaping both the *Keratella* community and the rotifer community as a whole. Unfortunately, this survey represents only a fraction of all lakes within the U.S., the majority of which were sampled only once. Thus, species present in the U.S. may have been missed in lakes that were not sampled or in lakes sampled outside of peak productivity. Additionally, it has been acknowledged that net filtration may be inefficient at capturing all zooplankton species compared with other sampling methods such as sedimentation and quantitative sampling (Bottrell *et al*. 1976). Species in littoral areas of lakes were also excluded from this study. Genetic investigation into a potential cryptic species complex (*K. cochlearis*) was not performed although it is likely that several cryptic species of *K. cochlearis* exist within the U.S. Despite these limitations, results of this analysis provide a useful resource for comparison in future studies on *Keratella* and rotifer biogeography, and the maps in this study may be referenced as current distribution patterns for individual species of *Keratella* within the conterminous U.S.

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