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4 **Feeding, survival, and reproduction of two populations of *Eurytemora* (Copepoda)**

5 **exposed to local toxic cyanobacteria**

6

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25

26 Abbreviated running title: Effects of cyanobacteria on *Eurytemora*

27 **Abstract**

28 Understanding lower food web interactions in the Laurentian Great Lakes can be furthered by
29 experimental comparisons among locations with similar ecological stresses, such as harmful
30 algal blooms. Here we compare responses to toxic cyanobacteria by crustacean copepods of
31 the genus *Eurytemora* from eutrophic coastal regions of Lake Michigan and the Baltic Sea.
32 We measured grazing, survivorship, reproduction, and juvenile (nauplius) size, incubating
33 females in experimental treatments holding good food and mixtures of good food with either
34 cyanobacteria or cyanobacteria filtrate. Animals tested were from Green Bay, Lake Michigan
35 and Gulf of Finland, Baltic Sea. Results showed similarities between copepods in the two
36 study locations; when fed mixtures of cyanobacteria and good food there were no effects in
37 either location on survivorship, grazing rates, or fecundity, but copepods in both sites were
38 most sensitive to good food combined with cyanobacteria filtrate (in absence of
39 cyanobacteria cells). Filtrate exposure significantly reduced grazing by animals in both
40 locations and decreased adult survival and nauplius size in the Baltic experiment, suggesting
41 animals responded to toxins or other compounds entering the water. These responses may be
42 due to direct effects on females or indirect effects from changes in good food quality. Our
43 results also demonstrated a significant trade-off between offspring quantity and quality, being
44 more pronounced when food quality was manipulated by the presence of cyanobacteria cells.
45 These findings further our knowledge of how a widely distributed group like *Eurytemora* can
46 succeed in the face of changing local selection pressures from natural and anthropogenic
47 stressors.

48

49 **Key words**

50 *Microcystis*, *Nodularia*, HAB, feeding, survival, cyanotoxin

51

52 **Introduction**

53 The expansion and persistence of cyanobacterial blooms are increasing globally due to
54 human-induced eutrophication (Paerl and Otten, 2013). Such eutrophication of coastal areas
55 is occurring in diverse locations including the Laurentian Great Lakes (Binding et al., 2015),
56 Lake Baikal (Timoshkin et al., 2016), the Baltic Sea (Andersen et al., 2017) and estuaries
57 worldwide (Bricker et al., 2008). Climate change, acting via global warming and changes in
58 factors such as precipitation, salinity and pH, further promote the development and frequency
59 of cyanobacteria mass-occurrences (O'Neil et al., 2012). Effects of cyanobacterial blooms on
60 lower food web interactions are especially important due to the intimate connections between
61 zooplankton and phytoplankton (Ger et al., 2014). Cyanobacteria have traditionally been
62 considered low quality food for zooplankton due to low manageability, minor nutritional
63 quality, and toxin content (Porter and Orcutt, 1980; Lampert, 1987; DeMott and Moxter,
64 1991). A large meta-analysis of published studies on the effects of cyanobacteria on
65 zooplankton population growth and survivorship reaffirmed the importance of nutritional
66 effects, but also highlighted the lack of work examining how fitness of copepods is affected
67 by cyanobacteria (Wilson et al., 2006). More recent work has focused on key traits that
68 zooplankton rely on to improve their fitness, including physiological tolerance (such as
69 detoxification pathways), and avoidance of poor quality food by selective feeding behaviors.
70 Such characteristics allow calanoid copepods to co-exist with toxic cyanobacteria, and even
71 become more tolerant of blooms over time through local selection and adaptation (Ger et al.,
72 2014, 2016).

73 Work on cyanobacteria-zooplankton interactions studying the calanoid copepod
74 *Eurytemora affinis* and toxic algae indicate contrasting results (Ger et al., 2016). Previous
75 studies have shown low egg production and survival when copepods are fed toxic
76 cyanobacteria (Koski et al., 1999; Kozlowsky-Suzuki et al., 2003). However, Schmidt and

77 Jónasdóttir (1997) observed that while the cyanobacteria *Microcystis aeruginosa* was
78 inadequate as a single food source due to poor nutritional quality, it was beneficial in small
79 doses as a supplement to the main diatom food source *Thalassiosira weissflogii*. Vehmaa et
80 al. (2013) also observed a positive effect of the cyanobacterium *Nodularia spumigena* on
81 reproduction of copepods when provided with a mixed diet. The main negative effects of
82 toxic cyanobacteria on zooplankton are expected to arise from direct dietary exposure, such
83 as ingestion of toxic cells and poor nutritional impacts (Ger et al., 2014). Dissolved toxins
84 derived from cyanobacteria during bloom conditions, such as the hepatotoxin microcystin
85 produced by *M. aeruginosa* and many other species, are not considered a main threat for
86 copepods like *E. affinis* because typical field concentrations of microcystin are orders of
87 magnitude below lab-determined lethal levels (Ger et al., 2009, 2016). Additional studies
88 with *E. affinis* also suggest that toxicity may not be the most important factor governing
89 zooplankton-cyanobacteria interactions. For instance, when availability of good food is
90 reduced *Eurytemora* are able to feed more readily on toxic *Nodularia spumigena* despite high
91 amounts of dissolved toxins (Gorokhova and Engström-Öst, 2009). However, in addition to
92 toxins, cyanobacteria produce other compounds during blooms that may have more direct
93 negative effects on zooplankton (Ger et al., 2016). Also, morphological traits such as
94 filament or colony size, and history of co-occurrence are important factors, as smaller
95 filaments and long co-evolutionary history may favor feeding on toxic strains when good
96 food is scarce. As predicted by optimal foraging theory, selective feeders like copepods
97 should discriminate more strongly against low-quality algae when food concentrations are
98 high (cf., DeMott 1989). These findings stress the need to further investigate effects of toxic
99 algae on copepods, such as those in the *Eurytemora* group.
100 *Eurytemora affinis* is a common species in coastal areas, estuaries, and marshes of the
101 Northern Hemisphere where it is a major link in the lower food web, feeding on both algae

102 and toxic cyanobacteria (Engström et al., 2000) and providing food for young fish (Ger et al.,
103 2010). It also expanded into freshwater habitats such as the Laurentian Great Lakes during
104 the middle of the 20th century (Lee, 1999), and recent studies even consider clades in the
105 Great Lakes a separate species (*E. carolleeae*; Alekseev and Souissi, 2011). Lee et al. (2013)
106 demonstrated that high food availability was likely a central factor allowing *E. affinis* to
107 spread from brackish water to freshwater areas, and research in the St. Lawrence estuary
108 where both invasive and non-invasive clades coexist indicate that feeding behaviors differ
109 among the clades (Favier and Winkler, 2014; Cabrol et al., 2015). Studying the feeding
110 ecology of invasive copepods like *Eurytemora* can provide insight into how zooplankton
111 might respond to new feeding conditions following range expansion into novel habitats.
112 Local adaptation of *Eurytemora* to feeding on phytoplankton in eutrophic regions
113 involves responses to toxic cyanobacteria blooms, a major result of eutrophication. Both
114 study areas in the current work, Green Bay (Lake Michigan, USA) and the Gulf of Finland
115 (Baltic Sea), suffer from annual cyanobacterial mass-occurrences. The main source of toxic
116 blooms in the Gulf of Finland is a filamentous species, *Nodularia spumigena*, producing a
117 hepatotoxin called nodularin (Suikkanen et al., 2010). In Green Bay the dominant
118 cyanobacteria species is now *Microcystis aeruginosa*, which can grow either as solitary cells
119 or colonies. It is now common in many Great Lakes regions, producing microcystin, a
120 hepatotoxin closely related to nodularin (Mur et al., 1999; De Stasio et al., 2014). Comparing
121 the responses of *Eurytemora* to these local cyanobacteria and their toxins can help determine
122 potential changes in food web interactions in the face of increasing eutrophication.
123 The aim of this study was to compare the effects of toxic cyanobacteria on *Eurytemora*
124 from Green Bay and the Baltic Sea using a comparable experimental design in parallel
125 experiments. We examined survivorship, feeding, egg production, and size of nauplii
126 produced by animals from the two sites in response to manipulation of representative species

127 of toxic cyanobacteria, dissolved substances produced by the cyanobacteria and high quality
128 algal food similar to local food resources. Given the known sensitivity of *Eurytemora* to
129 cyanobacteria in the native range we hypothesized that feeding, survivorship, and egg
130 production would be negatively affected by the presence of toxic cyanobacteria. We also
131 expected there would be separate effects of extracellular toxins and other metabolites released
132 by cyanobacteria distinguishable from nutritional effects due directly to feeding on the
133 cyanobacteria cells. Furthermore, because of the relatively recent invasion of the Great Lakes
134 by *Eurytemora* and the short time period of exposure to freshwater cyanobacteria species, we
135 expected animals from Green Bay to be more sensitive to toxic cyanobacteria due to the
136 briefer history of selection under local environmental conditions.
137

138 **Methods**

139 *Study sites*

140 Green Bay, Lake Michigan is the largest freshwater estuary of the Laurentian Great Lakes,
141 and exhibits high primary productivity and strong physical and trophic gradients (Richman et
142 al., 1984; De Stasio et al., 2008). In the shallow southern region there is no persistent
143 stratification due to frequent mixing (Qualls et al., 2007). The southern bay is also influenced
144 by high nutrient and sediment loading entering from the lower Fox River resulting in
145 considerable primary productivity throughout the summer months (Stoermer, 1978; LaBuhn
146 and Klump, 2016). As a result, Green Bay suffers from nuisance phytoplankton blooms. In
147 addition, the phytoplankton community in the bay has shifted to increased dominance by
148 cyanobacteria (*Anabaena, Aphanizomenon, Microcystis*) following a zebra mussel invasion
149 (De Stasio et al., 2014). Concentrations of the hepatotoxin microcystin typically range from
150 0.3 to 1.7 µg/L (McDermott et al., 1995; B. De Stasio, unpublished data) in the southern bay.

151 The Gulf of Finland is the most eastern region of the Baltic Sea and is fairly shallow
152 (average depth 38 m), with limited vertical mixing due to slow water exchange. This creates
153 horizontal gradients of salinity (surface: 0.2 - 5.8) and temperature (summer SST: 15 - 17 ºC)
154 (Myrberg et al., 2006; Suikkanen et al., 2013). The Gulf of Finland is predominantly nitrogen
155 limited during the productive season (spring and late summer), which allows intensive
156 nuisance blooms, mainly of non-toxic N-fixing *Aphanizomenon* sp. and hepatotoxic
157 *Nodularia spumigena.* Concentrations of nodularin, the main toxin produced by *Nodularia*,
158 typically vary between 0.5 and 2.6 μg/L (Kankaanpää et al., 2001). Cyanobacteria blooms in
159 the Baltic Sea have been common since at least the 19th century, but have increased due to
160 human-induced impacts (Bianchi et al., 2000).
161
162 *Study species*
163 *Eurytemora affinis* is native to northern temperate coastal areas around the world but was
164 introduced to North American freshwater systems and first documented in 1880 (Lee, 1999).
165 It was later reported in Lake Michigan by Robertson (1966), and since the late 1960s the
166 species is found in both littoral areas and pelagic plankton communities in Green Bay
167 (Gannon, 1974). The species complex has spread multiple times independently from brackish
168 water to freshwater, and established clades in different parts of the world due to its ability to
169 adapt locally (Dodson et al., 2010). There are at least six recognized clades of *E. affinis*; one
170 clade in Asia, one in Europe, and four in America (Lee, 2000), with recent studies indicating
171 that North American clades represent a separate species (*E. carolleeae*; Alekseev and Souissi,
172 2011; Vasquez et al., 2016). *Eurytemora* in Lake Michigan waters is most common between
173 July and November, being rare in winter and spring (Torke, 2001). The work by Vasquez et
174 al. (2016) has shown that all previous samples from the Great Lakes (including from Green
175 Bay) were *E. carolleeae* and we assume that all animals employed in our Green Bay

176 experiment were *E. carolleeae* resulting from the freshwater invasion of the Great Lakes
177 during the late 1950s (Lee, 1999; Lee et al., 2013).
178 In the Baltic Sea *E. affinis* has its abundance peak in June and July (Viitasalo, 1992)
179 and is native to the system (Lee, 1999). *E. affinis* occurs both in pelagic and coastal areas in
180 the Baltic Sea (Viitasalo, 1992). There is also evidence of recent invasions into the Baltic Sea
181 by clades from North America (Sukhikh et al., 2013). It is unknown if the *Eurytemora* we
182 collected at the Tvärminne Zoological Station were exclusively *E. affinis*, but because *E.*
183 *carolleeae* were found in other areas of the Gulf of Finland we refer to animals used in our
184 Baltic Sea experiment as *Eurytemora* sp.

Insert Table 1 near here

185 *Experimental Design*
186 We compared the response of *Eurytemora* to toxic cyanobacteria by conducting parallel
187 experiments with a common design but using animals from either the Baltic Sea or Green
188 Bay (Table 1). Food treatments were created so that all animals received the same quantity
189 (500 µg C/L) of a food source representative of local algae and of sufficient quality to
190 provide good growth and survivorship conditions during the experiment. This concentration
191 was intended to keep food resources above limiting levels, resulting in maximum ingestion
192 rates on good food. In the "good food" treatment (GF) this was the only source of nutrition.
193 The "cyanobacteria" treatment (CYAN) contained the good food and additional cultured
194 toxic cyanobacteria to achieve external toxin concentrations similar to local conditions. In the
195 Baltic experiment this amounted to adding 100 µg C/L of *Nodularia spumigena* and for
196 Green Bay 50 µg C/L of *Microcystis aeruginosa* (Table 1; see below for cyanobacteria strain
197 information). The third treatment received the same amount of good food plus a volume of
198 filtrate from the cyanobacteria source equivalent to the volume of cyanobacteria solution
199 added in the CYAN treatment. Consequently the CYAN and FILT treatments differed only

200 in the presence or absence of cyanobacteria cells, and the GF and FILT treatments differed
201 only in extracellular components added with the cyanobacteria cells.
202 *Strain cultivation*
203 Four different phytoplankton species were used as food sources in the experiments: the
204 cryptophyte *Rhodomonas salina* and the toxic cyanobacterium *Nodularia spumigena* in the
205 Baltic Sea, and the chlorophyte *Scenedesmus quadricauda* and the toxic cyanobacterium
206 *Microcystis aeruginosa* in Green Bay. In the Baltic Sea experiment the *Rhodomonas salina*
207 culture (Cryptophyceae; 07B6; obtained from Dr. Anke Kremp, Finnish Environment
208 Institute) was grown using f/2 medium at 18°C in ~10 μmol photons/m^2/s with a 16 : 8 h light
209 : dark regime and a salinity of 6‰ in aged seawater. The *Nodularia spumigena* culture (strain
210 AV1, a potent nodularin producer) was obtained from Prof. Kaarina Sivonen, University of
211 Helsinki and grown in Z8-N nutrient solution (Sivonen et al., 1989) with modified salinity
212 (6‰) at 18°C in a 16 : 8 h light : dark regime. Cell counts for initial culture concentrations in
213 the Baltic Sea experiments were performed using the Ütermöhl method with transect counts
214 using an eyepiece micrometer. *Nodularia* filament lengths (64.04 ± 5.86 μm, mean ± SE)
215 were converted into cell density, while individual cell counts were determined directly for
216 *Rhodomonas* (length: 19.8 ± 0.61 μm). To determine food concentration in μg C/L, cell
217 densities were converted with biovolume estimates and carbon conversion factors
218 (Montagnes et al., 1994; Olenina et al., 2006). Appropriate volumes of algae and
219 cyanobacteria from exponentially growing cultures were added to each treatment bottle with
220 FSW, filtered seawater (0.2 μm pore size, Sartobran 300 filters, Sartorius Stedim Biotech
221 GmbH. Göttingen, Germany) to obtain desired initial concentrations of food (used within
222 ~24h). Filtrate from the *Nodularia* cultures for use in the experiment was obtained by
223 filtering (GF/C, Whatman, nominal pore size 1.2 μm) the appropriate amount of culture.

224 For Green Bay experiments both cultures were grown in freshwater media and were
225 initiated from batch cultures. The *S. quadricauda* culture (Carolina Biological Supply,
226 Burlington, NC) received Bristols solution made with Milli-Q water (125 mL/L) to optimize
227 growth rates. The toxic *Microcystis aeruginosa* strain (PCC 7820; Pasteur Institute, Paris,
228 France) was grown in Cyanobacteria BG-11 Freshwater Solution (C3061; Sigma Chemical,
229 St. Louis, MO) at a concentration of 20 mL/L in Milli-Q water. The *S. quadricauda* culture
230 was kept gently aerated and maintained under exponential growth conditions at room
231 temperature (20°C) in direct sunlight near a window. The *M. aeruginosa* also was kept at
232 room temperature in moderate, but indirect, sunlight and stirred gently on a shaker table.
233 *Microcystis* grew in this culture as a mixture of single and bicells. Prior to use in the
234 experiment each culture was centrifuged (3000 rpm, 30 min) to separate cells from growth
235 medium, which can be toxic to animals in high concentrations. Pelleted cells were
236 resuspended in filtered lake water (GF/C, Whatman) and then enumerated for use in the
237 experiment. To determine the amount of food to be added in the treatments, cell densities of
238 algal and cyanobacteria cultures were estimated with a hemocytometer counting chamber and
239 converted to µg C/L based on mean cell size (*Scenedesmus*: 12.7 um, *Microcystis*: 4.1 um)
240 and biovolume estimates obtained at 400X and a carbon content conversion factor of 0.2 pg
241 C/μm^3 (Reynolds, 1984; Rocha and Duncan, 1985). Filtrate from *Microcystis* cultures for use
242 in the experiment was obtained by filtering (GF/C, Whatman) the appropriate amount of
243 resuspended culture.
244
245 *Field sampling*
246 In the Baltic Sea experiment, seawater was collected at 5 m depth off the Tvärminne
247 Zoological Station at the entrance to the Gulf of Finland (N 59.8556°, E 23.2617°) using a
248 Limnos water sampler (Hydrobios, Germany). Immediately after collection, the seawater

249 sample was filtered (0.2 µm pore size, Sartobran 300 filters) and allocated into treatment
250 bottles, stored at 17°C, and used within 24 h of preparation.
251 *Eurytemora sp.* was sampled over a three-day period (3-6 August 2012) from 25 m depth
252 with four vertical tows, using a 200-µm mesh net (0.485 m diameter) with cod-end collection
253 cup. Samples were gently transferred to a 40-L container with seawater from below the
254 thermocline, and immediately transported to a temperature climate chamber with 16 : 8 h
255 light : dark cycle. Sorting of tow samples took place upon return to the laboratory and desired
256 number of adults were placed in treatment bottles with filtered seawater, inoculated with the
257 treatment algal conditions and stored at 17°C.
258 Surface water was collected from Green Bay at Little Sturgeon Bay, a small
259 embayment along the southeast shore of the bay (N 44.8452º, W 87.5584º), and transferred
260 via carboy to the lab where it was double filtered (Whatman #1 qualitative filter followed by
261 Whatman GF/C filter). Filtered water was stored at room temperature in the laboratory and
262 used within ~24h. Animals were collected between sunset and midnight on 1 October 2013
263 by horizontal tows between the surface and ~3 m depth near a boat dock, using a 250-µm
264 mesh net (0.5 m diameter) with cod-end collection cup. Samples were transferred to 6-L
265 buckets containing Little Sturgeon Bay water, transported on ice to the lab, and stored at 17ºC
266 with 16 : 8 h light : dark cycle. *Eurytemora* were transferred individually to beakers of
267 filtered lake water containing treatment food conditions.
268
269 *Baltic experiments*
270 *Grazing* - Copepods were subjected to the three treatments, consisting of different mixtures
271 of laboratory cultures of *Nodularia* and *Rhodomonas* (Table 1): 1) Good Food (GF)
272 consisting of only *Rhodomonas*, 2) Cyanobacteria addition (CYAN) with *Rhodomonas* and
273 *Nodularia,* 3) Filtrate addition (FILT) containing *Rhodomonas* and filtrate from the

274 *Nodularia* culture (corresponding to the volume of *Nodularia* added in CYAN). Volumes of
275 algae and cyanobacteria cultures added to the treatments were determined through cell counts
276 of each culture as described above under *Strain cultivation*.

277 Adult *Eurytemora* sp. females carrying egg sacs were sorted from samples and then
278 acclimated in each treatment condition for 60 h. Treatment solutions were made fresh and
279 replaced daily. Following the acclimation period male *Eurytemora* sp. were sorted from fresh
280 samples and added to bottles with the females that had dropped their egg sacs during the
281 acclimation period. This ensured that new eggs were produced by females acclimated to each
282 treatment condition. Each treatment had three replicates with *Eurytemora* sp. (12 females
283 and 3 males per bottle) and three control replicates without animals, carried out in 1.2 L
284 Pyrex glass flasks with a screw cap. This number of animals ensured sufficient feeding to
285 allow measurement of grazing rates without excessive depletion of food. Males were
286 included to fertilize females so reproduction rate and offspring size could be determined.
287 Closed bottles were incubated in a 17°C climate chamber and gently mixed by inversion
288 twice during the experiment to reduce settling of food.

289 Replicate samples of initial conditions were collected from each bottle for chlorophyll *a*,
290 cell counts and toxin concentration (see below) before addition of copepods. The experiment
291 was run for 24 h, after which final samples were collected. Container contents were then
292 gently filtered through a 63-µm mesh cup to check for adult survivorship, number of females
293 carrying egg sacs, and nauplii produced. Changes in chlorophyll concentration during the
294 experiment were used to determine ingestion rates (µg C/individual/h) according to the
295 standard procedures of Frost (1972) and assuming a 50:1 carbon conversion from chlorophyll
296 (Reynolds 1984).

297 *Reproduction* - Females carrying egg sacs after the completion of the grazing experiment
298 (GF:23, CYAN:24, FILT:21) were transferred into individual wells of 12-well tissue culture

299 plates containing the same treatment solution. Tray contents were checked daily and after all
300 eggs in a plate had hatched, acid Lugol's solution was added to preserve nauplii for later
301 counting and measurement. Individual females were assessed for number of eggs produced to
302 determine egg ratios. Fecundity was estimated as egg production rate (eggs/female/day) by
303 dividing egg ratios by egg development time (in days) according to temperature of the
304 experiment using the relationship from Andersen and Nielsen (1997). Development time was
305 1.92 days at the experimental temperature of 17°C. Lengths of first stage nauplii from each
306 replicate (10 minimum) were measured at 100 X magnification under an inverted microscope
307 with an eyepiece micrometer.
308
309 *Green Bay experiments*
310 *Grazing* - Food manipulation in the Green Bay experiment included analogous treatment
311 conditions as employed in the Baltic Sea experiment but using species more representative of
312 local conditions (Table 1): 1) Good food (GF) consisting of *Scenedesmus*, 2) Cyanobacteria
313 addition (CYAN) including *Scenedesmus* and *Microcystis*, and 3) Cyanobacteria culture
314 filtrate addition (FILT) with *Scenedesmus* and filtrate corresponding to the same volume of
315 *Microcystis* culture added in CYAN. *Scenedesmus* concentration added was kept constant in
316 all treatments and equal to that used in the Baltic Sea experiment (500 µg C/L). Volume of
317 *Microcystis* added was intended to achieve similar toxin concentrations as in the Baltic Sea
318 experiments, resulting in an increased carbon content of 10% compared to that from
319 *Scenedesmus*.
320 Female *E. carolleeae* carrying egg sacs were acclimated to the experimental treatment
321 conditions for 41 h. Females without eggs sacs at the end of the acclimation period were
322 moved to triplicate beakers (250 mL) containing fresh treatment conditions. Triplicate
323 beakers without animals served as controls. Each beaker with *Eurytemora* contained 12-14

324 females to achieve measurable feeding rates but not deplete food resources during the
325 experiment. Beakers were held in a 15°C incubator with no light and covered with a sheet of
326 plexiglass to reduce evaporation. Beakers were gently stirred twice during the experiment to
327 reduce settling of algae. During the 22-h grazing experiment survivorship was also recorded.
328 At the end of the grazing experiment, all copepods were removed by gently filtering beaker
329 contents through a mesh cup (128-µm mesh), counting animals and preserving them in
330 formalin (4% buffered). Initial and final samples were taken to assess chlorophyll *a*, cell
331 densities, and toxin concentrations. Cell densities were determined using the same counting
332 procedures employed for quantifying cell cultures and conversion to carbon units (see *Strain*
333 *Cultivation*). Ingestion rates (µg C/individual/h) were calculated according to the standard
334 procedures of Frost (1972) and carbon conversion as employed in the Baltic experiment.
335 A separate experiment to determine background starvation rates of *Eurytemora* from
336 Green Bay was conducted along with the grazing experiment. Twelve female *E. carolleeae*
337 previously acclimated to GF conditions as above were placed into separate wells of a tissue
338 culture plate holding 4.5 mL of filtered water (Whatman GF/C). Animals were held at 20°C
339 and checked at 12 h intervals for five days. Water was replaced with freshly filtered water
340 and fraction of females alive was determined at each time point.
341
342 *Reproduction* - Fifty females with egg sacs were placed in 250-mL beakers containing the
343 same three treatment conditions as above and acclimated for 40 h at 15°C in constant dark.
344 At the end of the acclimation period females that were not carrying egg sacs were combined
345 in new beakers with males (mean of 7 males per beaker). Three days after the start of the
346 reproduction experiment, females carrying egg sacs (GF:10, CYAN:7, FILT:18) were placed
347 in separate covered petri dishes (35 mm diameter) with fresh treatment food solutions
348 changed daily until all eggs hatched. Individual females were assessed for number of eggs

349 produced to determine egg ratios. Fecundity was estimated as egg production rate
350 (eggs/female/day) by dividing egg ratios by egg development time (in days) as described
351 above for the Baltic Sea experiment. For the GB experiment, development time was 2.19
352 days at 15°C. Hatched nauplii were counted daily for each female. After all nauplii hatched,
353 the sample was preserved with acid Lugol's solution for later analysis. Nauplius length
354 measurements were conducted at 100 X magnification on a inverted microscope with an
355 eyepiece micrometer.
356
357 *Analytical procedures*
358 Samples for chlorophyll analysis were filtered onto GF/C Whatman filters and frozen at -
359 20ºC until measurement. For the Baltic Sea experiments, samples were extracted in ethanol
360 according to Jespersen and Christoffersen (1987) and analyzed using a Shimadzu UV-2501
361 PC spectrophotometer. In the Green Bay experiment samples were extracted using alkalized
362 acetone according to Wetzel and Likens (1991) and analyzed using a scanning
363 spectrophotometer (Cary Model 50) with 50 mm path-length cuvette.
364 Samples for extracellular toxins in filtrates were collected from water passed through
365 a GF/C Whatman filter and stored at -20ºC. Toxin concentrations for both experiments were
366 analyzed by ELISA, using a microcystin plate kit (EnviroLogix, Portland, ME, USA),
367 according to standard kit instructions. This ELISA kit measures both microcystin and
368 nodularin (cf. Gorokhova and Engström-Öst, 2009); consequently, data for both experiments
369 are reported as microcystin-LR equivalents. Each experiment only contained one kind of
370 toxic algae, so ELISA data represent concentrations of toxin specific to each type of
371 cyanobacteria employed (i.e., nodularin or microcystin).
372
373 *Statistical analyses*

374 Data distributions were examined for normality prior to statistical analysis with the
375 Paleontological Statistics (PAST) software package (Hammer et al., 2001). Transformations
376 were successfully applied to some data to meet normality expectations; percentage data were
377 arcsine transformed before analysis whereas LOG or square root transformations were
378 employed for others, followed by back-transformation before reporting. Analysis of Variance
379 (ANOVA) and Tukey-Kramer multiple comparison tests were used when data met parametric
380 considerations. If data were not normally distributed following transformation original data
381 were tested with non-parametric exact permutation tests (9,999 permutations) or Kruskal-
382 Wallis and Mann-Whitney tests with Bonferroni corrections. Relationships of nauplius size
383 and fecundity were examined with linear regression analysis, followed by a Generalized
384 Linear model using the *G* statistic test of slopes equaling zero. Significance was assumed for
385 all tests if *p* values were 0.05 or smaller.

386

387 **Results**

388 As intended, our manipulations of diets exposed animals from both populations to
389 similar amounts of extracellular toxins (Table 1). There was no significant difference in toxin
390 levels, all measured as microcystin-LR, between CYAN or FILT treatments in the Baltic
391 experiment or the Green Bay FILT treatment. Concentrations of nodularin in the Baltic Sea
392 experiment were approximately 0.75 µg/L of microcystin-LR equivalents, while mean
393 microcystin-LR toxin level in the GB FILT treatment was 0.80 µg/L. Toxin in the Green Bay
394 CYAN treatment was slightly lower at 0.52 µg/L, and significantly different than in the other
395 CYAN or FILT treatments (Table 1). Toxin levels in the GF treatments were below detection
396 limits in both experiments, and consequently there was an overall significant effect of
397 treatment on toxin concentrations ($F_{3,14}$=12.08, p=0.00004).

398 *Baltic Sea Experiments*

Insert Figure 1 near here

399 Survivorship varied among feeding treatments in the Baltic Sea experiment (Fig. 1A).
400 Survival was high for copepods that fed on the algae *Rhodomonas* in the GF treatment (mean
401 =94.2%). Supplementing food with the cyanobacteria *Nodularia* in the CYAN treatment did
402 not significantly alter survivorship (Q=0.29, df=6, p=0.978) and the 95% confidence interval
403 of both GF and CYAN include 100% survivorship. Animals in the FILT treatment, which
404 were fed good food but also exposed to cyanobacterium culture filtrate, had significantly
405 lower survivorship compared to animals in GF or CYAN treatments with an average of 40%
406 of the animals surviving (GF comparison: Q=4.95, p=0.03; CYAN comparison: Q=5.24,
407 p=0.02). This survivorship rate is lower than the expected rate of 87% following starvation
408 conditions based on previously published data for this population (Koski et al., 1999).

409 Animals in the Baltic Sea experiment seemed varied their feeding depending on the
410 food treatment (Fig. 1B). Mean ingestion rate was highest when feeding on only good food
411 (GF mean =0.53 μg C/copepod/h). Mean rate for the CYAN treatment was lower than for
412 GF, and animals in the FILT treatment essentially did not feed (Fig. 1B). There was no
413 significant difference between the GF and CYAN ingestion rates (t-test exact permutation
414 test: p=0.30) but ingestion in FILT was significantly lower than in GF (p=0.05).

415 Manipulation of feeding conditions did not significantly affect reproductive output of
416 *Eurytemora* sp. from the Baltic Sea (Fig. 1C; $F_{2,64}$=1.41, p=0.252). Females in the GF
417 treatment produced an average of 5.55 eggs/female/day during the experiment. Fecundity of
418 animals in the CYAN treatment appeared decreased compared to the other two treatments,
419 but variability within treatments was high leading to non-significant differences overall.

420 Mean size of first stage nauplii was significantly decreased by the feeding treatments
421 (Fig. 1D; $F_{2,304}$=32.75, p<0.0001). Nauplii in the GF treatment averaged 0.145 mm and were
422 significantly larger than those in CYAN (mean=0.137 mm; Q=4.51, p=0.004). The smallest

423 nauplii occurred in the FILT treatment with a mean of 0.125 mm, significantly smaller than
424 the other treatments (GF comparison: Q=11.88, p<0.0001; CYAN comparison: Q=7.36,
425 p<0.0001).

Insert Figure 2 near here

426 *Green Bay Experiments*

427 *Eurytemora* from Green Bay survived well in the feeding experiments, with no significant
428 differences among the treatments (Fig. 2A; $F_{2,6}$=1.34, p=0.331). Over 94.2% of animals
429 survived on average in the GF treatment, while survival in the CYAN treatment exhibited a
430 mean of 89.4%. In the FILT treatment animals survived at a rate of over 92.7%. In
431 comparison to these values, animals in the separate starvation experiment exhibited a mean
432 survival rate of 85.1% (SE= 0.9%) for an equal period of time. Overall, starvation rates were
433 linearly related to time with a mortality rate of 0.68% per hour (SE = 0.06%; R^2 = 0.977, t= -
434 11.25, p = 0.002).

435 Animals ingested significantly different amounts of carbon depending on the
436 treatment manipulations (Fig. 2B; $F_{2,6}$=13.5, p=0.006). Mean ingestion in GF conditions was
437 0.79 µg C/copepod/h, not significantly different than the mean of 1.08 in the CYAN
438 treatment (Q=4.91, p=0.312). When exposed to filtrate from the cyanobacterium culture
439 (FILT) copepods showed significantly reduced ingestion rates with a mean of 0.30 µg
440 C/copepod/h (p<0.05 for both comparisons).

441 Similar to results from the Baltic Sea experiment, *E. carolleeae* from Green Bay did
442 not exhibit different egg production based on feeding treatments (Fig. 2C; $F_{2,32}$=0.62,
443 p=0.546). Mean fecundity values ranged from 5.02 (FILT) to 6.32 eggs/female/day (CYAN)
444 with animals in the GF treatment producing an average of 5.15 eggs/female/day. These
445 fecundity values were essentially the same as those obtained in the Baltic Sea experiment for
446 GF and FILT treatments (see Fig. 1C).

447 In contrast to the Baltic Sea results, nauplius size was not affected significantly in the
448 Green Bay experiment (Fig. 2D; $F_{2,113}$=0.24, p=0.791). Mean nauplius length was
449 approximately 0.09 mm for all feeding treatments. Interestingly, mean length of nauplii in the
450 Green Bay GF (Fig. 2D; 0.090 mm) was significantly smaller than mean length in GF of the
451 Baltic Sea experiment (Fig. 1D; 0.145 mm; t=31.738, df=172, p<0.0001). These differences
452 in nauplius length in GF reflected the trend for adult body length between the two
453 populations. Adult female *E. carolleeae* from Green Bay were significantly smaller (t=13.16,
454 df=75, p<0.0001) than the *Eurytemora* sp. used in the Baltic Sea experiment (mean prosome
455 length +/- 1 SE: GB =0.780 +/- 0.008 mm; Baltic= 0.950 +/- 0.010 mm).

456 *Comparison of nauplius length and Eurytemora fecundity*

457 Significant negative relationships between average size of nauplii and number of eggs
458 produced occurred in both experiments (Fig. 3). Slope of the linear relationship for Baltic Sea
459 copepods in GF conditions was -0.0015 (SE=0.0004) and was significantly less than zero
460 (G=12.54, p=0.0004). Slope for animals fed *Nodularia* in the CYAN treatment was even
461 more negative (mean= -0.0023, SE= 0.0009) and was also significantly lower than zero
462 (G=6.13, p=0.013). In the FILT conditions there was no significant relationship (Fig. 3A). A
463 similar result was obtained in the Green Bay experiment, but only the relationship for the
464 CYAN treatment was significant (Fig. 3B; G=8.04 p=0.004) with a slope of -0.001
465 (SE=0.0004).

Insert Figure 3 near here

466

467 **Discussion**

468 There has been conflicting evidence reported in studies of copepod feeding on cyanobacteria
469 concerning the separate effects of dissolved toxins and other compounds produced during
470 bloom conditions compared to nutritional impacts of ingested cells. We have shown that

471 substances dissolved in filtrates negatively affected the copepod *Eurytemora* from both the
472 Baltic Sea and Green Bay. Ingestion rates for both populations were significantly reduced in
473 the FILT treatment, where filtrate was added to good food, compared to feeding just on good
474 food. In addition, these effects were reduced if animals were fed a mixed diet of good food
475 and cyanobacteria. In addition, both populations exhibited a trade-off between offspring size
476 and reproductive rates when feeding on the mixed diets of cyanobacteria and good food.
477 Animals from the two locations responded differently in terms of other traits
478 measured. *Eurytemora* sp. from the Baltic Sea exhibited decreased survivorship and smaller
479 nauplius length in the FILT treatment whereas animals from Green Bay did not. Overall,
480 *Eurytemora carolleeae* from Green Bay was not more sensitive to the effects of
481 cyanobacteria than *Eurytemora* sp. from the Baltic Sea despite having invaded this freshwater
482 estuary relatively recently.
483 *Grazing*
484 Ingestion rates were significantly reduced for both populations of *Eurytemora* in the FILT
485 treatments compared to good food treatments (Fig. 1B and Fig. 2B). In the Green Bay feeding
486 experiment, ingestion by *E. carolleeae* was also significantly lower in FILT compared with
487 the treatment with good food and cyanobacteria cells together (CYAN). While copepods in
488 the FILT treatment had only the good food available as a source of nutrition, they were
489 simultaneously exposed to cell-free filtrate of toxic cyanobacteria. Although there were no
490 differences in quantity of good food available in the FILT treatments, *Eurytemora* could have
491 been indirectly affected via changes in good food cell quality caused by the cyanobacterial
492 filtrate, thereby leading to decreased ingestion by the copepod. A similar phenomenon has
493 been documented in other studies. Cell-free filtrates of the haptophyte *Prymnesium* were
494 shown to indirectly affect ingestion and growth of the rotifer *Brachionus* via negative effects
495 of filtrate on the cryptophyte *Rhodomonas* (Barreiro et al., 2005). Similarly, Suikkanen et al.

496 (2006) showed that the algae *Rhodomonas* was affected by *Nodularia* filtrate, but not
497 specifically by purified nodularin.
498 Another possible explanation for the decreased ingestion rates in FILT is that
499 *Eurytemora* changed its feeding behavior due to toxins or other secondary metabolites
500 released from cyanobacteria cells into the filtrate solution (Barreiro et al., 2005; Sopanen et
501 al., 2008). This is a plausible explanation as survivorship and nauplius size also were
502 decreased in the Baltic Sea FILT treatment. Similarly, female *Acartia* sp. showed decreased
503 condition factors during post-bloom conditions in the Baltic Sea (Engström-Öst et al., 2015),
504 suggesting that disrupting blooms (comparable to what occurred in our filtrate preparation)
505 can be harmful. Many copepods are considered selective feeders and able to continue feeding
506 on good food in the presence of toxins (DeMott and Moxter, 1991; Ger et al., 2016), but other
507 crustacean zooplankton often stop feeding (Ger et al., 2014). While some populations of
508 *Eurytemora* have been shown to tolerate microcystin (Ger et al., 2009, 2010, 2014) and
509 perhaps the closely related nodularin (Engström-Öst et al., 2002), it does not mean that
510 feeding in our experiments decreased due to toxin exposure, per se. Other secondary
511 metabolites may be likely to have caused the effects observed in the FILT treatments. Even
512 though cyanobacteria toxins are studied a great deal (recent reviews by Rastogi et al., 2014;
513 Pearson et al., 2016), other metabolites have attracted far less attention, and many of them are
514 not even known to science. Rapid progress is being made on characterizing these substances
515 (Mazard et al., 2016), but this area still needs further research.
516 In the Green Bay experiment, although the copepods did feed quite actively on
517 *Microcystis* in the CYAN treatment (approximately 40% of total ingestion; data not shown),
518 they showed high survival and reproduction in this particular treatment, suggesting they
519 benefitted from this food mixture despite the presence of toxins and other extracellular
520 components in solution (cf. Vehmaa et al., 2013; Hogfors et al., 2014). Similarly, in the

521 Baltic Sea experiment there was no significant difference between ingestion rates of
522 *Eurytemora* sp. provided with just good food or with the mixed diet. This finding that
523 *Eurytemora* sp. can utilize *Nodularia* is consistent with previous studies showing that Baltic
524 *Eurytemora* actively ingests *N. spumigena* (Engström-Öst et al., 2002, 2011) as well as
525 studies showing that calanoid copepods can manipulate and feed on filaments that are
526 relatively straight (Vanderploeg et al., 1998), like the *Nodularia* used in our experiment.
527 *Survival*
528 *Eurytemora* from the two populations exhibited different survivorship responses to FILT
529 treatments. The Baltic Sea population survived well when feeding on the mixed diet of
530 *Rhodomonas* and *Nodularia* (Fig. 1A). This result is supported by Reinikainen et al. (2002)
531 who also found negligible effects of *Nodularia* or nodularin on *E. affinis* survival. On the
532 other hand, in the Baltic experiment, *Eurytemora* survivorship was significantly reduced in
533 the treatment with *Rhodomonas* and *Nodularia* filtrate. This survivorship rate was much
534 lower than rates expected based on starvation conditions previously determined for this same
535 population (Koski et al. 1999) indicating that something in the filtrate caused increased
536 mortality. This result is consistent with data presented in Sopanen et al. (2008) where
537 stronger negative effects were observed in filtrate than in mixtures when 'good' and 'bad'
538 food were present. Given the likelihood that our *Nodularia* filaments were disrupted
539 following filtration and intracellular contents entered the filtrate treatment, *Eurytemora* may
540 have responded strongly (i.e., with increased mortality) to either released toxin or other
541 metabolites (cf. Sopanen et al., 2008). Decreased condition of the animals most likely also
542 had consequences for feeding rates, as evidenced by decreased ingestion in the filtrate
543 treatment.
544 Survivorship in the Green Bay experiment was not significantly altered between the
545 treatments. This suggests that this population of *Eurytemora* is tolerant to toxic

546 cyanobacteria, consistent with an earlier study showing this population feeds well on late
547 summer phytoplankton from this location (Richman et al., 1980). Ger et al. (2010) also found
548 no changes in mortality of *Eurytemora* exposed to toxic *Microcystis*, consistent with our
549 results. This tolerance to toxic algae is likely related to previous exposure to *Microcystis*,
550 possibly leading to decreased sensitivity to dissolved microcystin (Sarnelle and Wilson, 2005;
551 Ger et al. 2016). A large meta-analysis by Wilson et al. (2006) found only marginal effects of
552 microcystins on zooplankton survival in general. This documented tolerance to *Microcystis*
553 by the Green Bay population could indicate that there has been strong selection for this
554 tolerance since the relatively recent invasion of the system a little more than 50 years ago.
555 However, it is also possible that tolerance to the effects of *Microcystis* is more general than
556 expected and already existed in the invasive clade before it invaded the Great Lakes. The
557 close similarity and evolutionary origin of nodularin and microcystin also supports this
558 possibility (Mur et al., 1999). Further studies comparing differences among *Eurytemora*
559 clades in tolerance to toxic cyanobacteria would be useful, as would studies of the evolution
560 of tolerance within populations.
561 *Reproductive output*
562 In the present study, female *Eurytemora* produced eggs at approximately the same rate in
563 both study areas (~5 eggs/female/day; Fig. 1C and 2C), but no differences were detected
564 between treatments in either of the experiments. Our results are consistent with those of
565 Sopanen et al. (2008) who found no changes in egg production rates of *Eurytemora* in either
566 algal mixtures containing toxic *Prymnesium parvum*, or in cell-free filtrates. On the other
567 hand, Nejstgaard and Solberg (1996) reported that toxins excreted by *Prymnesium* decrease
568 egg production of a common copepod (*Acartia clausi*), which did not prey upon *Prymnesium*.
569 The most plausible reason for *Eurytemora* continuing to produce eggs during low quality
570 food conditions is that females allocated energy reserves to offspring production during low

571 or unfavorable feeding conditions, which could have long-term consequences for their
572 condition or survival (as perhaps was observed in the Baltic FILT treatment). Ger and co-
573 authors (2009) show that *E. affinis* egg production was not affected by the ingestion of
574 *Microcystis*, but was highly dependent on the abundance of high quality food available (in
575 that case *Cryptomonas*) for sustaining egg production rates. Such findings suggest animals
576 can exhibit changes in energy allocation depending on feeding conditions.

577 *Trade-offs in egg production*

578 In the data presented here, nauplius length was negatively correlated with female egg
579 production rate for both populations examined, with the strongest effects observed when
580 animals were fed the mixed diets (Fig. 3, CYAN treatments). A reasonable body of evidence
581 supports a trade-off between offspring size and number, where increasing offspring quality is
582 constrained by the number of offspring produced (Roff, 1992), which our findings support.
583 Size is commonly considered a good indicator of offspring quality in ecological studies (Roff,
584 1992). In our experiment with animals from the Baltic Sea, nauplius size differed
585 significantly between treatments, decreasing when animals were fed either the mixture of
586 'good food' and cyanobacteria, or 'good food' and cyanobacteria filtrate. These differences
587 indicate negative maternal effects of the cyanobacteria treatment on offspring size because
588 lengths were determined for offspring in the first naupliar stage, which is a non-feeding stage.
589 Contrary to the current study, Vehmaa et al. (2013) revealed a positive trade-off between egg
590 quantity and quality for *Acartia* in the Baltic Sea; she and her coauthors showed that females
591 were able to allocate more resources to eggs when feeding on mixtures of good food and 20%
592 *Nodularia*, increasing both quality and viability of eggs. However, the good food source in
593 that experiment (*Brachiomonas*) was of lower nutritional quality than the *Rhodomonas*
594 employed in our experiments, and *Nodularia* therefore became an important nutritional
595 supplement.

596 In the Green Bay experiment, nauplius size was also negatively related to number of
597 eggs produced, but only significantly so for the CYAN treatment (Fig. 3B). This relationship
598 was observed even though there were no significant effects on ingestion rates, fecundity, or
599 mean nauplius size for this treatment, possibly indicating the independent nature of this trait
600 from the others measured. It is interesting that this same negative relationship was obtained
601 even though Green Bay *E. carolleeae* on average produced smaller offspring than the
602 *Eurytemora* sp. from the Baltic Sea. The largest nauplii produced in Green Bay are barely the
603 size of the smallest nauplii from the Baltic Sea. This size difference is consistent with adult
604 size differences between the two populations. Females from Green Bay were significantly
605 smaller (mean prosome length 0.78 mm) than females from the Gulf of Finland (on average
606 0.95 mm). It is unclear what causes these size differences, but temperature has been shown
607 generally to have a strong negative effect on copepod body size (Brun et al., 2016; Horne et
608 al., 2016), and specifically on *E. affinis* in the Baltic Sea (Viitasalo et al., 1995). Brun et al.
609 (2016) show that productivity and size selective predation have more complex relationships
610 with copepod body size, varying both between species and on a local scale. Further research
611 on factors affecting body size and other traits important for survival of copepods in the Great
612 Lakes would be fruitful in light of these findings.
613
614 *Conclusion*
615 To conclude, despite using what are now considered different species of *Eurytemora* and
616 different local cyanobacteria, both populations responded similarly to the food manipulations
617 in terms of feeding. There were significant negative effects on ingestion rates of the filtrate
618 derived from cyanobacteria cultures, and those effects were reduced when animals were fed a
619 mixed diet. Both populations also exhibited some degree of tolerance to toxic cyanobacteria
620 as survivorship and egg production were unaffected when animals were fed the mixed diets.

621 In addition, a negative relationship between nauplius length and fecundity of females was
622 documented for both groups. However, effects on survivorship and nauplius size were
623 different between the populations tested. Baltic *Eurytemora* sp. survival was significantly
624 reduced in the cyanobacteria filtrate treatment, and smaller nauplii were produced, likely a
625 result of a maternal effect due to reduced ingestion by mothers and/or lower allocation to egg
626 production. These traits of Green Bay animals were not significantly affected by food
627 treatments.

628 Tolerance to abiotic factors may facilitate dispersal success (Dodson et al., 2010;
629 Hirsch et al., 2016), suggesting that tolerance to cyanobacteria could also promote the
630 invasion of *Eurytemora* to new ecosystems, as seen in Lake Michigan. Lee et al. (2013)
631 showed that high food availability and tolerance to lower salinity also promote invasions by
632 *Eurytemora* to new systems. In Green Bay, *Eurytemora* may be forced to remain in dense
633 blooms because the system is eutrophic and shallower (De Stasio et al., 2008), whereas in the
634 Gulf of Finland, zooplankton can escape blooms by migrating deep (Almén et al., 2014). The
635 use of lab-cultured food sources may limit the generality of our results, and caution must be
636 exercised in drawing conclusions about how these two populations would respond to a more
637 natural assemblage of food resources. Even given these limitations, our results are consistent
638 with the conclusion that *E. carolleeae* in Green Bay may be more resistant to blooms and
639 cyanobacteria toxicity than *Eurytemora* sp. from the Baltic Sea. The Green Bay population
640 likely has undergone local adaptation following selection based on a number of novel
641 conditions during its introduction and subsequent persistence in the Laurentian Great Lakes.
642 Their tolerance to the cyanobacteria tested indicates the potential for future population
643 expansion as blooms increase in the Great Lakes, or for successful secondary dispersal to
644 inland eutrophic lakes where blooms occur.

645

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655

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874 laboratory experiments. Limnol. Oceanogr. 51, 1915-1924.

875 Table 1. Details of experiments on effects of toxic cyanobacteria on *Eurytemora*. Animals
876 were collected from the Baltic Sea in August 2012 or Green Bay, Lake Michigan in October
877 2013. Both experiments included treatments with good food (GF), good food plus
878 cyanobacteria (CYAN), or good food plus a volume of filtrate from cyanobacteria cultures
879 equivalent to the volume of cyanobacteria culture added (FILT). Baltic animals were fed
880 *Rhodomonas salina* (Rhod) and *Nodularia spumigena* (Nod) whereas Green Bay animals
881 were fed *Scenedesmus quadricauda* (Scen) and *Microcystis aeruginosa* (Mic). Mean
882 extracellular toxin concentration was measured as microcystin-LR equivalents (1 SE in
883 parentheses). Lower limit of toxin detection was 0.16 µg/L. Results of Tukey-Kramer
884 multiple comparison tests of toxin concentrations are indicated; values followed by the same
885 letter are not significantly different at the p=0.05 level.

886

			Treatment		
Location	Date	Treatment Conditions	GF	CYAN	FILT
Baltic Sea	Aug 2012	Rhod (µg C/L)	500	500	500
		Nod (µg C/L)	0	100	0
		Nod Filtrate (equiv. vol.; µg C/L)	---	---	100
		Replicates (n)	3	3	3
		Toxin (µg/L)	<0.16	0.75 (0.06)a	0.77 (0.06)a
Green Bay	Oct 2013	Scen (µg C/L)	500	500	500
		Mic (µg C/L)	0	50	0
		Mic Filtrate (equiv. vol.; µg C/L)	---	---	50
		Replicates (n)	3	3	3
		Toxin (µg/L)	<0.16	0.52 (0.02)b	0.80 (0.04)a

887

888 **Figure Captions**

889 Figure 1. Baltic Sea *Eurytemora* feeding experiment results for animals fed *Rhodomonas*
890 (GF), *Rhodomonas* and *Nodularia* (CYAN) or *Rhodomonas* with filtrate from *Nodularia*
891 cultures (FILT) during 2012. A) Mean percent survivorship of animals during experiment,
892 B) Mean ingestion rates, C) Mean fecundity as eggs produced/female/day, and D) Mean
893 nauplius length. Error bars represent ±1 standard error of the mean.

894

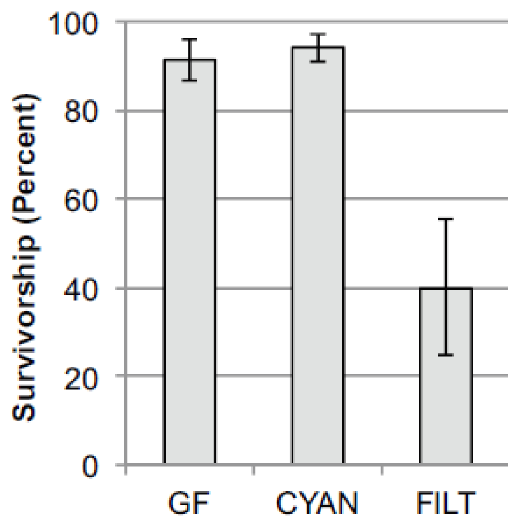
895 Figure 2. Green Bay *Eurytemora* feeding experiment results for animals fed *Scenedesmus*
896 (GF), *Scenedesmus* and *Microcystis* (CYAN) or *Scenedesmus* with filtrate from *Microcystis*
897 cultures (FILT) during 2013. A) Mean percent survivorship of animals during experiment,
898 B) Mean ingestion rates, C) Mean fecundity as eggs produced/female/day, and D) Mean
899 nauplius length. Error bars represent ±1 standard error of the mean.

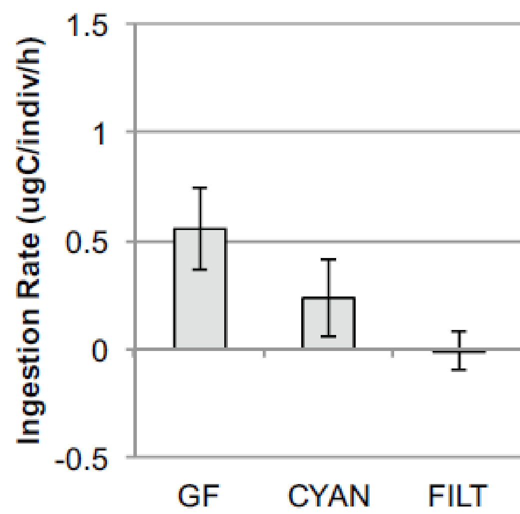
900

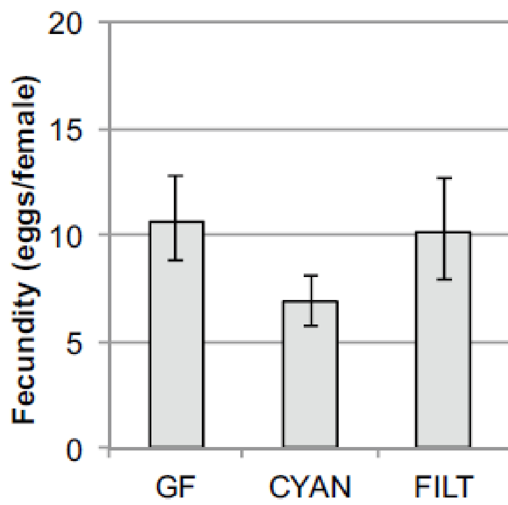
901 Figure 3. Relationships between nauplius length (mm) and fecundity (eggs
902 produced/female/day) for *Eurytemora* in A) Baltic Sea experiment in 2012 and B) Green Bay
903 experiment in 2013. Separate regressions are plotted for animals in good food (GF), good
904 food and cyanobacteria (CYAN), and good food with cyanobacteria filtrate (FILT)
905 treatments. Note differences in nauplius length axes between experiments. See text for
906 regression statistics.

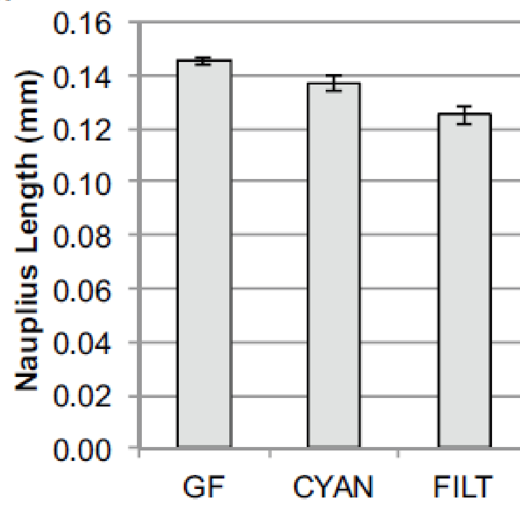
907

908 Fig. 1.









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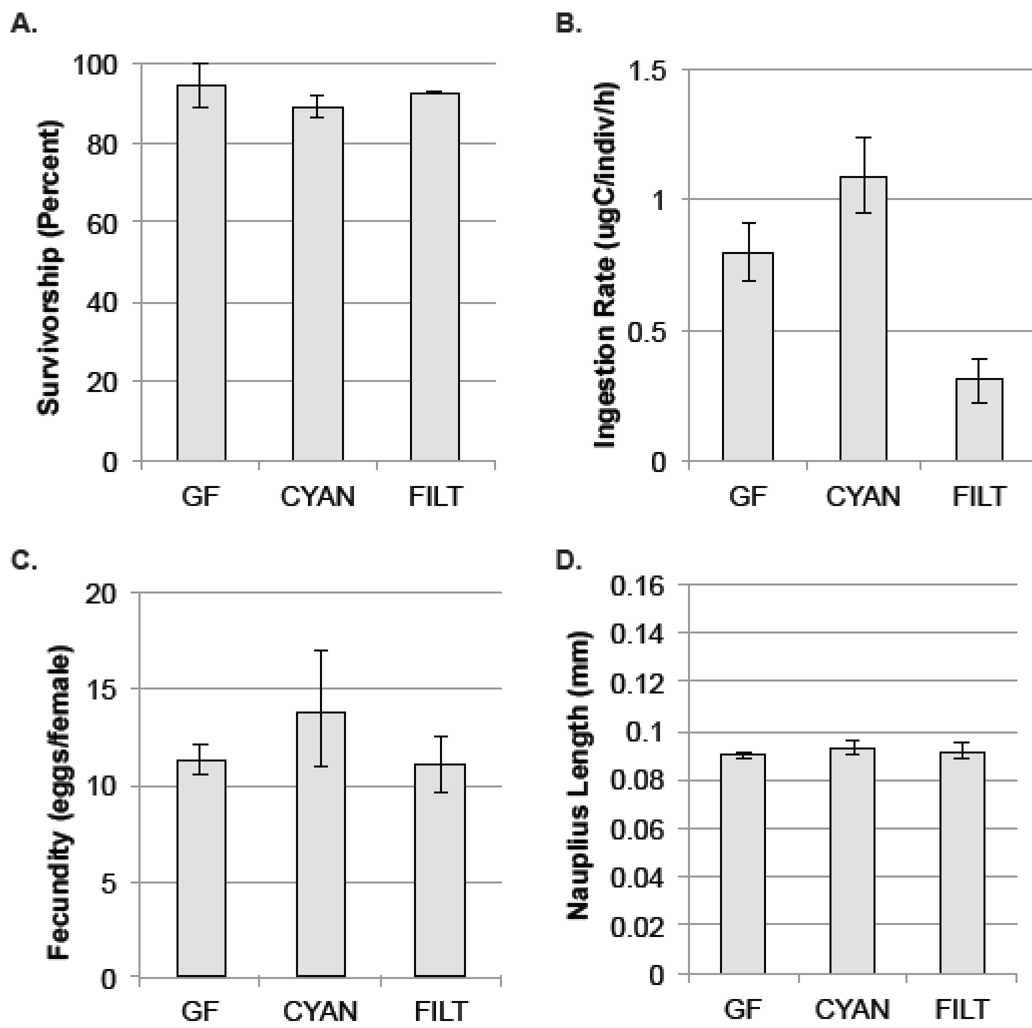
911 Fig. 2.



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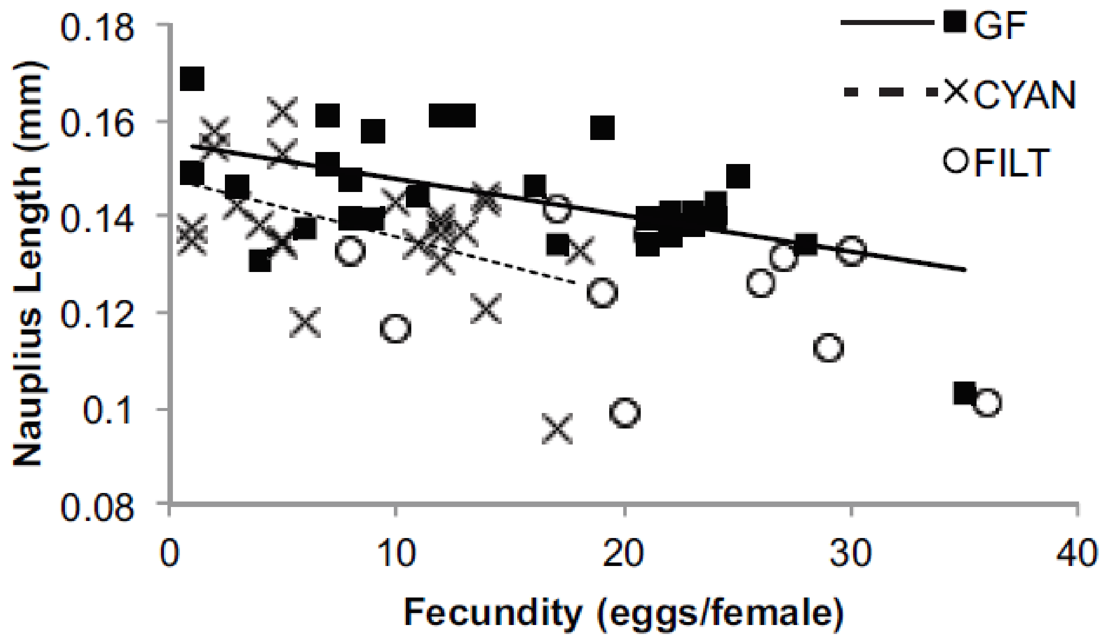
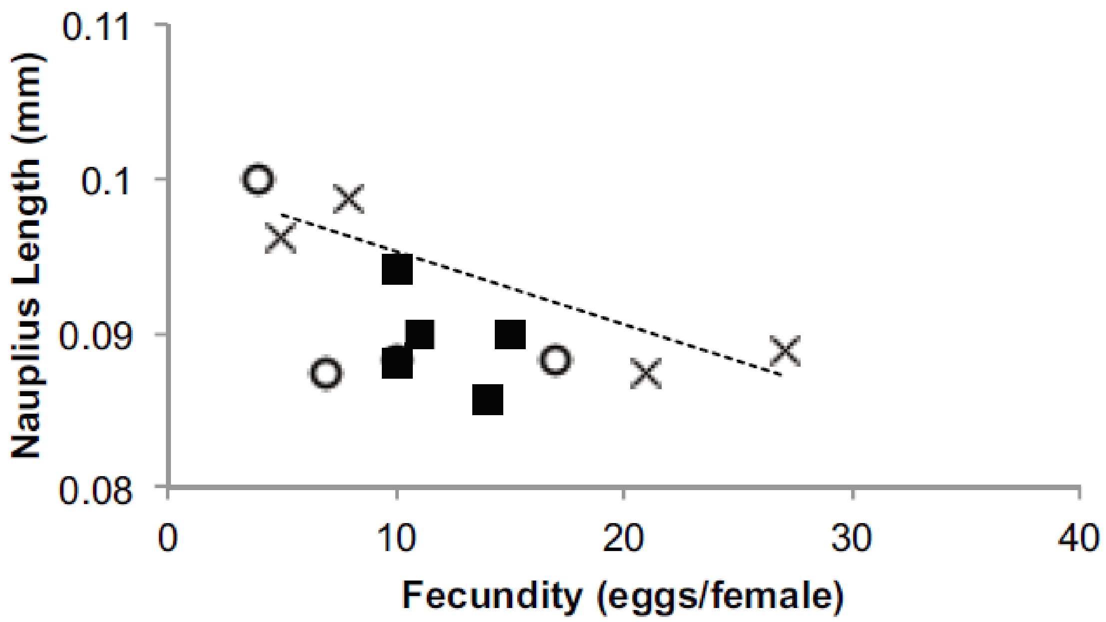
914 Fig. 3.





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