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SHALLOW LAKES RESEARCH

Predation by *Acanthocyclops americanus* (Copepoda: Cyclopoida) in the hypertrophic shallow waterbody, Lake Albufera (Spain): field and laboratory observations

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Abstract We quantified the predation of *Acanthocyclops americanus* from the shallow Mediterranean lake Albufera, using gut contents from field collections and laboratory feeding tests. For functional response studies, we used *Brachionus plicatilis* (at 6 concentrations, 400–4000 ind. 40 ml^{-1}) and *Diaphanosoma mongolianum* (at 2–20 ind. 40 ml^{-1}). Copepod feeding rates were also estimated using different proportions of rotifer prey and lake seston (0–67.5% of seston + 40 individuals of *B. plicatilis*). Prey selection studies were conducted using five zooplankton species: *Brachionus angularis, Brachionus plicatilis, Keratella tropica, Daphnia magna* and *Diaphanosoma mongolianum*. Gut contents of field-collected adult *Acanthocyclops* contained filamentous algae and cyanobacteria and 16 zooplankton species (*Keratella cochlearis*, unspined and spined forms, *K. tropica, Brachionus plicatilis, Brachionus calyciflorus, Brachionus angularis, Brachionus variabilis, Asplanchna girodi, Polyarthra vulgaris, Synchaeta pectinata, Lepadella rhomboides,* unidentified bdelloids, *Alona rectangula, Chydorus sphaericus, Bosmina longirostris, D. magna, Ceriodaphnia dubia* and copepod nauplii). When fed *B. plicatilis* or *D. mongolianum*, female *A. americanus* had higher prey consumption rates than males. Increased proportion of lake seston caused reduced consumption of brachionid prey. Our data suggest that *A. americanus* is omnivorous in nature.

Keywords Predation · Copepoda · Phytoplankton · Zooplankton · Gut contents · Functional response

Introduction

Predation is one of the most significant forces in structuring freshwater plankton communities (Gliwicz, 2003). Many groups of invertebrates such as cyclopoid copepods (Gliwicz & Umana, 1994; Brandl, 2005), cnidarians (e.g., *Hydra*: Link & Keen, 1995), and rotifers (e.g. *Asplanchna*: Sarma & Nandini, 2007) feed on plankton. Rotifers and cladocerans are common prey items for most genera of cyclopoids during the adult stages (Roche, 1987; Garcia-Chicote et al.,

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Maria Rosa Miracle—Deceased.

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2007). Although there exists a positive relationship between the size of the copepods and their prey size (Roche, 1990), many cyclopoids are capable of feeding on a wide range of prey size (Williamson, 1983). For example, members of Cyclopinae and Eucyclopinae with a body size of 2000 µm are known to kill and feed on prey items an order of magnitude larger than their biomass (e.g., large daphniids, insect larvae etc.: Dieng et al., 2003) and as small as 1/100th of their body size (smaller ciliates and rotifers: Brandl, 2005).

Generally, *Acanthocyclops, Eucyclops*, and *Mesocyclops* are among the most voracious predators of rotifers and cladocerans (Hołyńska et al., 2003; Reid & Williamson, 2009). For example, *Mesocyclops* is capable of killing and consuming as many as 70 individuals of rotifer prey per hour per predator (Sarma et al., 2013). Cyclopoid copepods are known to exert significant predation pressure on rotifers in some Mediterranean waterbodies (Lapesa et al., 2002). There are also some reports of invasive copepods in these waterbodies (Frisch et al., 2006). *Acanthocyclops* is widely distributed in Spanish waterbodies including the Lake Albufera, a shallow water lagoon in the Gulf of Valencia (Miracle et al., 2013). Cyclopoid predation, primarily by the genus *Acanthocyclops* is a crucial biotic factor affecting the abundance and diversity of cladocerans and rotifers in this water body (Oltra et al., 2001). However, quantitative studies on the impact of *Acanthocyclops* on zooplankton are not available. Seasonal studies on zooplankton from the Lake Albufera also revealed a correlation between cladoceran abundance and the fertility of *Acanthocyclops* (Miracle et al., 2013) suggesting that cladocerans and possibly rotifers form an important part of the diet of this predatory copepod (Brandl, 2005; Hopp & Maier, 2005). Information on the gut contents of field-collected *Acanthocyclops* through seasons from this water body is, however, not available.

Most works from literature reveal sporadic collections of zooplankton and/or gut content analysis of copepods only during certain months of the year (Gophen, 1977). Thus, it becomes difficult to infer the predation pressure by copepods on their nauplii and other zooplankton through seasons (Boersma et al., 2014). Most carnivorous adult cyclopoids consume microalgae in addition to zooplankton (Kiørboe, 2011a, b). This omnivory of cyclopoids is partly explained by the feeding interference by colonial or filamentous algae and/or higher densities of single algal cells which enter the gut of copepods through their prey items (Seckbach, 2007). Laboratory predation experiments do not involve the use of algae along with the zooplankton as prey for copepods; even if such tests are conducted, only one algal species is used or the quantity of algae consumed is rarely quantified (Sarma et al., 2013). Therefore, the role of alternative diets in copepod gut satiation is not evident from laboratory tests. Field studies complement this aspect.

The quantity of prey consumed by a predator is a function of the quantity of food available in the medium (Case, 2000). Most predatory copepod show increased consumption with increasing prey availability until an asymptote is reached; beyond this, the increase in prey density does not result in higher consumption. This response, Holling’s type II curve, is often documented for cyclopoids (Enríquez-García et al., 2013). The prey density at the asymptote varies among different genera and species of cyclopoids (Monakov, 2003). Prey size has a significant influence on the nature of the asymptote. Usually, for smaller prey items such as rotifers, higher numbers are needed to satiate a predatory copepod as compared to larger prey such as daphniids (Sarma et al., 2013). However, this aspect requires more investigation for different taxa of copepods, including *Acanthocyclops*.

Prey selectivity is yet another important ecological variable affecting survival and reproduction of copepods. It is known that copepods, in general, avoid feeding on toxic or nutritionally inadequate diet including cyanobacteria and gelatinous zooplankton such as *Asplanchna* (Rollwagen-Bollens et al., 2013; Heuschele & Selander, 2014), but select cladocerans and loricate rotifers (Enríquez-García et al., 2013). Although cyclopoids capture and ingest large items, for some prey species including *Daphnia* and *Diaphanosoma*, only fragments (e.g., postabdomen) are found in the gut. Therefore, identification of prey up to species level in the gut can be uncertain and hinders the use of prey selectivity indexes.

Female cyclopoid density in nature is generally higher than that of males. This is possibly because experimental studies on the lifespan suggest that males live less than females (García et al., 2011). However, this is not the only factor to explain the skewed ratio of male and female copepods in nature (Kiørboe, 2011b). The quantity of prey consumed by males and females also differs. A few published works have shown that females consume higher prey biomass than males (see Hirst & Kiørboe, 2014). It is not known if this holds true for *Acanthocyclops*.

In this study, we quantified the seasonal changes in the gut contents of *Acanthocyclops americanus* from Albufera and the prey (rotifers and cladocerans) consumption by males and females of this predatory copepod through functional response and prey selectivity experiments. We hypothesized that females would consume more prey than the males and that they would feed on small (< 500 µm)- rather than large (> 1000 µm)-sized prey in the field.

Materials and methods

Field collections

Lake Albufera is hypertrophic shallow waterbody on the Mediterranean coast of Spain near Valencia. The main characteristics of the lake can be found elsewhere (Miracle et al., 2013). Phytoplankton is dominated by cyanobacteria (Onandia et al., 2014) and *Acanthocyclops americanus* is the dominant zooplankton species during the whole year (Oltra & Miracle, 1984, 1992; Oltra et al., 2001). Monthly zooplankton samples were collected from Albufera for one full year (Dec. 2010–Nov. 2011) from each of 4 sites of the waterbody. Quantitative samples were collected by filtering the contents of a 2.7 l Ruttner bottle through 25-µm nytal filter. Samples were immediately fixed in 4% neutral formalin, and all individuals of zooplankton were later counted using an inverted microscope.

The gut content analysis of the predatory copepod *Acanthocyclops americanus* was carried out to estimate prey density and diversity. For this, we separated 15 adult females of *A. americanus* from the zooplankton net collection and carefully dissected each one under a stereomicroscope. Later, prey items from the gut contents were identified, as far as possible, to species level and quantified using a compound microscope. Based on the zooplankton identified from the gut contents and from the quantitative plankton samples, the prey selectivity indices were derived using Manly's alpha: α r_i/n_i $(1/\Sigma(r_j/n_j))$, where α_i is Manly's alpha for prey type i; r_i, r_j are proportions of prey type i or j in the diet (i and $j = 1, 2, 3 \ldots m$); n_i, n_j are the proportions of prey type i or j in the environment; m is the number of prey species tested. When $\alpha_i = 1/m$ feeding is not selective; $\alpha_i > 1/m$, then prey species i is preferred in the diet, and $\alpha_i < 1/m$, prey species i is avoided in the diet (Krebs, 1999).

Laboratory experiments

The prey species as well as the predators were collected from the Albufera Lake kept in lake water and used within 1 or 2 days in the experiments. Collections were made from 12 to 23 July 2013. At the time of collection, chlorophyll *a* varied from 150 to 200 µg l^{-1}. Experiments were conducted separately for females and males of *A. americanus* at 22 ± 1°C and in darkness. Before each test, the copepods were individually starved for 2 h in filtered (through 0.4-µm Whatman glass fiber filter) lake water. We used 50-ml test jars, each containing 40 ml filtered lake water and with two pre-starved adult females (non-ovigerous) or males. Four replicates were used for each treatment.

For prey selection experiments, we used three species of Rotifera and two species of Cladocera at three concentrations, to match roughly the natural densities of the lake and then half and double of these densities. We used: 20 individuals of *Brachionus plicatilis*, 10 *Keratella tropica*, 10 *Brachionus angularis*, four *Diaphanosoma mongolianum*, and four *Daphnia magna* 40 ml (proportions found in the lake), except for the addition of *D. magna,* which was not common at that time, but included to be able to test for selection between the two cladoceran species.

For functional response studies (conducted separately for each zooplankton prey), we used the most abundant rotifer and cladoceran species at the time of collection. These were *B. plicatilis* at densities of 400, 800, 1600, 2400, 3200, and 4000 individuals in 40 ml of the medium and *D. mongolianum* at 2, 4, 8, 12, 16, and 20 ind. 40 ml^{-1}. To test the effect of algae on the copepod feeding rates, we used *B. plicatilis* as prey but suspended in different dilutions of Albufera lake water from the collection site, filtered through 45 µm, to take out zooplankton but to keep most phytoplankton. Dilution was made with GF/F filtered Albufera lake water. Tested percentages of Albufera water with seston were 0% (GF/F filtered water only), 25, 50, and 67.5%. Controls contained the same concentrations of prey and seston but without the predators. Experiments were done with 40 individuals of *B. plicatilis* per test jar (at more or less natural rotifer densities) at the same conditions as in the other experiments, except that the test jars were gently shaken on a horizontal shaker.

Following the initiation of the feeding experiments, the copepods were allowed to feed for 1 h after which the test jar contents were fixed in 4% formalin. Later, the uneaten prey items were quantified using a stereomicroscope. The difference in the initial and final prey numbers from the test jars was considered as the number consumed by the predators. In the experiment with algae, Lugol solution was used a fixative and phytoplankton was counted in sedimentation chambers with an inverted microscope.

Data from prey selection tests were used to calculate Manly's α for experiments in which prey numbers were declining (Krebs, 1999): $\ln p_i / \sum_i^m \ln p_j$, where p_i, p_j are proportions of prey i or j remaining at the end of the experiments and n is the number of prey types. Data from the functional response experiments were transformed using linear regression model or rectangular hyperbola (Michaelis–Menten equation), following the statistical procedure described in Trexler et al. (1988): $V_0 = V_{\max} S/ Km + S$, where V_0 is the consumption rate, $V_{\max}$ is the saturation value of the consumption rate, S is availability of prey, and Km is prey concentration at which $V_{\max}/2$ is reached.

Results

Gut content analysis

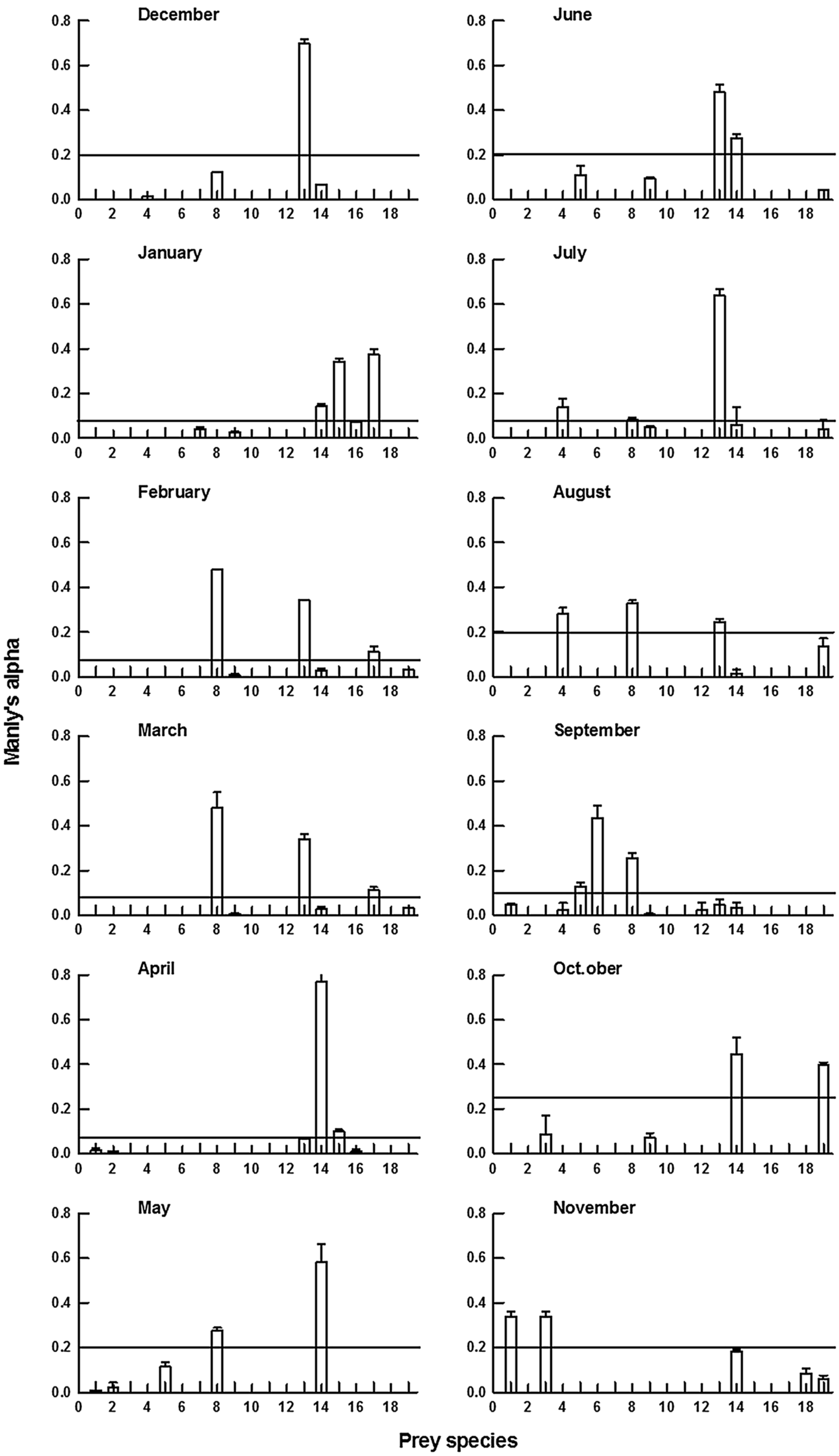
Gut contents of adult *Acanthocyclops* indicated more than 16 zooplankton species including *Keratella cochlearis* (unspined and spined forms), *K. tropica, Brachionus. plicatilis, B. calyciflorus, B. angularis, B. variabilis, Asplanchna girodi, Polyarthra vulgaris, Synchaeta pectinata, L. rhomboides,* bdelloids, *Alona rectangula, Chydorus sphaericus, Bosmina longirostris, Daphnia magna*, and *Ceriodaphnia dubia*. Changes in zooplankton prey selectivity of *Acanthocyclops americanus* from the lake Albufera through different seasons are presented in Fig. 1. *Acanthocyclops* also consumed large quantities of phytoplankton, including filamentous cyanobacteria, which could not be quantified, although they were identified in the samples (Table 1). The animal diet of this cyclopoid consisted of rotifers, cladocerans, and their own nauplii, the numbers of which varied depending on the season.

Fig. 1 Monthly patterns of prey selectivity (mean ± standard ▶ errors) by female *Acanthocyclops americanus* using Manly's α. The prey species as bars above the horizontal line indicate preferred items and those below were not preferred by the copepods. Numbers on *X*-axis indicate the prey items recorded from field samples: 1. *Keratella cochlearis* (unspined), 2. *K. cochlearis* (spined), 3. *K. tropica,* 4. *B. plicatilis,* 5. *B. calyciflorus,* 6. *B. angularis,* 7. *B. variabilis,* 8. *Asplanchna girodi,* 9. *Polyarthra vulgaris,* 10. *Synchaeta pectinata,* 11. *L. rhomboids*, 12. Bdelloids, 13. *Alona rectangula*, 14. Nauplii, 15. *Chydorus sphaericus,* 16. *Bosmina longirostris,* 17. *Daphnia magna,* 18. *Ceriodaphnia dubia*, and 19. Copepodites

Manly's α indicated that *Alona rectangula* was positively selected for six out of 12 sampling months while, *Asplanchna girodi* was selected for 4 months. Copepod nauplii were also the selected prey for *Acanthocyclops* but only during 5 months. The other prey species were positively selected only for 1 or 2 months during the study period. Mean annual prey selectivity indicated that as many as seven prey species (three rotifer species and four crustaceans) were included in the predator's diet (Fig. 2). *Asplanchna girodi* among rotifers and *Alona rectangula* among cladocerans were positively selected. However, the most abundant rotifer *Polyarthra vulgaris* was not selected by *Acanthocyclops*. Among daphniids, *D. magna* and *Ceriodaphnia dubia* were positively selected by the predators.

Functional response

There was no difference in the number of prey individuals in controls before and after the experiment. When *B. plicatilis* was used as prey, the consumption of rotifers by both male and female *Acanthocyclops* increased with increasing abundance in the medium until the offered density of 80 ind. ml^{-1} (= 3200 ind. 40 ml^{-1}). This relation was linear for female *Acanthocyclops*. Females also ate nearly twice the prey numbers as compared to males at comparable prey densities. Compared to rotifers, the number of *D. mongolianum* consumed by the copepods was much lower. For both male and female copepods, there was an increase in the prey consumption with increasing prey


December
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0.0
0.2
0.4
0.6
0.8
0
2
4
6
8
10
12
14
16
18
Manly's alpha
Prey species

Table 1 List of plankton commonly observed during the study period from the lake Albufera

- Phytoplankton
  - Bacillariophyceae
    - *Ceratoneis arcus* Kütz
    - *Cyclotella comensis* Grunow
    - *Cymbella* sp.
    - *Fragilaria capucina* Demazières
- *Gomphonema lanceolatum* Ehr.
    - *Navicula cryptocephala* Kützing
    - *Nitzschia palea* (Kütz.) W. Smith
  - Chlorophyta
    - *Ankistrodesmus densus* Kors
    - *Chlamydomonas* sp.
    - *Chlorella vulgaris* Beij.
    - *Chlorococcum* sp.
    - *Coelastrum microporum* Näg.
    - *Dictyosphaerium pulchellum* Wood
    - *Monoraphidium arcuatum* (Korš.) Hind.
    - *Oocystis marssonii* Lemm.
    - *Pediastrum boryanum* (Turp.) Menegh.
    - *Scenedesmus quadricauda* (Turp.) Bréb.
    - *Ulothrix subtilissima* Rabenhorst
  - Conjugatophyceae
    - *Closterium acutum* Bréb in Ralfs
    - *Cosmarium laeve* Rabenhorst
  - Cyanobacteria
    - *Anabaena* sp.
    - *Aphanizomenon* sp.
    - *Chroococcus minutus* (Kützing) Nägeli
    - *Cylindrospermopsis raciborskii* (Woloszynska) Subba Rayu
    - *Coelomoron pusillum* (Van Goor) Komárek
    - *Microcystis aeruginosa* Kützing
    - *Planktothrix agardhii* (Gomond) Anagnostidis et Komárek
    - *Pseudanabaena* sp.
    - *Spirulina* sp.
  - Cryptophyta
    - *Cryptomonas erosa* Ehrbg.
    - *Plagioselmis lacustris* (Pasch. & Ruttn.) Javorn.
  - Dinophyta
    - *Gymnodinium* sp.
    - *Peridinium cinctum* (Müller) Ehr.
  - Euglenophyta
    - *Euglena agilis* Carter
    - *Phacus longicauda* (Ehr.) Duj.

Table 1 continued

- Zooplankton
  - Rotifera
  - Bdelloidea
    - *Rotaria neptunia* (Ehrenberg, 1830)
    - *Rotaria rotatoria* (Pallas, 1766)
    - Unidentified bdelloids
  - Epiphanidae
    - *Proalides tentaculatus* de Beauchamp, 1907
  - Brachionidae
    - *Brachionus angularis* Gosse, 1851
    - *Brachionus bidentatus* Anderson, 1889
    - *Brachionus calyciflorus* Pallas, 1766
    - *Brachionus leydigii* Cohn, 1862
    - *Brachionus plicatilis* Müller, 1786
    - *Brachionus quadridentatus* Hermann, 1783
    - *Brachionus urceolaris* Müller, 1773
    - *Brachionus variabilis* Hempel, 1896
    - *Keratella cochlearis* (Gosse, 1851)
    - *Keratella lenzi* Hauer, 1953
    - *Keratella quadrata* (Müller, 1786)
    - *Keratella tropica* (Apstein, 1907)
    - *Notholca acuminata* (Ehrenberg, 1832)
    - *Notholca squamula* (Müller, 1786)
  - Euchlanidae
    - *Euchlanis dilatata* Ehrenberg, 1832
  - Lepadellidae
    - *Colurella obtusa* (Gosse, 1886)
    - *Lepadella patella* (Müller, 1773)
    - *Lepadella rhomboides* (Gosse, 1886)
  - Lecanidae
    - *Lecane bulla* (Gosse, 1851)
    - *Lecane closterocerca* (Schmarda, 1859)
    - *Lecane furcata* (Murray, 1913)
    - *Lecane hamata* (Stokes, 1896)
    - *Lecane papuana* (Murray, 1913)
  - Notommatidae
    - *Cephalodella forfícula* (Ehrenberg, 1831)
    - *Cephalodella gibba* (Ehrenberg, 1832)
    - *Eosphora najas* Ehrenberg, 1830
  - Trichocercidae
    - *Trichocerca elongata* (Gosse, 1886)
  - Synchaetidae
    - *Synchaeta oblonga* Ehrenberg, 1832
    - *Synchaeta pectinata* Ehrenberg, 1832
    - *Polyarthra vulgaris* Carlin, 1943
  - Asplanchnidae

Table 1 continued

Asplanchna girodi de Guerne, 1888
Conochilidae
Conochilus sp.
Testudinellidae
Testudinella patina (Hermann, 1783)
Cladocera
Alona rectangula Sar, 1862
Bosmina longirostris (O.F. Müller)
Ceriodaphnia dubia Richard, 1894
Chydorus sphaericus (O.F. Müller, 1776)
Daphnia magna Straus, 1820
Daphnia pulicaria Forbes, 1893
Diaphanosoma mongolianum Uéno, 1938
Ilyocryptus sordidus (Liéven, 1848)
Leydigia acanthocercoides (Fischer, 1854)
Moina micrura Kurz, 1874
Copepoda
Acanthocyclops americanus (Marsh, 1893)
Mixodiaptomus kupelwieseri (Brem, 1907)

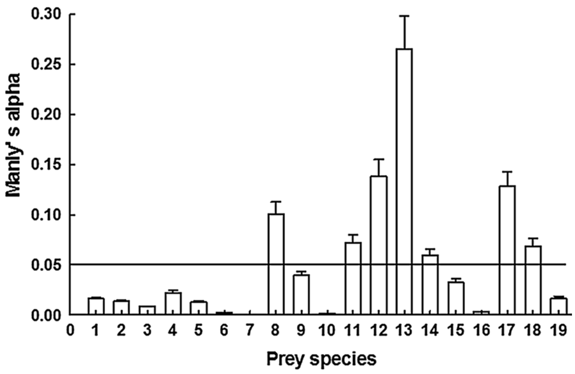

Fig. 2 Mean annual prey selectivity (mean ± standard errors) by female *A. americanus* using Manly's α. The prey species as bars above the horizontal line indicate preferred items and those below were not preferred by the copepods. Numbers on *X*-axis indicate the prey items recorded from field samples as shown in Fig. 1

availability in the medium (Fig. 3). Although male *Acanthocyclops* continued to feed linearly when fed *D. mongolianum,* in general, they consumed lower number of prey than females when the offered cladoceran density was lower (8–16 prey items 40 ml^{-1}). Statistically, the prey consumption by *A. americanus* was significantly influenced by the sex of the copepod and the prey density in the medium ($P < 0.001$) (Table 2, Fig. 3). However, the interaction of copepod sex x prey density was significant only for *B. plicatilis* but not for *D. mongolianum.*

Lake seston-rotifer mixed diet

Figure 4 shows the effect on prey (*Brachionus plicatilis*) consumption by *A. americanus* (females) in relation to the proportion in the medium of the natural seston of the lake. Water filtered through 45 µm contained abundant phytoplankton composed of filamentous (mainly *Pseudanabaena*), and colonial cyanobacteria. Prey consumption by female copepods clearly decreased with increasing proportion of seston in the medium. Thus, in the absence of seston, *A. americanus* had an ingestion rate of about 6 brachionid prey per copepod, which decreased to nearly half when the diet contained 67.5% seston.

Prey selectivity

When both male and female *Acanthocyclops* were offered the same prey composition, prey selection by the copepods varied not only depending on the prey concentration but also the sex of the copepods. *Keratella tropica* and *Brachionus angularis* were positively selected by both males and females in 5 of 6 treatments (Fig. 5). At the low and natural prey densities, males selected *B. plicatilis*; however, at high prey density, this rotifer was not preferred.

Regardless of prey density, *D. magna* was not selected by the males. In addition, under natural prey densities, males simultaneously preferred 4 out of 5 prey items supplied in the diet. Female copepods preferred *D. magna* at low and natural prey densities, but not at high densities. Similar to the males, females too preferred *Diaphanosoma mongolianum* but only at natural prey availability. Some daphniids in the prey selection test jars (after the test period) were dead, and the carapace was partly compressed.

During the predation experiments, we separately made some behavioral observations on the feeding aspects of *A. americanus*. The predator attacked the cladoceran prey (*Diaphanosoma mongolianum*) individually and could not trap more than one prey item at a time. Once captured, the prey was transferred to a gap between the thoracic appendages of the copepod where the cladoceran was squeezed to death, and soft

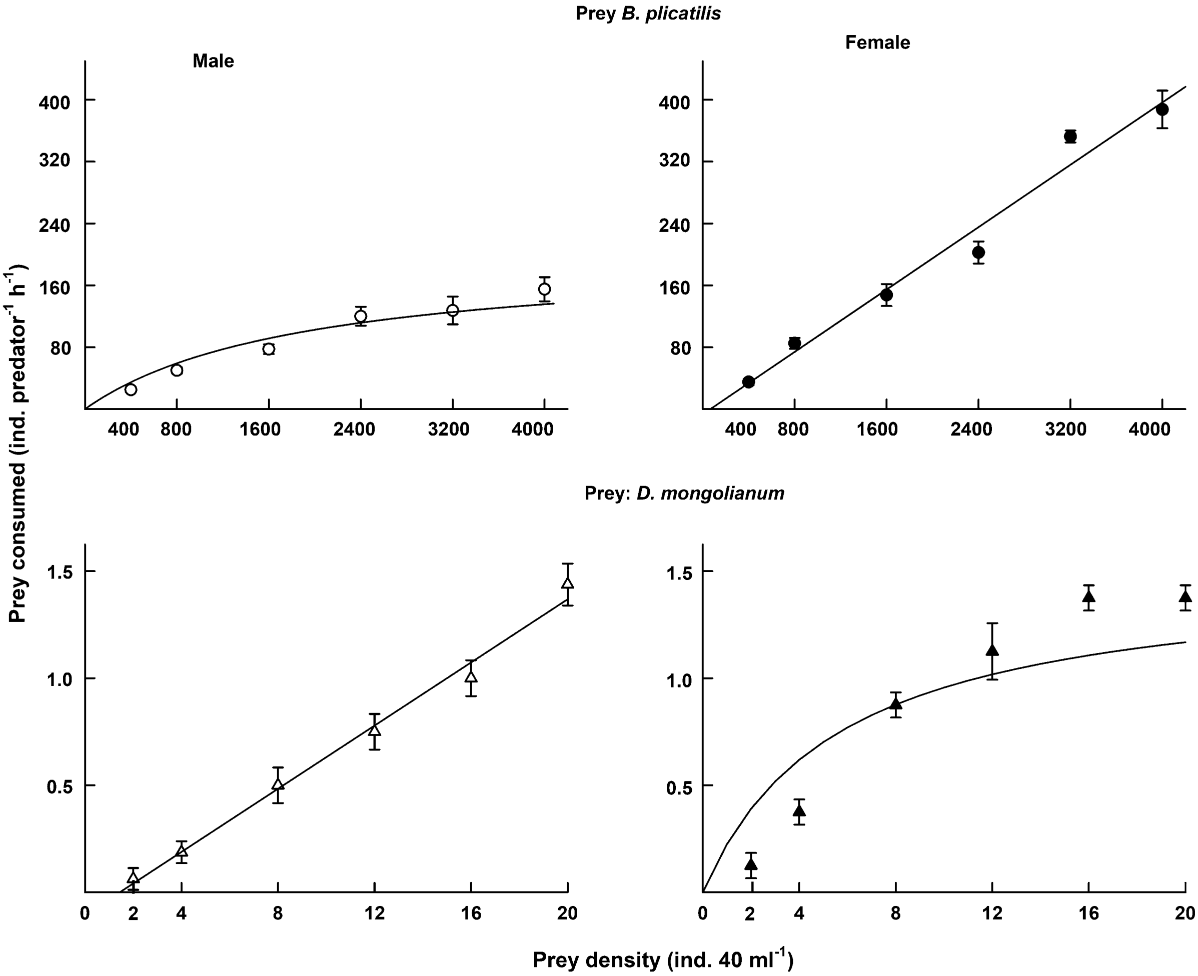

Fig. 3 Functional responses of male and female *Acanthocyclops americanus*. Shown are mean ± standard error for each prey concentration

Table 2 Results of two-way ANOVA between the prey (*B. plicatilis* and *D. mongolianum*) consumption between males and females in relation to different prey densities

Source of variation	DF	SS	MS	*F*	*P*
B. plicatilis					
Male vs female (A)	1	1430.08	1430.08	173.64	< 0.001
Prey density (B)	5	3639.17	727.83	88.37	< 0.001
A × B interaction	5	924.17	184.83	22.44	< 0.001
Error	36	296.50	8.24		
D. mongolianum					
Male vs female (A)	1	9.19	9.19	16.33	< 0.001
Prey density (B)	5	167.69	33.54	59.62	< 0.001
A × B interaction	5	5.688	1.14	2.02	0.099
Error	36	20.25	0.56		

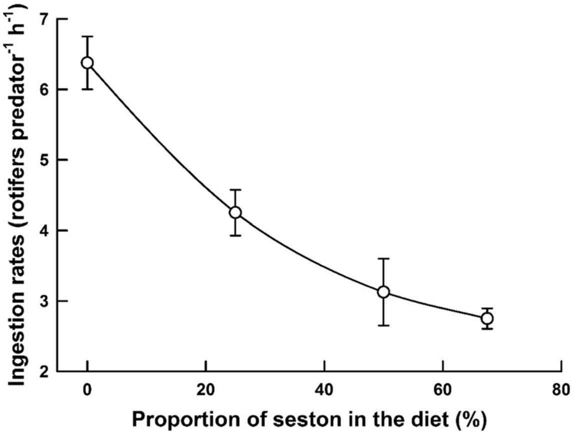

Fig. 4 Ingestion rates (mean ± standard errors) of *A. americanus* (females) fed *Brachionus plicatilis* in relation to the proportion of Albufera seston in the feeding suspension

parts were consumed by the predator. Sometimes empty loricas of brachionids were also pushed out by *A. americanus* in this way. Some rotifer prey became motionless in the water column when the predator attacked them. However, such prey, when sinking slowly, were attacked again by the predator and consumed.

Discussion

Cyclopoids constitute a dominant biomass among copepods in the Albufera (Oltra & Miracle, 1992). In most freshwater bodies, cyclopoids regulate the abundance and dynamics of both rotifers (Brandl, 2005) and cladocerans (Gliwicz & Umana, 1994). To assess the impact of cyclopoid predation on zooplankton from the lake Albufera, we first analyzed gut contents of *Acanthocyclops americanus* through seasons. From the gut of adult *A. americanus,* we were able to identify ten rotifer and five cladoceran species. Although bdelloids were present in the gut, they were unidentifiable. The process of identification (and more so of quantification) of prey items from the copepod gut contents was both difficult and laborious (Brandl, 2005). For prey items which were nearly intact in the copepod gut in the present work, this problem was minimal. Also, for certain prey, the partly digested body parts were sufficient for quantification of prey numbers. For example, a small part of the anterior lorica of brachionid rotifers (species of *Brachionus* and *Keratella*) is sufficient to identify and quantify the species (Koste, 1978). Foot opening or dorsal lorica of *Lepadella rhomboides* was often clear even when the lorica was nearly digested. For *Polyarthra*, the paddles were visible even when the entire soft body was digested. Bdelloids have typical ramate trophi while for *Asplanchna*, ramus is generally used to confirm the species (Sarma & Nandini, 2017). For *Synchaeta* which was also a soft-bodied rotifer, we relied on manubria of trophi (two manubria were considered as a single individual). For cladoceran species, postabdomen is generally resistant to digestion (Dumont & Negrea, 2002) and this was considered for both identification and quantification.

Most rotifer species of the genera *Brachionus*, *Keratella*, *Polyarthra*, *Synchaeta*, and *Asplanchna* are vulnerable to copepod predation (Roche, 1987; Brandl, 2005) as also observed here from the gut content studies. However, not all prey items found in the gut of a predator indicate selectivity (Jackson & Lenz, 2016). Thus, Manly's index of selectivity showed only up to three prey species which were positively selected at any sampling month. *Alona* was positively selected for 50% of the sampling period, while asplanchnids and nauplii were selected for 5 months. Brachionid rotifers of the genera *Brachionus* and *Keratella* were also selected for 5 months. These data indicated that both rotifers and cladocerans formed a significant diet for *A. americanus*. Our observations were in agreement with Williamson (1983) who concluded that soft-bodied rotifers (e.g., *Asplanchna*) are more vulnerable than hard-loricated species to copepod predation. In spite of its high abundance, *Polyarthra vulgaris* was not selected frequently by *Acanthocyclops*. This is because *Polyarthra* in general shows skipping escape responses, which prevents it from being captured by *Acanthocyclops* (Roche, 1987).

Different groups of phytoplankton including cyanobacteria were present in the gut of copepods. Previous reports also suggest that cyclopoids in general are never exclusively carnivores but in most cases, their diet consists of a significant portion of phytoplankton (García et al., 2011). This suggests that adult *Acanthocyclops americanus* is a generalist feeder. The presence of toxic cyanobacteria in the gut contents of *Acanthocyclops* in this study is noteworthy. Most studies have confirmed that

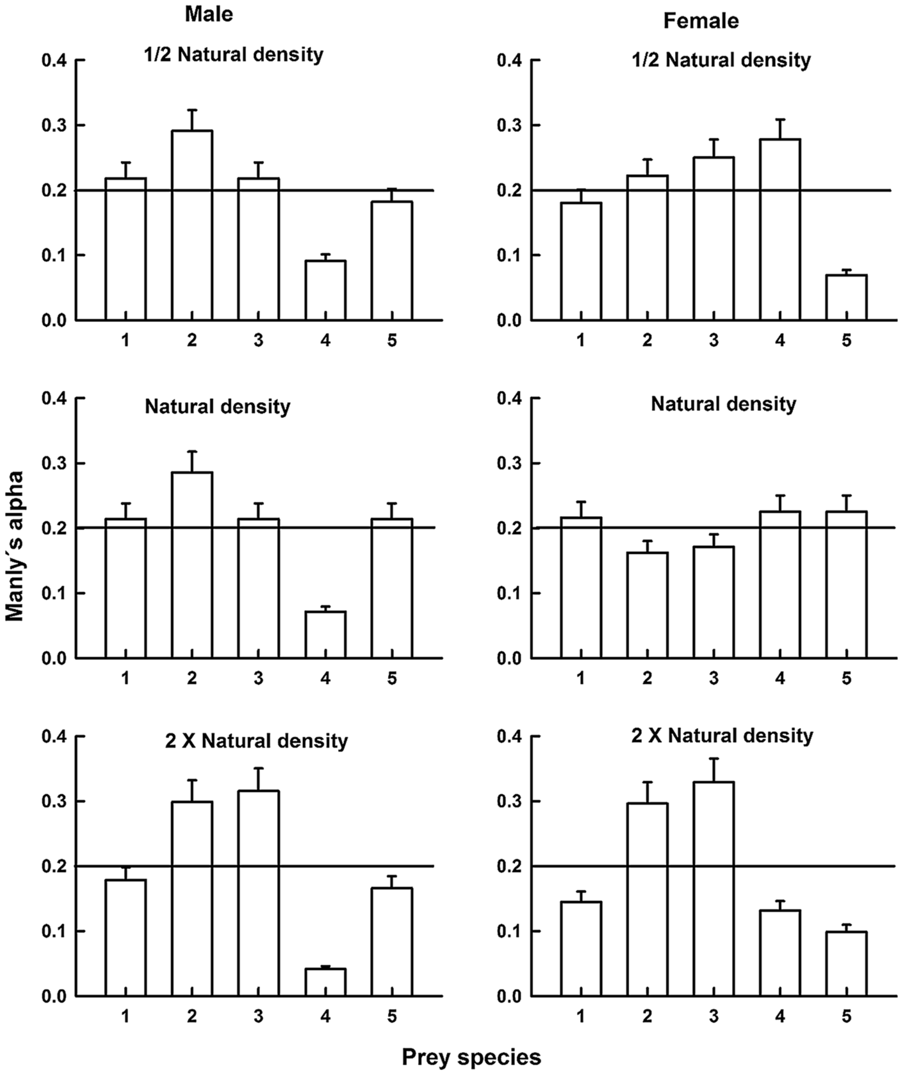

Fig. 5 Prey selectivity by male and female *A. americanus* using Manly's α. The prey species as bars above the horizontal line indicate preferred items and those below were not preferred by the copepods. Numbers on *X*-axis indicate the prey items offered under laboratory conditions: 1. *B. plicatilis,* 2. *K. tropica,* 3. *B. angularis,* 4. *D. magna*, and 5. *D. mongolianum*

copepods survive in ponds infested with toxic cyanobacteria including *Microcystis* by feeding on them or selectively choosing some other phytoplankton species (WHO, 1999; de Kluijver et al., 2012). In Lake Albufera, during winter months, the density of the edible fraction of phytoplankton is higher than that of *Microcystis* and probably this facilitates persistence of certain zooplankton including cladocerans (Oltra & Miracle, 1992). Possibly during this period, cyclopoids feed on both phytoplankton and zooplankton (Oltra & Miracle, 1984; Romo & Miracle, 1993).

Our laboratory tests showed that *A. americanus* consumed lower number of rotifers with an increase in the concentration of phytoplankton in mixed diets. In this experiment, there is some possibility that rotifers reduced the phytoplankton in the test jars. However, published data on the rotifer growth fed phytoplankton of different size fractions from Albufera do not support this (Miracle et al., 2014). It appears that zooplankton consumption by the predatory copepods decreases when phytoplankton biomass increases in the diet (Sarma et al., 2013).

Copepods, like many other predatory species, show increased prey consumption with an increase in prey availability until an asymptote is reached (Monakov, 2003). The asymptotic density varies depending on the prey type and the sex of the predator (Abrams, 2000). Cladocerans have higher biomass than rotifers by an order of magnitude (Bottrell et al., 1976) and hence only a few individuals are enough to bring down the predator's hunger level (DeMott, 1993). The consumption of a lower number of cladocerans in our test jars was also due to the higher handling time of the prey by the predator. Although we did not quantify this here, Roche (1990) showed that the handling time for a single brachionid prey varied from 1 to 3 min. while for cladocerans, it was much higher (8–12 min.). Thus, the higher consumption of brachionids in our functional response study was due to higher availability in the medium (leading to higher probability of encounters) and lower handling time per prey time (Roche, 1987, 1990). The maximum number of rotifers consumed by the female A. *americanus* peaked around a prey density of 80 ind. ml^{-1}. This is much lower than that reported for *Mesocyclops pehpeiensis* which had a maximal rotifer (*Brachionus rubens*) consumption at the prey density 200 ind. ml^{-1} (Sarma et al., 2013). For both the prey species (rotifers and cladocerans) tested here, male copepods consumed a significantly lower number of zooplankton, especially at low prey density, than females which indicates that female *A. americanus* is more voracious than males. This is consistent with available literature where females need higher energy levels to meet reproductive costs (Hirst & Kiørboe, 2014) and hence they are more predatory and consume significantly higher prey biomass than males (LeBlanc et al., 1997).

Prey selection by copepods is a complex process involving various physiological, ecological and ethological, and morphological factors of both prey and the predators. Cyclopoids are generally considered raptorial where they individually catch each prey item (Kiørboe, 2011a). When the prey is strongly armed with a protective case such as carapace, then copepods might just penetrate through the space between the valves and feed on soft tissues including developing embryos (Gliwicz & Umana, 1994). On the other hand, for certain rotifer genera with thick lorica such as *Keratella* and *Anuraeopsis*, copepods may simply squeeze out the prey using the thoracic appendages and feed on soft tissues. This was also observed in our work, although it was not quantified. In such cases, the entire prey is not ingested, and hence the identification of prey in the gut is based on other hard structures such as trophi (Brandl, 2005).

Conclusions

Our field study showed that *Acanthocyclops americanus* is a common predator of zooplankton of Lake Albufera. Gut content analysis of *A. americanus* showed the presence of rotifers, cladocerans, and copepod nauplii. Phytoplankton including cyanobacteria were also present in the guts of *A. americanus*. Prey selectivity data from the field samples showed that this predatory copepod selectively fed on *Alona rectangula* but avoided *Polyarthra vulgaris*. Laboratory tests indicated that female *A. americanus* had higher prey consumption rates than males. When lake seston was included in the diet containing rotifers, the female copepods consumed a lower quantity of zooplankton. Our study shows that *A. americanus* is an omnivorous copepod.

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References

Abrams, P. A., 2000. The evolution of predator-prey interactions: theory and evidence. Annual Review of Ecology and Systematics 31: 79–105.

Boersma, M., A. Wesche & H. J. Hirche, 2014. Predation of calanoid copepods on their own and other copepods' offspring. Marine Biology 161: 733–743.

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson & T. Weglenska, 1976. Review of some problems in zooplankton production studies. Norwegian Journal of Zoology 24: 419–456.

Brandl, Z., 2005. Freshwater copepods and rotifers: predators and their prey. Hydrobiologia 181: 475–489.

Case, T. J., 2000. An Illustrated Guide to Theoretical Ecology. Oxford University Press, London.

DeMott, W. R., 1993. Hunger-dependent diet selection in zooplankton. In Hughes, R. N. (ed.), Diet Selection: An Interdisciplinary Approach to Foraging Behaviour. Blackwell Scientific Publications, Oxford: 102–123.

de Kluijver, A., J. Yu, M. Houtekamer, J. J. Middelburg & Z. Liu, 2012. Cyanobacteria as a carbon source for zooplankton in eutrophic Lake Taihu, China, measured by ^{13}C labeling and fatty acid biomarkers. Limnology and Oceanography 57: 1245–1254.

Dieng, H., M. Boots, N. Tuno, Y. Tsuda & M. Takagi, 2003. Life history effects of prey choice by copepods: implications for biocontrol of vector mosquitoes. Journal of the American Mosquito Control Association 19: 67–73.

Dumont, H. J. & S. V. Negrea, 2002. Introduction to the class Branchiopoda. In Dumont, H. J. (ed.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, Vol. 19. Backhuys Publishers, Leiden.

Enríquez-García, C., S. Nandini & S. S. S. Sarma, 2013. Feeding behavior of *Acanthocyclops americanus* (Marsh) (Copepoda: Cyclopoida). Journal of Natural History 47: 853–862.

Frisch, D., H. Rodríguez-Pérez & A. J. Green, 2006. Invasion of artificial ponds in Doñana Natural Park, southwest Spain, by an exotic estuarine copepod. Aquatic Conservation: Marine and Freshwater Ecosystems 16(5): 483–492.

García, C. E., S. Nandini & S. S. S. Sarma, 2011. Demographic characteristics of the copepod *Acanthocyclops americanus* (Sars, 1863) (Copepoda: Cyclopoida) fed mixed algal (*Scenedesmus acutus*)-rotifer (*Brachionus havanaensis*) diet. Hydrobiologia 666: 59–69.

Garcia-Chicote, J., C. Rojo & M. A. Rodrigo, 2007. *Acanthocyclops robustus* feeding: a case of cannibalism. Limnetica 26: 265–275.

Gliwicz, Z. M., 2003. Between Hazards of Starvation and Risk of Predation: The Ecology of Offshore Animals, Vol. 12. International Ecology Institute, Oldendorf/Luhe.

Gliwicz, Z. M. & G. Umana, 1994. Cladoceran body size and vulnerability to copepod predation. Limnology and Oceanography 39: 419–424.

Gophen, M., 1977. Food and feeding habits of *Mesocyclops leuckarti* (Claus) in Lake Kinneret (Israel). Freshwater Biology 7: 513–518.

Heuschele, J. & E. Selander, 2014. The chemical ecology of copepods. Journal of Plankton Research 36: 895–913.

Hirst A. G. & T. Kiørboe, 2014. Macroevolutionary patterns of sexual size dimorphism in copepods. Proceedings of the Royal Society B. Biological Sciences 281: 20140739.

Hołyńska, M., J. W. Reid & H. Ueda, 2003. Genus *Mesocyclops* Sars, 1914, pp. 12–213. In: H. Ueda & J. W. Reid (eds), Copepoda: Cyclopoida. Genera *Mesocyclops* and *Thermocyclop*s. Vol. 20. In H.J. Dumont (ed). Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. Backhuys, Leiden.

Hopp, U. & G. Maier, 2005. Implication of the feeding limb morphology for herbivorous feeding in some freshwater cyclopoids. Freshwater Biology 50: 742–747.

Kiørboe, T., 2011a. How zooplankton feed: mechanisms, traits and trade-offs. Biological Reviews of the Cambridge Philosophical Society 86: 311–339.

Kiørboe, T., 2011b. What makes pelagic copepods so successful? Journal of Plankton Research 33: 677–685.

Koste, W., 1978. Rotatoria. Gebruder Borntraeger, Berlin. 2 vols.

Krebs, J. R., 1999. Ecological Methodology, 2nd ed. Addison-Wesley Educational Publishers, Boston.

Jackson, J. M. & P. H. Lenz, 2016. Predator-prey interactions in the plankton: larval fish feeding on evasive copepods. Scientific Reports 6: 33585.

Lapesa, S., T. W. Snell, D. M. Fields & M. Serra, 2002. Predatory interactions between a cyclopoid copepod and three sibling rotifer species. Freshwater Biology 47(9): 1685–1695.

LeBlanc, J. S., W. D. Taylor & O. E. Johannsson, 1997. The feeding ecology of the cyclopoid copepod Diacyclops thomasi in Lake Ontario. Journal of Great Lakes Research 23: 369–381.

Link, J. & R. Keen, 1995. Prey of deep-water *Hydra* in Lake Superior. Journal of Great Lakes Research 21: 319–323.

Miracle, M. R., V. Alekseev, V. Monchenko, V. Sentandreu & E. Vicente, 2013. Molecular-genetic-based contribution to the taxonomy of the *Acanthocyclops robustus* group. Journal of Natural History 47: 863–888.

Miracle, M. R., E. Vicente, S. S. S. Sarma & S. Nandini, 2014. Planktonic rotifer feeding in hypertrophic conditions. International Review of Hydrobiology 99: 141–150.

Monakov, A. B., 2003. Feeding of freshwater Invertebrates. Kenobi Productions, Ghent.

Oltra, R. & M. R. Miracle, 1984. Comunidades zooplanctónicas de la Albufera de Valencia. Limnetica 1: 51–61.

Oltra, R. & M. R. Miracle, 1992. Seasonal succession zooplankton populations in the hypertrophic lagoon: Albufera of Valencia (Spain). Archiv für Hydrobiologie 124: 187–204.

Oltra, R., M. T. Alfonso, M. Sahuquillo & M. R. Miracle, 2001. Increase of rotifer diversity after sewage diversion in the hypertrophic lagoon, Albufera of Valencia, Spain. Hydrobiologia 446(447): 213–220.

Onandia, G., M. R. Miracle & E. Vicente, 2014. Primary production under hypertrophic conditions and its relationship with bacterial production. Aquatic Ecology 48: 447–473.

Reid, N. W. & C. E. Williamson, 2009. Copepoda. In Thorp, J. H. & A. P. Covich (eds), Ecology and Classification of North American Freshwater Invertebrates, 3rd ed. Academic Press, New York: 829–899.

Roche, K., 1987. Post-encounter vulnerability of some rotifer prey types to predation by the copepod *Acanthocyclops robustus*. Hydrobiologia 147: 229–233.

Roche, K., 1990. Some aspects of vulnerability to cyclopoid predation of zooplankton prey individuals. Hydrobiologia 198: 153–162.

Rollwagen-Bollens, G., S. M. Bollens, A. Gonzalez, J. Zimmerman, T. Lee & J. Emerson, 2013. Feeding dynamics of the copepod *Diacyclops thomasi* before, during and following filamentous cyanobacteria blooms in a large, shallow temperate lake. Hydrobiologia 705: 101–118.

Romo, S. & M. R. Miracle, 1993. Long term periodicity of *Planktothrix agardhii*, *Pseudanabaena galeata* and *Gleiterinema sp*. in a shallow hypertrophic lagoon, the Albufera of Valencia, Spain. Archiv für Hydrobiologie 126: 469–486.

Sarma, S. S. S. & S. Nandini, 2007. Small prey size offers immunity to predation: a case study on two species of *Asplanchna* and three brachionid prey (Rotifera). Hydrobiologia 593: 67–76.

Sarma, S. S. S. & S. Nandini, 2017. Rotíferos Mexicanos (Rotifera). Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Iztacala, México, Estado de México.

Sarma, S. S. S., J. Jiménez-Contreras, R. Fernández, S. Nandini & G. García-García, 2013. Functional responses and feeding rates of *Mesocyclops pehpeiensis* Hu (Copepoda) fed different diets (rotifers, cladocerans, alga and cyanobacteria). Journal of Natural History 47: 841–852.

Seckbach, J. (ed.), 2007. Algae and Cyanobacteria in Extreme Environments. Springer, New York.

Trexler, C. J., C. E. McCulloch & J. Travis, 1988. How can the functional response best be determined? Oecologia 76: 206–214.

WHO, 1999. World Health Organization: Toxic cyanobacteria in water. In Chorus, I. & J. Bartram (eds), A guide to their public health consequences, monitoring and management. St Edmundsbury Press, Suffolk.

Williamson, C. E., 1983. Invertebrate predation on planktonic rotifer. Hydrobiologia 104: 385–396.