Diversity of wetland zooplankton in the Lachlan River catchment, New South Wales, Australia

Tsuyoshi Kobayashi[1,2*], Jan Miller[3], Russell J. Shiel[4], Hendrik Segers[5] and Simon J. Hunter[1]

[1]Science, Economics and Insights Division, Department of Planning and Environment, PO Box 29, Lidcombe, NSW 1825, Australia

[2]School of Agriculture, Environmental and Veterinary Sciences, Charles Sturt University, Albury, NSW 2640, Australia

[3]Cumberland Flora & Fauna Interpretive Services, 13 Park Road, Bulli, NSW 2516, Australia

[4]R.J. Shiel & Assoc., Environmental Consultants, 3 Hillcrest Ave, Crafers West, SA 5152, Australia

[5]Royal Belgian Institute of Natural Sciences, OD Nature, Vautierstraat 29, B-1000 Brussels, Belgium

*Author for correspondence: Yoshi.Kobayashi@environment.nsw.gov.au

ABSTRACT

Inland wetlands are areas of high biodiversity, providing various ecosystem services. In this study, we assessed the species diversity of wetland zooplankton in the Lachlan River catchment. Biodiversity sampling is labour-intensive and attained sample size is often not large enough to detect all species present. Therefore, we applied integrated rarefaction and extrapolation sampling curves and asymptotic analysis to estimate species richness. We observed 103 species of rotifers, 29 species of cladocerans and 13 species of copepods, with further 14 rotifers, three cladocerans and four copepods identified at higher than species level, totalling 117 taxa of rotifers, 32 taxa of cladocerans and 17 taxa of copepods from 36 wetland sites across the catchment. The observed zooplankton species included the first record of the rotifer *Trochosphaera solstitialis* in Australia, and the first record of the rotifers *Brachionus lyratus tasmaniensis, Keratella shieli, Lepadella tyleri, Notholca salina* and *N. squamula* and the cladoceran *Alona setuloides* in NSW. Based on the integrated sampling curves and asymptotic analysis of species richness in wetlands of the Lachlan River catchment, greater additions of rotifers (estimated asymptote: ≈ 145) are more likely to be realised with increasing sample size than those of cladocerans (estimated asymptote: ≈ 36) and copepods (estimated asymptote: ≈ 18).

Key words: biogeography, bootstrap sampling, incidence matrix, range extension

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Introduction

Biodiversity in freshwater ecosystems including inland wetlands has received renewed attention recently (e.g. Flitcroft *et al.* 2019; Tickner *et al.* 2020). This is due to concern that natural and anthropogenic stresses and habitat destruction are accelerating biodiversity loss across the Earth's ecosystems, particularly among freshwaters which are likely the most threatened (Dudgeon *et al.* 2006; Kingsford *et al.* 2016; Alahuhta *et al.* 2019; Gozlan *et al.* 2019; Ragavan *et al.* 2021). Maintaining biodiversity in freshwater ecosystems is critically important as biodiversity provides a range of ecosystem services (Mace *et al.* 2012; Albert *et al.* 2021). Inventory of biodiversity is one of the fundamental requirements for effective management of species and the associated structural and functional services they provide in freshwater ecosystems including wetlands (Mitsch *et al.* 2015; Cantonati *et al.* 2020).

For inland wetlands of Australia, the species diversity of vertebrate fauna such as birds and fish, especially in Ramsar wetlands including the Macquarie Marshes and the Gwydir Wetlands in New South Wales (NSW), have been well studied (e.g. Kingsford and Thomas 1995; Rayner *et al.* 2009; Wilson *et al.* 2010; Galego de Oliveira *et al.* 2019). The species diversity of other inland wetland fauna, especially microinvertebrates such as zooplankton, is still understudied apart from those in billabongs (oxbows or cut-off meanders) and temporary floodplain waters in the River Murray catchment where diverse species of zooplankton occur (Shiel 1978, 1983; Shiel *et al.* 1998). Zooplankton occupy a critical niche in facilitating energy and nutrient transfer from bacteria and primary producers (e.g. algae) to higher consumers (e.g. larval fish) (Boon and Shiel 1990; Gehrke 1992). In this study, we assessed the species composition and species richness of wetland zooplankton (rotifers, cladocerans and copepods including calanoids and cyclopoids) in the Lachlan River catchment in southeast NSW, Australia. The Lachlan River and its catchment support eight nationally significant wetlands listed in the Directory of Important Wetlands (Environment Australia 2001) and nine regionally significant wetlands (DPI 2018). Biodiversity conservation in the Lachlan River catchment has been a focus of current water resources management (Dyer *et al.* 2019, 2021; DPIE 2020; Ryan *et al.* 2021).

Biodiversity sampling for biota including zooplankton is labour-intensive and attained sample size may not be sufficient to detect all the species present (Dumont and Segers 1996; Gotelli and Colwell 2011). To overcome likely sampling deficiency that can significantly underestimate true biodiversity, various mathematical and statistical modelling approaches have been developed (e.g. Dumont and Segers 1996; Gotelli and Colwell 2011; Chao *et al.* 2020 and references therein). In this study, we estimated the species richness of zooplankton, in addition to the observed species richness, based on integrated rarefaction and extrapolation sampling curves and asymptomatic analysis of species richness (Chao *et al.* 2014, 2020).

Study area and study sites

The Lachlan River catchment covers a total area of ≈ 84, 700 km², located in southeast NSW, Australia (Fig. 1). The Lachlan River itself rises on the Breadlebane Plain on the tablelands of the Great Dividing Range and flows westward for ≈ 1,300 km before terminating in the Great Cumbung Swamp near Oxley (DPI 2018). The river historically flowed through the Great Cumbung Swamp and into the Murrumbidgee River, but rarely does so now. Average annual rainfall in the Lachlan River catchment varies from 1,100 mm yr^{-1} in the eastern part of the catchment to less than 300 mm yr^{-1} in the far west, with 400 to 600 mm yr^{-1} in the middle part of the catchment (DPI 2018).

Total area of wetlands in the Lachlan River catchment is estimated to be ≈ 4,700 km², of which floodplain wetlands comprise ≈ 95 % of the total wetland area (Kingsford *et al.* 2004). The wetlands in the Lachlan

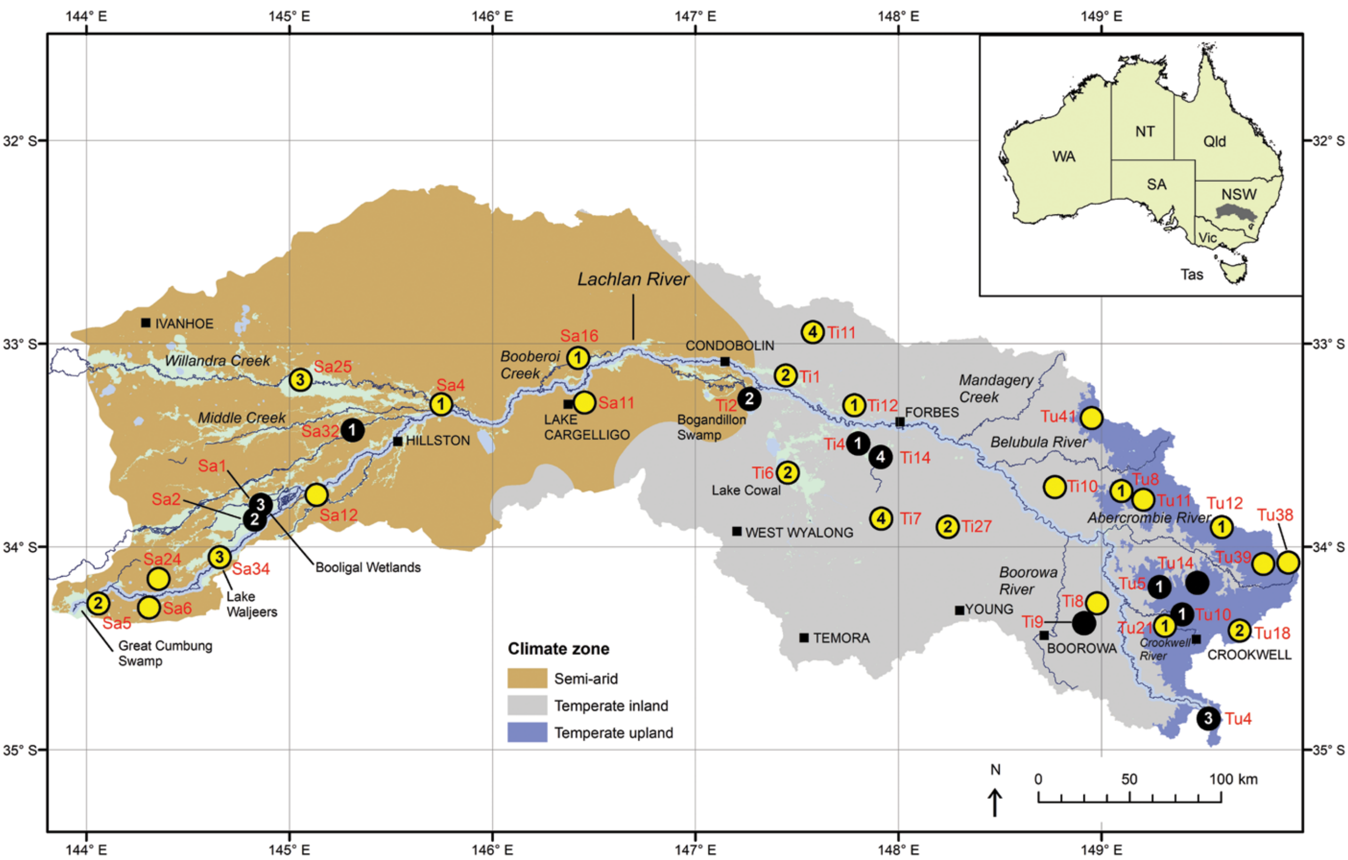

Figure 1. Lachlan River catchment and survey sites. The yellow and black-filled circles indicate the survey site locations. The number in the filled circle indicates the endemic species richness recorded at that survey site. The black-filled circles indicate the site locations of newly recorded species in Australia (*Trochosphaera solstitialis* at site Ti4) or in NSW (*Brachionus lyratus tasmaniensis* at sites Sa1 and Ti2; *Keratella shieli* at site Ti14; *Lepadella tyleri* at sites Sa2 and Sa32; *Notholca salina* at sites Ti9, Tu10 and Tu14; *Notholca squamula* at sites Ti9, Tu5 and Tu14; *Alona setuloides* at site Tu4). WA: Western Australia, NT: Northern Territory, SA: South Australia, Qld: Queensland, Vic: Victoria, Tas: Tasmania, NSW: New South Wales.

River catchment provide habitats for a range of water-dependent species including birds such as straw-necked ibis (*Threskiornis spinicollis*), glossy ibis (*Plegadis falcinellus*), egrets (*Ardea/Egretta* sp.), Australian painted snipe (*Rostratula australis*) and blue-billed duck (*Oxyura australis*), and a range of native fish including Murray cod (*Maccullochella peelii*), golden perch (*Macquaria ambigua*), silver perch (*Bidyanus bidyanus*), the endangered Murray-Darling Basin population of eel-tailed catfish (*Tandanus tandanus*) and the endangered western NSW population of olive perchlet (*Ambassis agassizii*) (DPI 2018; Commonwealth of Australia 2021). These wetlands also feature areas of water-dependent native vegetation including river red gum (*Eucalyptus camaldulensis*) forest and woodlands, black box (*Eucalyptus largiflorens*) woodland and lignum (*Duma florulenta*). The lower Lachlan floodplain is home to nine nationally important wetlands, including Lake Brewster, the Booligal Wetlands and the Great Cumbung Swamp. The Great Cumbung Swamp, covering an area of ≈ 160 km^2, contains one of the largest remnant examples of common reed (*Phragmites australis*) swamps and stands of river red gums in NSW (MDBA 2012; Commonwealth of Australia 2021).

Materials and methods

Site selection

Following the NSW monitoring, evaluating and reporting wetland typology (Claus *et al.* 2011), we divided the Lachlan River catchment into three zones: semi-arid, temperate inland and temperate upland (Fig. 1). In each zone, twelve sampling wetland sites were randomly selected based on the NSW wetlands dataset (Kingsford *et al.* 2004) and RiverStyles® spatial dataset (https://water.dpie.nsw.gov.au/science-data-and-modelling/surface-water/monitoring-changes/river-styles-in-nsw).

Field sampling and laboratory analyses

Field sampling was conducted at a total of 36 sites (Fig. 1), of which 12 sites located in the semi-arid zone and 12 sites located in the temperate inland zone were visited once between September and December 2013, and 12 sites located in the temperate upland zone were visited once between June and July 2014. Photos of selected sampling sites are shown in Appendix I. At each site, three to four water samples (10 L each) were collected using a plastic bucket to produce one composite water sample (on average ≈ 700 m apart between each water sample collected at each site). The composite water sample was filtered through a 63-μm mesh net and zooplankton retained on the net were backwashed into a 300 mL plastic jar and preserved in 70% ethanol. In a laboratory, all zooplankton specimens from each composite sample were sorted, examined and identified under a Leica M80 stereomicroscope at a magnification of × 20 to × 50. Small specimens (< 1–2 mm in size) were identified under a Leica DM 2500 compound microscope at a magnification of ×100 to × 1000, with bright-field, differential interference contrast or phase-contrast illumination. For the identification of rotifer species, the morphology of their trophi was also examined following the sodium hypochlorite extraction method (Shiel 1995). Juvenile calanoid or cyclopoid copepods were counted as one taxon in the samples that contained no adult calanoids or cyclopoids. The primary taxonomic literature used included Smirnov and Timms (1983), Morton (1985, 1990), Bayly (1992), Holyńska and Brown (2002), Segers (1995, 2007), Shiel (1995), and Sinev (2015). Photomicrographs of some species of zooplankton (including the trophi of rotifers) were taken using a Leica DFC 480 digital camera attached to the microscope and Leica Image Manager (IM) Version 4.0 image analysis software (Leica Microsystems, Germany).

Estimates of species richness and sample completeness

In this study, we estimated species richness through rarefaction and extrapolation following Chao *et al.* (2014, 2020). The method is a comprehensive statistical framework for the analysis of biodiversity data, based on Hill numbers (Hill 1973; Chao *et al.* 2014). It employs a bootstrap method for constructing confidence intervals around Hill numbers and the proposed estimators are said to be accurate for both rarefaction and short-range extrapolation with the extrapolated sample size up to twice the reference (or realised) sample size (Chao *et al.* 2014). We used R package iNEXT (Hsieh *et al.* 2016) in the statistical computing-and-graphics environment 'R' (ver. 4.2.1, R Foundation for Statistical Computing, Vienna, Austria, see https://www.r-project.org/), to compute and plot the integrated rarefaction and extrapolation sampling curves for species richness and sample completeness parameterised by a diversity order of $q = 0$ in the Hill number family (i.e. for incidence/presence data) (Hill 1973; Chao *et al.* 2014). Extrapolation sampling curves were constructed with an endpoint corresponding to doubling of a realised sample size ($36 \times 2 = 72$ in this study). When $q = 0$, sample completeness is simply the ratio of the observed species richness to the estimated true species richness (observed plus undetected) (Chao *et al.* 2020). An asymptotic analysis in the R package iNEXT was undertaken and used for comparison of estimated asymptotic species richness with observed species richness. For the estimated sampling curve, asymptotic species richness, and sample completeness, 95% lower and upper confidence limits were computed with a bootstrap method (1000 resamples used) (Hsieh *et al.* 2016). Initial input data consisted of a species (i)-by-sampling unit (j) incidence matrix (incidence raw data) with S rows and T columns, where S denotes the number of species (or taxa) ($S = 166$ in this study) and T denotes the sample size (number of sampling sites) (T = 36 in this study). The value of the element of the incidence matrix was 1 if species i (or taxon) was present at site j and 0 if species was absent. The incidence raw data were transformed to incidence frequencies data as iNEXT input data format, using the function "as.incfreq" (Hsieh *et al*. 2016).

Mapping of species distributions

The spatial distribution of surveyed site locations including newly recorded species in Australia and NSW and the endemic species richness recorded at each survey location were mapped using ArcGIS ArcMap 10.4 software (Environmental Systems Research Institute, Redlands California).

Results

Overall, 103 species of rotifers, 29 species of cladocerans and 13 species of copepods were identified, with further 14 rotifers, three cladocerans and four copepods identified at higher than species level, totalling 117 taxa of rotifers, 32 taxa of cladocerans and 17 taxa of copepods in this study (Appendix II). Of these, the rotifer *Trochosphaera solstitialis* was recorded for the first time in Australia and the rotifers *Brachionus lyratus tasmaniensis*, *Keratella shieli*, *Lepadella tyleri*, *Notholca salina* and *N. squamula* and the cladoceran *Alona setuloides* were recorded for the first time in NSW (Table 1 and Fig. 2). The site locations of these newly recorded species are shown in Fig. 1. The endemic species richness (defined as the observed number of the species with their known distributions restricted to Australia) was six for rotifers, four for cladocerans and six for copepods (Table 1). This equated about 5 % of rotifers, 9 % of cladocerans and 35 % of copepods against the total richness for each group. The greatest endemic species richness (four) was observed in the temperate-inland climate zone (Ti7, Ti11 and Ti14) (Fig. 1).

Table 1. Records of the Australian-endemic zooplankton found in wetlands of the Lachlan River catchment and their distributions in Australian inland waters (Koste 1979; Smirnov and Timms 1983; Morton 1985, 1990; Shiel and Koste 1985; Julli 1986; Koste and Shiel 1986, 1987; Timms and Morton 1988; Maly and Bayly 1991; Smirnov 1992; Maly *et al*. 1997; Jocqué *et al*. 2007; Sommer *et al*. 2008; R.J. Shiel, pers. observe.; present study). WA: Western Australia, NT: Northern Territory, SA: South Australia, Qld: Queensland, Vic: Victoria, Tas: Tasmania, NSW: New South Wales. Species presence coded as 1 and species absence coded as 0.

Species	WA	NT	SA	Qld	Vic	Tas	NSW	Recorded site in this study (see Fig.1 for location)
Rotifera								
Asplanchna asymmetrica	0	0	0	1	1	0	1	Ti4, Ti7
Brachionus keikoa	0	0	1	1	1	0	1	Sa34, Ti6, Ti14
Brachionus lyratus tasmaniensis	0	0	0	0	0	1	1	Sa1, Ti2
Filinia grandis	0	0	1	1	1	1	1	Ti6, Ti14
Keratella shieli	1	0	1	0	0	0	1	Ti14
Lepadella tyleri	0	0	0	0	0	1	1	Sa2, Sa32
Cladocera								
Alona rigidicaudis	1	1	1	1	1	1	1	Sa2, Sa4, Sa5, Sa34, Ti11
Alona setuloides	1	0	0	0	0	0	1	Tu4
Biapertura kendallensis	1	1	0	1	1	0	1	Tu10, Tu12, Tu18
Macrothrix breviseta	1	1	0	1	1	0	1	Sa5, Ti7, Ti8, Ti11, Tu4, Tu8
Copepoda								
Australocyclops australis	1	1	1	1	1	1	1	Sa1, Sa25, Sa34, Ti1, Ti7, Ti11, Ti12, Ti27, Tu5
Boeckella fluvialis	1	0	0	1	1	0	1	Sa25, Ti1, Ti2, Ti11, Ti27
Boeckella pseudochelae	0	0	0	0	1	1	1	Tu21
Calamoecia canberra	1	1	1	1	0	0	1	Ti7, Ti14
Eucyclops australiensis	1	1	1	1	1	1	1	Sa1, Sa16, Sa25, Tu4
Eucyclops nichollsi	0	1	1	1	1	1	1	Tu18

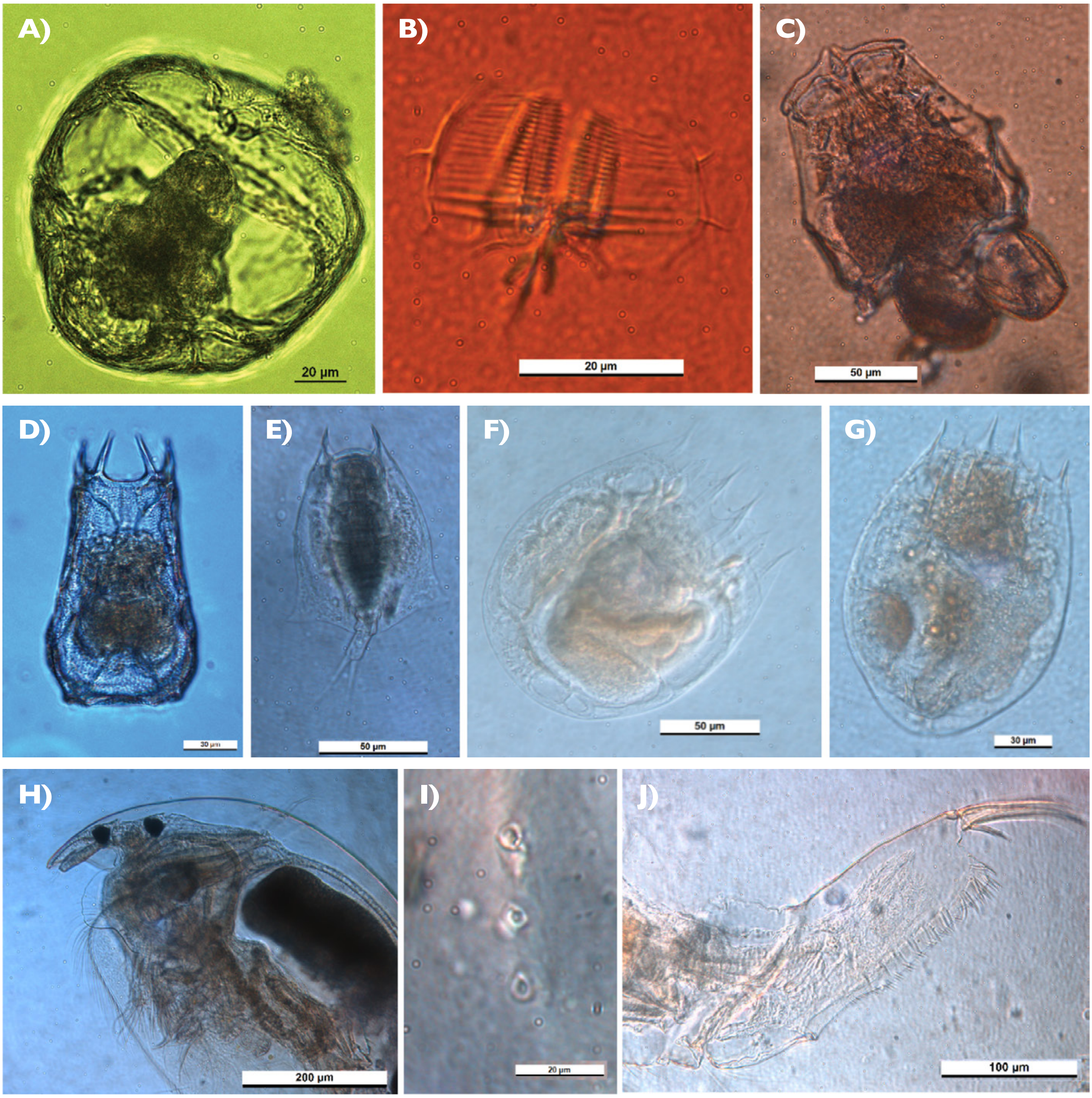

Figure 2. Photomicrographs of zooplankton in wetlands of the Lachlan River catchment. A) *Trochosphaera solstitialis*, B) trophi of *Trochosphaera solstitialis*, C) *Brachionus lyratus tasmaniensis*, D) *Keratella shieli*, E) *Lepadella tyleri*, F) *Notholca salina*, G) *Notholca squamula*, H) *Alona setuloides*, I) three head pores of *Alona setuloides*, and J) postabdomen of *Alona setuloides*. Photomicrographs: T. Kobayashi.

A plot of the integrated rarefaction and extrapolation sampling curve against sample size is shown with a 95% confidence interval (CI) for rotifers, cladocerans and copepods in Figs. 3A, 3C and 3E respectively. The estimated asymptotic species richness was 147.3 (CI: 119.9 to 174.7) for rotifers, 36.4 (CI: 19.1 to 53.7) for cladocerans and 18.1 (CI: 9.5 to 26.7) for copepods. A plot of the estimated sample completeness against sample size showed that the realised sample size (n = 32) in this study was effective to cover 89.3 (CI: 86.4 to 92.2), 95.4 (92.7 to 98.2) and 96.5 (92.2 to 100.0) % of the sample completeness for rotifers, cladocerans and copepods (Figs. 3B, 3D and 3F) respectively.

Discussion

Diverse zooplankton were observed in inland wetlands of the Lachlan River catchment, including the first record of the rotifer *Trochosphaera solstitialis* in Australia and the first record of the rotifers *Brachionus lyratus tasmaniensis*, *Keratella shieli*, *Lepadella tyleri*, *Notholca salina* and *N. squamula* and the cladoceran

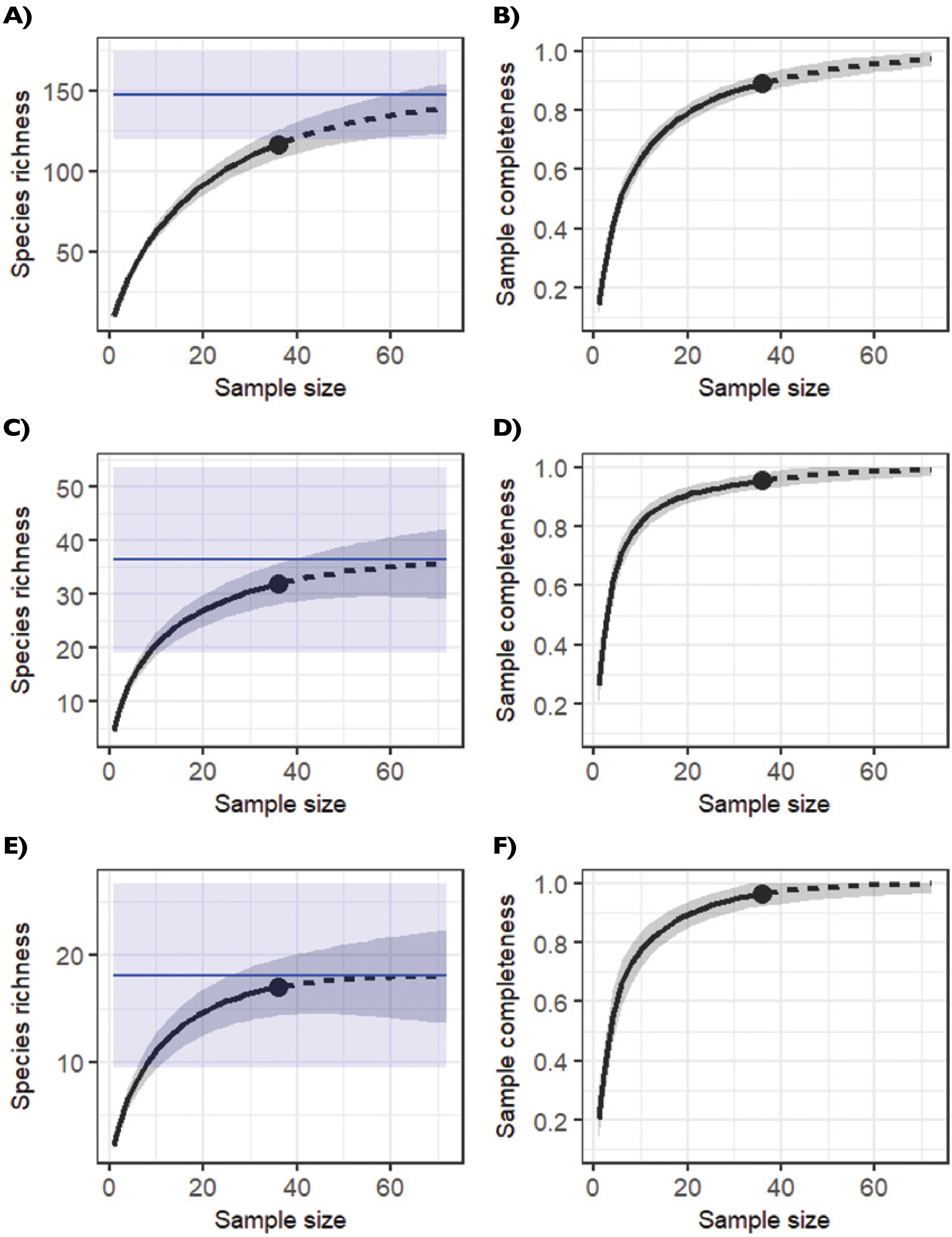

Figure 3. Observed and estimated species richness and sample completeness (estimated proportion of species collected) as a function of sample size. A) and B) rotifers, C) and D) cladocerans, and E) and F) copepods. For each zooplankton group, the rarefaction curve is shown by solid lines and the extrapolation curve is shown by dotted lines, with the observed species richness by closed dots. The asymptotic estimate of species richness is shown by blue horizontal lines. Blue and grey shaded areas represent the respective 95% confidence intervals based on bootstrap method (1000 times).

Alona setuloides in NSW. They make further additions to the recent new record of zooplankton, both endemic and non-endemic species, in inland wetlands of NSW (Table 2); they underscore the importance of a broader scale inventory of aquatic biodiversity including microinvertebrates for inland wetlands.

Trochosphaera solstitialis is a rare, thermophilous species, even though the species is widespread globally (Nogrady and Segers 2002). In the genus *Trochosphaera*, two species are currently known, with *T. aequatorialis* the other species. Morphologically, the two *Trochosphaera* species are distinguished by the position of corona, situated in anterior third of body for *T. solstitialis* and in equatorial position for *T. aequatorialis*. The trophi of *T. solstitialis* have two pairs of proximal unci teeth that are significantly greater than the distal unci teeth, whereas all unci teeth are similar in *T. aequatorialis*. The morphological features of the Lachlan River catchment *Trochosphaera* specimen clearly conform to those of *T. solstitialis* (Figs. 2A and 2B). *Brachionus lyratus tasmaniensis* and *L. tyleri* were both first reported and described from specimens collected in Tasmania (Koste and Shiel 1986, 1987). In Tasmania, both species occur in acidic waters (Koste and Shiel 1986, 1987). Morphologically *Brachionus lyratus tasmaniensis* (subspecies rank of *B. lyratus*) may be separated from *Brachionus lyratus* based on the facettation patterns on the dorsal surface, in addition to currently known differences between them, thus justifying its species status as *Brachionus tasmaniensis* (Koste & Shiel, 1986) (R.J. Shiel, pers. observe. 2020). Further comprehensive taxonomic analyses (both morphological and genetic) of *B. lyratus* and *B. lyratus tasmaniensis* are warranted. *Notholca salina* has been reported from the Lake Muir region in south-western Western Australia (Pinder *et al.* 2004), and *N. squamula* has been reported from multiple locations in Victoria and Tasmania (Green 1981; Koste *et al.* 1988). These two species of *Notholca* occur predominantly in alkaline waters (Koste *et al.* 1988). Among the cladocerans, the first record of *Alona setuloides* in NSW is intriguing as it is the only record of this species outside north-western Western Australia where the specimen was first collected and described as a new species (Smirnov and Timms 1983). *Alona setuloides* is said to be similar to *Alona setulosa* Megard, 1967 in external appearance (Smirnov and Timms 1983). However, *A. setuloides* is larger and has a much longer postabdomen than *A. setulosa*. In addition, the three main head pores of *A. setuloides* are clearly separated as found in this study (Fig. 2G), while those of *A. setulosa* have interrupted connections (Megard 1967; Sinev 2009). Because of the recent relocation of many *Alona* species including *A. setulosa* to the genus *Ovalona* (Sinev 2015), further detailed studies are warranted to clarify the taxonomic position of *A. setuloides* (A. Sinev, pers. comm. 2019, 2022).

For freshwater zooplankton, the degree of endemism is said to be similar or even higher in Australia (especially southwest Western Australia), relative to other regions of the world (Bayly and Morton 1978; Dumont 1983; Smirnov and Timms 1983; Korovchinsky 1996; Sinev and Shiel 2012). Some of the species previously endemic to Australia have now been recorded in other countries (e.g. *Keratella slacki* and *Mesocyclops australiensis* in New Zealand; *Ovalona pulchella* in China) (Sanoamuang and Stout 1983; Duggan and Pullan 2017; Gu *et al.* 2022). Based on this study, the degree of endemism seems to be relatively low for the zooplankton community

Table 2. Recent new records of inland wetland zooplankton in New South Wales. [M]Macquarie Marshes (147.531 °E/30.659 °S) (Kobayashi *et al.* 2007), [J]Jibbon Lagoon (151.165 °E/34.087 °S) (Kobayashi *et al.* 2012; Sinev and Kobayashi 2012), [T]Thirlmere Lakes (150.568 °E/34.229 °S) (Kobayashi *et al.* 2020), [*]Present study. **First record in Australia.

	Australian-endemic	Non-endemic
Rotifera	*Brachionus lyratus tasmaniensis* Koste & Shiel, 1986[*]	*Keratella javana* Hauer, 1938[T]
	Keratella shieli Koste, 1979[*]	*Lecane rhytida* Harring & Myers, 1926[T]
	Lepadella tyleri Koste & Shiel, 1987[*]	*Lecane shieli* Segers & Sanoamuang, 1994[M,**]
		Notholca salina Focke, 1961[*]
		Notholca squamula (Müller, 1786)[*]
		Notommata saccigera Ehrenberg, 1832[T,**]
		Rousseletia corniculata Harring, 1913[T]
		Trochosphaera solstitialis Thorpe, 1893[*,**]
Cladocera	*Acanthalona willisi* (Smirnov, 1989)[J]	
	Alona setuloides Smirnov & Timms, 1983[*]	-
	Miralona victoriensis Sinev, 2004[T]	
Copepoda	*Hemiboeckella searli* Sars, 1912[J]	-

in the Lachlan catchment. The community with low endemicity may not necessarily mean that it has low conservation values because there are other factors such as rarity and relictness of species to be considered (Arponen *et al.* 2005; Sahuquillo and Miracle 2015). In the Lachlan catchment, four of the observed Australian-endemic species (*K. shieli*, *A. setuloides*, *B. pseudochelae* and *E. nichollsi*) were rare, each occurring at a single site. Thus, these endemics may be highly vulnerable to local disappearance (extirpation). Geographically, the Australian-endemic species recorded in this study show a significant latitudinal-range (about 1,000 km) extension of the rotifers *B. lyratus tasmaniensis* and *L. tyleri* (Koste and Shiel 1986, 1987) and a significant longitudinal-range (over 3,000 km) extension of the cladoceran *A. setuloides* (Smirnov and Timms 1983; Griggs *et al.* 1999). These range extensions are based on the biogeographically disjunct records of the species. Such disjunct range extensions have been reported for other zooplankton species in Australia and elsewhere (e.g. Kobayashi *et al.* 2007; Altindağ *et al.* 2009; Sinev and Kobayashi 2012). They may chiefly reflect insufficient sampling and species identification efforts to determine true biogeographical distributions and the underlying dispersal and isolation mechanisms (Shiel *et al.* 1998; Griggs *et al.* 1999; Cox and Moore 2010).

Species richness is widely used as a measure of biodiversity (Magurran and McGill 2011). However, often it is not a straightforward task to gain accurate information on species richness in any local assemblages, due to constraints on sampling and statistical procedures (Gotelli and Colwell 2011). It is therefore necessary to employ methods to reduce the inherent under-sampling bias in observed species richness (Dumont and Segers 1996; Gotelli and Cholwell 2011). Based on the integrated sampling curves and asymptotic analysis of species richness (Chao *et al.* 2014, 2020), greater additions of species of rotifers (observed: 117 taxa; estimated asymptote: ≈ 147 taxa) are more likely to be realised with increasing sample size than those of cladocerans (observed: 32 taxa; estimated asymptote: ≈ 36 taxa) and copepods (observed: 17 taxa; estimated asymptote: ≈ 18 taxa) in wetlands of the Lachlan River catchment.

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APPENDIX I

APPENDIX I Photos of selected sampling sites in the Lachlan River catchment. The sites are listed from low to high longitudes (westerly to easterly direction) (see Fig. 1). Photos by Jan Miller.

Semi-arid zone

Site Sa5: Great Cumbung Swamp near Maud - floodplain wetland, uncleared grazing. Photo taken 15/08/2013.

Site Sa25: Willandra Creek at Willandra National Park - floodplain wetland, previously uncleared grazing, currently conservation. Photo taken 23/09/2013.

Site Sa16: Booberoi Creek near Euabalong - floodplain wetland, mix of uncleared grazing, cleared grazing and cropping. Photo taken 10/10/2013.

APPENDIX I

Temperate inland zone

Site Ti6: Lake Cowal - lake, lakebed cropping when dry, cattle grazing. Photo taken 2/11/2013.

Site Ti12: Goobang Creek at Lachlan Valley National Park - swampy meadow, formerly uncleared grazing and state forest, currently conservation. Photo taken 31/10/2013.

Site Ti4: Bundaburrah Cowal - floodplain wetland, mix of cleared and uncleared grazing. Photo taken 30/10/2013.

APPENDIX 1

Temperate upland zone

Site Tu5: Burwood Creek - swampy meadow, cleared grazing. Photo taken 19/06/2014.

Site Tu12: Hackney Creek - swampy meadow, cleared grazing/recently cultivated adjacent to pine plantation in forestry land. Photo taken 30/05/2014.

Site Tu39: Hooseberry Gully - swampy meadow, conservation reserve. Photo taken 21/06/2014.

APPENDIX 2

APPENDIX II Wetland zooplankton in the Lachlan River catchment.

ROTIFERA (n = 117)

1 *Ascomorpha saltans* Bartsch, 1870
2 *Asplanchna asymmetrica* Shiel & Koste, 1985
3 *Asplanchna brightwellii* Gosse, 1850
4 *Asplanchna priodonta* Gosse, 1850
5 *Asplanchna sieboldii* (Leydig, 1854)
6 *Asplanchnopus multiceps* (Shrank, 1793)
7 *Brachionus angularis* Gosse, 1851
8 *Brachionus bidentatus* Anderson, 1889
9 *Brachionus budapestinensis* Daday, 1885
10 *Brachionus calyciflorus* Pallas, 1766
11 *Brachionus keikoa* Koste, 1979
12 *Brachionus lyratus* Shephard, 1911
13 *Brachionus lyratus tasmaniensis* Koste & Shiel, 1986
14 *Brachionus* cf. *nilsoni* Ahlstrom, 1940
15 *Brachionus novaezealandiae* Morris, 1913
16 *Brachionus quadridentatus* Hermann, 1783
17 *Brachionus quadridentatus melheni* Barrois & Daday, 1894
18 *Brachionus rubens* Ehrenberg, 1838
19 *Brachionus urceolaris* Müller, 1773
20 *Brachionus* cf. *variabilis* Hempel, 1896
21 *Brachionus* spp.
22 *Cephalodella catellina* (Müller, 1786)
23 *Cephalodella gibba* (Ehrenberg, 1830)
24 *Cephalodella misgurnus* Wulfert, 1937
25 *Cephalodella* spp.
26 *Colurella adriatica* Ehrenberg, 1831
27 *Colurella obtusa* (Gosse, 1886)
28 *Colurella uncinata bicuspidata* (Ehrenberg, 1832)
29 *Colurella uncinata deflexa* (Ehrenberg, 1834)
30 *Conochilus dossuarius* Hudson, 1885
31 *Conochilus natans* (Seligo, 1900)
32 *Conochilus unicornis* Rousselet, 1892
33 *Dicranophoroides caudatus* (Ehrenberg, 1834)
34 *Dicranophorus epicharis* Harring & Myers, 1928
35 *Dissotrocha hertzogi* Hauer, 1939
36 *Encentrum uncinatum* (Milne, 1886)
37 *Encentrum* sp.
38 *Eosphora najas* Ehrenberg, 1830
39 *Eosphora thoides* Wulfert, 1935
40 *Epiphanes brachionus* (Ehrenberg, 1837)
41 *Epiphanes clavulata* (Ehrenberg, 1832)
42 *Epiphanes daphnicola* (Thompson, 1892)
43 *Epiphanes* cf. *macroura* (Barrois & Daday, 1894)
44 *Epiphanes spinosa* (Rousselet, 1901)

APPENDIX 2

45	*Epiphanes* sp.
46	*Euchlanis dilatata* Ehrenberg, 1832
47	*Euchlanis meneta* Myers, 1930
48	*Filinia australiensis* Koste, 1980
49	*Filinia grandis* (Koste & Shiel, 1980)
50	*Filinia longiseta* (Ehrenberg, 1834)
51	*Filinia pejleri* Hutchinson, 1964
52	*Filinia terminalis* (Plate, 1886)
53	*Hexarthra intermedia* (Wiszniewski, 1929)
54	*Hexarthra mira* (Hudson, 1871)
55	*Horaella brehmi* Donner, 1949
56	*Itura aurita* (Ehrenberg, 1830)
57	*Itura myersi* Wulfert, 1935
58	*Itura* sp.
59	*Keratella australis* Berzinś, 1963
60	*Keratella cochlearis* (Gosse, 1851)
61	*Keratella procurva* (Thorpe, 1891)
62	*Keratella shieli* Koste, 1979
63	*Keratella slacki* Berzinś, 1963
64	*Keratella tropica* (Apstein, 1907)
65	*Lecane arcuata* (Bryce, 1891)
66	*Lecane bulla* (Gosse, 1851)
67	*Lecane closterocerca* (Schmarda, 1859)
68	*Lecane curvicornis* (Murray, 1913)
69	*Lecane elsa* Hauer, 1931
70	*Lecane flexilis* (Gosse, 1886)
71	*Lecane hamata* (Stokes, 1896)
72	*Lecane ludwigii* (Eckstein, 1883)
73	*Lecane luna* (Müller, 1776)
74	*Lecane lunaris* (Ehrenberg, 1832)
75	*Lecane pyriformis* (Daday, 1905)
76	*Lepadella discoidea* Segers, 1993
77	*Lepadella oblonga* (Ehrenberg, 1834)
78	*Lepadella ovalis* (Müller, 1786)
79	*Lepadella patella* (Müller, 1773)
80	*Lepadella triptera* (Ehrenberg, 1830)
81	*Lepadella tyleri* Koste & Shiel, 1987
82	*Lepadella* sp.
83	*Lindia* sp.
84	*Lophocharis salpina* (Ehrenberg, 1834)
85	*Lophocharis* cf. *turanica* Mirabdullaev, 1992
86	*Lophocharis* sp.
87	*Monommata* sp.
88	*Notholca salina* Focke, 1961
89	*Notholca squamula* (Müller, 1786)
90	*Notommata copeus* Ehrenberg, 1834
91	*Notommata* sp.

APPENDIX 2

92 *Platyias quadricornis* (Ehrenberg, 1832)
93 *Polyarthra* cf. *dolichoptera* Idelson, 1925
94 *Polyarthra* cf. *longiremis* Carlin, 1943
95 *Proales* cf. *pejleri* De Smet, Van Rompu & Beyens, 1993
96 *Proales* sp.
97 *Scaridium longicaudum* (Müller, 1786)
98 *Sinantherina socialis* (Linnaeus, 1758)
99 *Squatinella lamellaris* (Müller, 1786)
100 *Synchaeta oblonga* Ehrenberg, 1832
101 *Synchaeta pectinata* Ehrenberg, 1832
102 *Testudinella patina* (Hermann, 1783)
103 *Testudinella* sp.
104 *Trichocerca bicristata* (Gosse, 1887)
105 *Trichocerca bidens* (Lucks, 1912)
106 *Trichocerca longiseta* (Schrank, 1802)
107 *Trichocerca porcellus* (Gosse, 1851)
108 *Trichocerca pusilla* (Jennings, 1903)
109 *Trichocerca rattus* (Müller, 1776)
110 *Trichocerca ruttneri* Donner, 1953
111 *Trichocerca similis* (Wierzejski, 1893)
112 *Trichocerca tenuior* (Gosse, 1886)
113 *Trichocerca* spp.
114 *Trochosphaera solstitialis* Thorpe, 1893
115 *Trichotria tetractis* (Ehrenberg, 1830)
116 *Wolga spinifera* (Western, 1894)
117 unidentified bdelloids

CLADOCERA (n = 32)

118 *Alona rigidicaudis* (Smirnov, 1971)
119 *Alona setuloides* Smirnov & Timms, 1983
120 *Alonella clathratula* Sars, 1896
121 *Armatalona macrocopa* (Sars, 1895)
122 *Biapertura kendallensis* (Henry, 1919)
123 *Bosmina meridionalis* Sars, 1904
124 *Ceriodaphnia* cf. *cornuta* Sars, 1885
125 *Ceriodaphnia* sp.
126 *Chydorus* spp.
127 *Daphnia carinata* King, 1853
128 *Daphnia lumholtzi* Sars, 1885
129 *Diaphanosoma unguiculatum* Gurney, 1927
130 *Dunhevedia crassa* King, 1853
131 *Flavalona setigera* (Brehm, 1931)
132 *Graptoleberis testudinaria* (Fischer, 1848)
133 *Ilyocryptus* sp.
134 *Leberis diaphanus* (King, 1853)
135 *Leydigia laevis* Gurney, 1927
136 *Leydigia leydigii* (Schoedler, 1863)

APPENDIX 2

137 *Macrothrix breviseta* Smirnov, 1976
138 *Macrothrix spinosa* King, 1853
139 *Moina australiensis* Sars, 1896
140 *Moina micrura* Kurz, 1874
141 *Moina tenuicornis* Sars, 1896
142 *Neothrix armata* Gurney, 1927
143 *Ovalona pulchella* (King, 1853)
144 *Oxyurella* cf. *tenuicaudis* (Sars, 1862)
145 *Pleuroxus inermis* Sars, 1896
146 *Scapholeberis kingi* Sars, 1903
147 *Simocephalus acutirostratus* (King, 1853)
148 *Simocephalus elizabethae* (King, 1853)
149 *Simocephalus exspinosus* (DeGeer, 1778)

COPEPODA (n = 17 including unidentified juvenile calanoids and cyclopoids)

150 *Acanthocyclops robustus* (Sars, 1863)
151 *Australocyclops australis* (Sars, 1896)
152 *Boeckella fluvialis* Henry, 1922
153 *Boeckella pseudochelae* Searle, 1912
154 *Boeckella triarticulata* (Thomson, 1883)
155 *Calamoecia ampulla* (Searle, 1911)
156 *Calamoecia canberra* Bayly, 1962
157 *Calamoecia lucasi* Brady, 1906
158 *Eucyclops australiensis* Morton, 1990
159 *Eucyclops nichollsi* Brehm, 1950
160 *Eucyclops* sp.
161 *Macrocyclops albidus* (Jurine, 1820)
162 *Mesocyclops australiensis* (Sars, 1908)
163 *Thermocyclops decipiens* (Kiefer, 1929)
164 *Tropocyclops* sp.
165 unidentified juvenile calanoids
166 unidentified juvenile cyclopoids