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Tardigrade eggs and exuviae in Antarctic lake sediments: insights into Holocene dynamics and origins of the fauna

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ABSTRACT

The preservation of tardigrade eggs and exuviae in Antarctic lake sediments provided an opportunity to assess post-glacial colonisation and Holocene tardigrade dynamics on the southern continent. Tardigrade eggs were recovered from five lakes, two from the maritime Antarctic and three from continental Antarctica. Eggs were identified from the following species: Dactylobiotus *cf.* ambiguus, Macrobiotus furciger, Macrobiotus blocki, Minibiotus weinerorum *and* Acutuncus antarcticus. *Other, unornamented eggs were also observed. The preservation of some of these eggs in exuviae allowed identification to at least genus. Significant variations were observed in egg abundance within the sediment of each lake, and in one lake a species (*Dactylobiotus *cf.* ambiguus*) became locally extinct, probably as the result of penguin-associated eutrophication. Tardigrades generally did not become abundant for a considerable period after the lakes' formation. The presence of an in-part endemic fauna is consistent with slow colonisation from Antarctic sources rather than wind transport from extra-continental sites. Tardigrade eggs appear to be abundant in high-latitude lake sediments, and greater use could be made of these records when evaluating tardigrade dynamics during the Holocene.*

Key words: colonisation, population dynamics, ecological change, Macrobiotus, Minibiotus, Acutuncus

1. INTRODUCTION

While there is evidence of tardigrades from the fossil record for the Cenozoic and Mesozoic periods (Cooper 1964; Bertolani & Grimaldi 2000), there is little information about tardigrade palaeobiogeography or population dynamics during more recent times. Modern biogeographical distribution can be interpreted in terms of early evolution of some genera (e.g., McInnes & Pugh 1998; Pilato & Binda 2001), but such data provide only a very broad temporal view of tardigrade population development. Scant information is available regarding the palaeodynamics of tardigrades during the Holocene (which covers the last 10000 years) that have ultimately determined the details of modern distributions.

For many organisms the study of remains preserved in lake sediments has led to many insights in terms of biogeography and also responses to environmental variability (Smol *et al*. 2001). Little attempt has been made to use this approach for tardigrades, largely as a result of the paucity of identifiable remains. In an exhaustive review, Frey (1964) listed only a few records from the classical scientific literature: eggs of *Macrobiotus echinogenitus* Richters, 1903 and *Macrobiotus islandicus* Richters, 1904 were recovered from European bogs, where they were common. More recently, tardigrade eggs have been reported from sediments from higher latitudes both in the northern (Jankovska 1991) and southern (Miller & Heatwole 2003) hemispheres.

In this paper we present data on the distribution of tardigrade egg and exuviae in the sediments of a series of Antarctic lakes, and discuss the implications for tardigrade dispersal and dynamics in this region over the last 10000 years.

Tardigrades are found throughout the ice-free areas of Antarctica, with >40 species recorded (see species list in Convey & McInnes 2005). Some of these species appear to be cosmopolitan, while others have far more limited distributions. However, the true nature of the fauna is uncertain, as many apparently cosmopolitan species may in fact be Antarctic endemics shoehorned into species from better-studied areas. For example, *Milnesium tardigradum* Doyère, 1840 has long been thought to be widespread in Antarctica, but it has recently been shown that at least one population is a new species, *Milnesium antarcticum* Tumanov, 2006 (Tumanov 2006). It is possible that other populations also differ at the species level (Dastych 1984). McInnes & Pugh (1998) concluded that most of the tardigrade fauna of Antarctica is of Gondwanan origin, and was in place prior to the start of the Pleistocene (1.8 Myr before present; BP) and probably much earlier. If so, there has been ample time for local speciation. How this fauna survived Pliocene and Pleistocene glaciations is uncertain, but probably involved survival of endemic Antarctic species in local refugia (relictual model)

Tab. 1. Details of the sediment cores used in this study for the isolation of tardigrade eggs. For two lakes a reference is given that contains further information about the collection of the cores.

Lake	Region	Latitude	Longitude	Date of collection	Length of core (m)	Reference
Boeckella	Hope Bay, Antarctic Peninsula	63° 24' S	57° 0' W	Nov 1987	2.95	Zale & Karlén 1989
Limnopolar	Livingston Island	62° 38' S	61° 4' W	Dec 2003	0.29	
Reid	Larsemann Hills	69° 23' S	76° 53' E	Dec 1997	1.15	Hodgson *et al.* 2001
Waterfall	Vestfold Hills	68° 33' S	78° 20' E	Dec 2003	2.04	
Terrasovoje	Amery Oasis	70° 33' S	68° 2' E	Dec 2003	3.04	

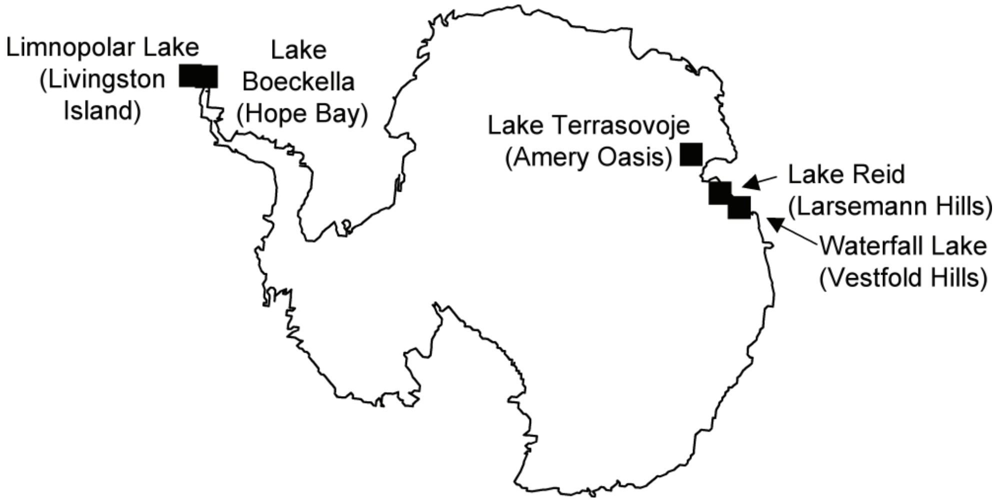

Fig. 1. Map of Antarctica showing the locations of the lakes sampled in this study.

rather than continental extinction followed by recolonisation from extra-continental locations during interglacial periods (dispersal model). Evidence for this conclusion comes from the presence of endemic Antarctic species, many of which have limited distributions (Dastych 1984).

2. METHODS

Sediment cores from the five fresh to slightly brackish Antarctic lakes listed in table 1 were obtained using piston or gravity corers (see also Fig. 1 for the locations of the lakes and other places named in the text). All cores were sectioned soon after collection and were subsequently stored at 4 °C. The core from Lake Boeckella was preserved with polyethyleneglycol (PEG) using the method of Tippet (1964) and stored at 4 °C.

In order to isolate tardigrade eggs and remains 1 g sub-samples of the sediment sections were dispersed in distilled water, and, after addition of a few drops of the stain Rose Bengal, allowed to stand overnight. This procedure also dissolved the PEG in the samples from the Lake Boeckella core. The samples were then washed through 200 µm, 100 µm and 40 µm sieves with pre-filtered water. All retained material was viewed under a dissecting microscope and tardigrade eggs and exuviae with or without eggs were counted. All abundances are recorded on a per gram dry weight basis (g_{dw}^{-1}). Samples were collected for both light and scanning electron microscopy (SEM).

The ages of the sediments were determined by radiocarbon dating. Age models for Lakes Reid and Boeckella have been presented elsewhere (Zale 1994; Hodgson *et al.* 2005). Details of age models for the other lakes are available from the corresponding author and will be discussed in more detail elsewhere.

Lake Boeckella is located in a small ice-free area at Hope Bay, close to the northern tip of the Antarctic Peninsula (Fig. 1). The basal sediments of Lake Boeckella were deposited circa 6000 yr BP. Limnopolar Lake is located on Byers Peninsula, Livingston Island, offshore from the western coast of the Antarctic Peninsula. The lake formed over 3000 yr BP (J. Gibson & A. Quesada, unpublished data), though core used in this study extended back to sediment deposited *ca* 2100 yr BP.

The other lakes studied were in East Antarctica. Waterfall Lake is a small, freshwater lake located in the Vestfold Hills, one of the largest ice-free areas (area >400 km^2) in East Antarctica. The basal sediment in Waterfall Lake was deposited over 6000 yr BP. Lake Reid is located in the Larsemann Hills, a small, coastal ice-free area about 100 km southwest of the Vestfold Hills. Lake Reid is one of very few Antarctic lakes that have been shown to have existed prior to the last glacial maximum 18000 yr BP. Hodgson *et al.* (2005) showed that sedimentation has occurred continuously in this lake since its formation >130000 yr BP. In contrast to all the other lakes in this study, Lake Terrasovoje (Amery Oasis) is located away from the modern-day

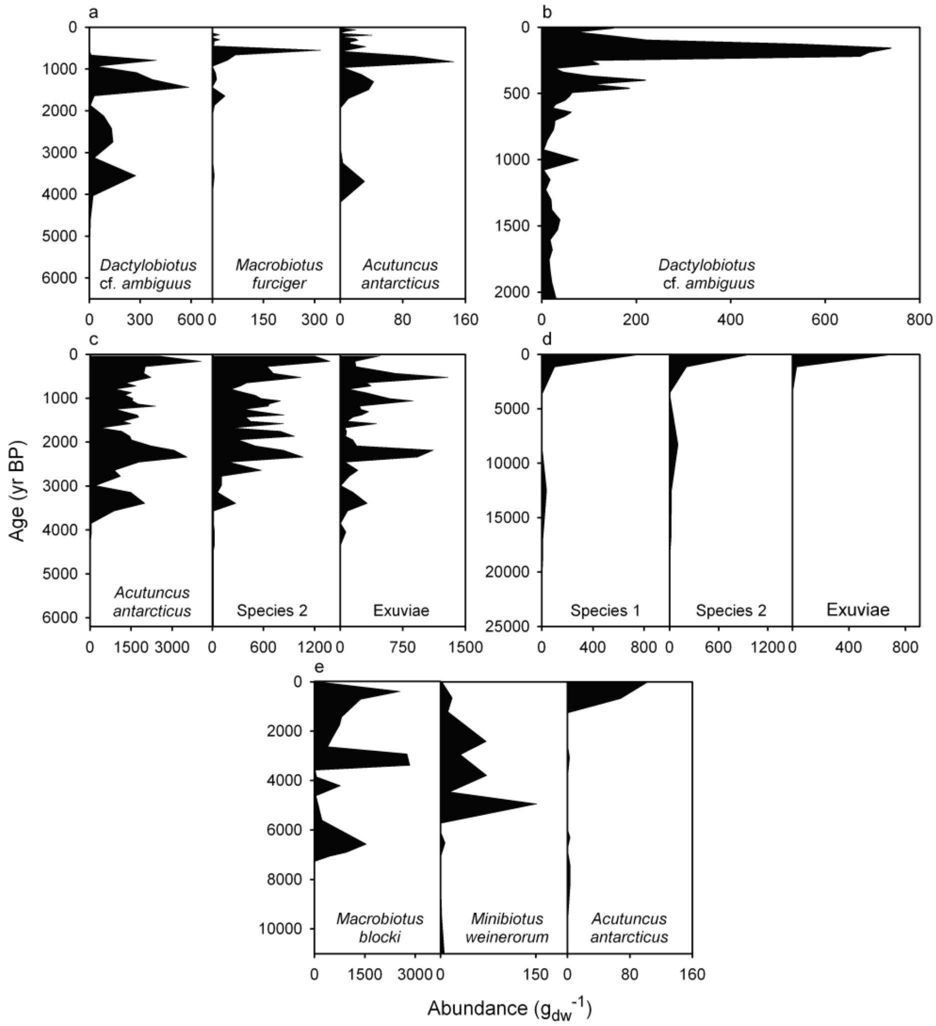

Fig. 2. Distribution of tardigrade eggs and exuviae in the sediments of five Antarctic lakes. **a**: Lake Boeckella. **b**: Limnopolar Lake. **c**: Waterfall Lake. **d**: Lake Reid. **e**: Lake Terrasovoje. The maximum ages plotted in figures (**a**), (**c**) and (**e**) are the estimated time for the onset of biological sedimentation in these lakes. The core from Limnopolar Lake did not reach glacial sediment, and only the period for which tardigrade remains were recorded is shown for Lake Reid.

coastline (Wagner *et al*. 2004). The Amery Oasis has been in part ice-free for up to 2 Ma (Fink *et al*. 2006), and is much older than the Vestfold or Larsemann Hills. The sediment record from Lake Terrasovoje spans the Holocene (Wagner *et al*. 2004).

3. RESULTS

Figure 2 shows the distributions of tardigrade eggs and exuviae in the sediment of the five Antarctic lakes included in this study.

3.1. Lake Boeckella

Tardigrade eggs were present throughout most of the Lake Boeckella core, though very few exuviae were recorded, and then from only the most recent sediments. Three common egg types were observed (Fig. 2a). The most abundant had features similar to eggs of the lacustrine species *Dactylobiotus ambiguus* (Murray 1907), and to the closely related species *Dactylobiotus caldarellai* Binda & Pilato, 1994, (Binda & Pilato 1994, 1999; McInnes & Pugh 1999), but could not be unambiguously assigned to either species. Here we refer to the species present in Lake Boeckella as *Dactylobiotus* cf. *ambiguus* (Fig. 3a). Eggs of *D.* cf. *ambiguus* first appeared close to the base of the sediment column, and were most abundant after the arrival of penguins in the area circa 2000 yr BP. No eggs were present in sediments deposited after 650 yr BP.

The second egg type belonged to the terrestrial species *Macrobiotus furciger* Murray 1907 (Fig. 3b), which is often associated with the nitrophilous alga *Prasiola crispa* (Lightfoot) Kützing 1843, but which may be present in the littoral zone of lakes (McInnes & Pugh 1999). This egg type was observed intermittently throughout the core at low abundance, though a sharp peak occurred soon after the disappearance of *D.* cf. *ambiguus* (Fig. 2a).

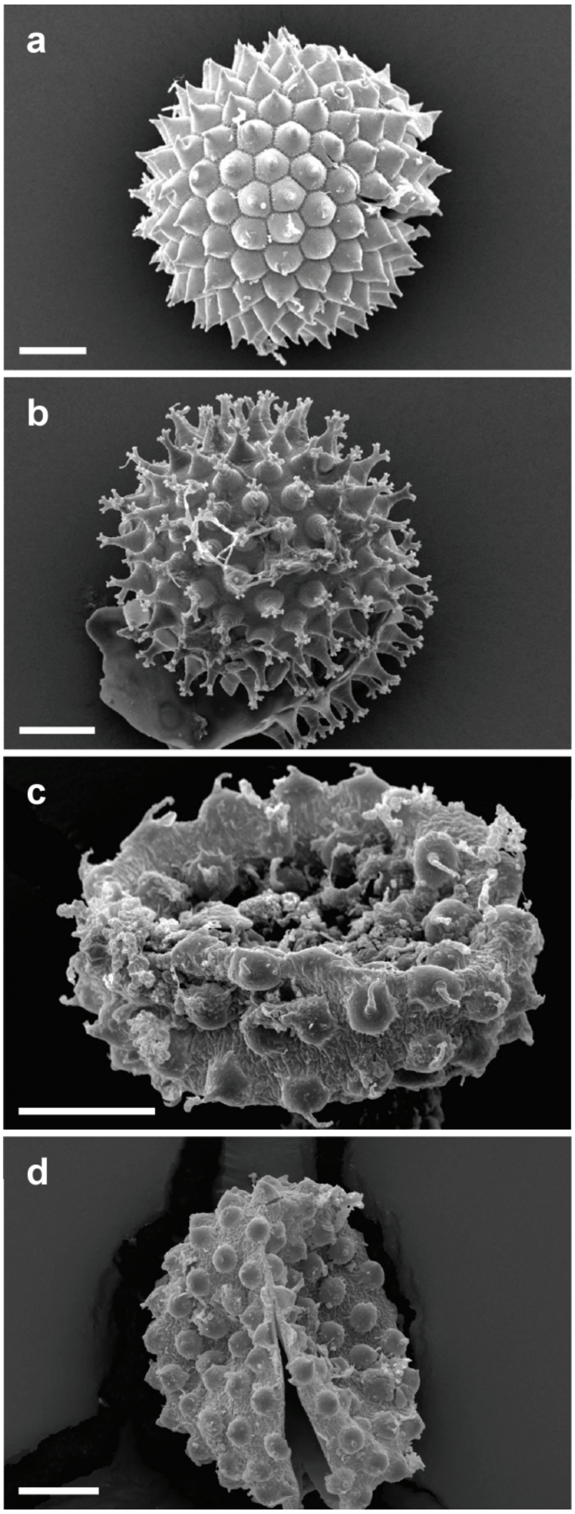

Fig. 3. Scanning electron micrographs of tardigrade eggs. **a**: *Dactylobiotus* cf. *ambiguus* (Lake Boeckella). **b**: *Macrobiotus furciger* (Lake Boeckella). **c**: *Macrobiotus blocki* (Lake Terrasovoje). **d**: *Minibiotus weinerorum* (Lake Terrasovoje). (Figs 3a and 3b from Gibson & Zale 2006). Scale bars = 20 µm.

The third egg type was identified as the widespread terrestrial and limnetic Antarctic species *Acutuncus antarcticus* (Richters 1904). These small eggs, which had the double outer membrane separated by short rods typical of this species (Pilato & Binda 1997), occurred sporadically throughout the sediment, first appearing circa 4000 yr BP. They were most abundant during the period when the lake was eutrophic.

3.2. Limnopolar Lake

Eggs of *D.* cf. *ambiguus* were recorded throughout the core from Limnopolar Lake, but generally in low numbers (<300 g_{dw}^{-1}) (Fig. 2b). However, abundance began to increase about 500 yr BP, and after an initial peak 400 yr BP reached a maximum (>700 g_{dw}^{-1}) late in the 18th century and throughout the 19th century. No other tardigrade eggs were observed in this core.

3.3. Waterfall Lake

Both tardigrade eggs and exuviae (many containing eggs) (Fig. 4) were common in the sediment of Waterfall Lake (Fig. 2c). The base of the sediment core was deposited approximately 6200 yr BP, but no tardigrade remains were recorded in sediment deposited prior to 5000 yr BP. The abundance of eggs and exuviae increased markedly from 4000 yr BP, reaching total abundance of over 4000 g_{dw}^{-1} in recent sediments.

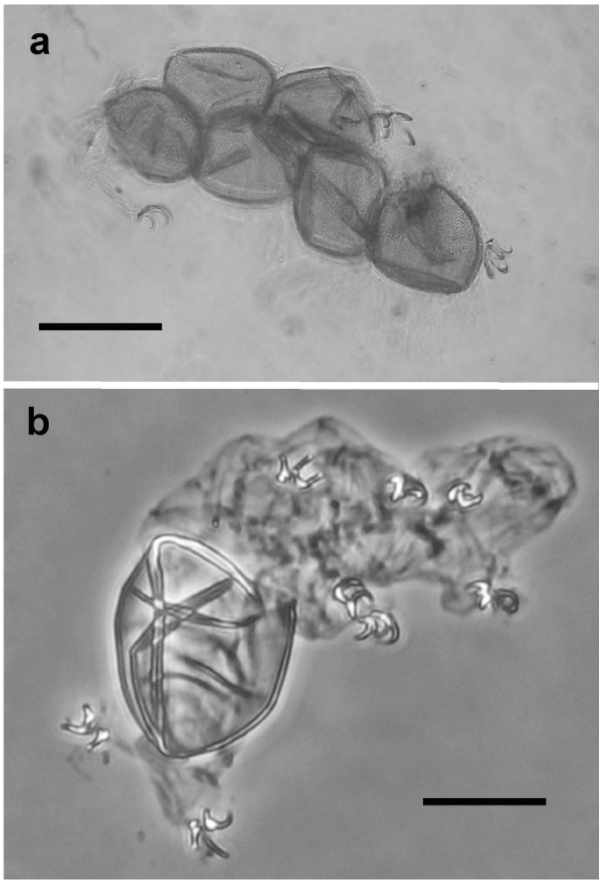

Fig. 4. Light micrographs (phase contrast) of unidentified tardigrade exuviae from the sediment of Waterfall Lake, Vestfold Hills. Note the presence of eggs in 4a. Scale bars = 50 µm.

Eggs of *A. antarcticus* were present at abundances up to 3500 g_{dw}^{-1}, with peaks occurring at about 3500, 2400 and 200 yr BP. Further species that produced smooth, featureless eggs were observed, but could not be identified. The claw structure of exuviae containing these eggs suggested that they were from the genera *Isohypsibius* and *Diphascon*. Both genera have previously been reported from the Vestfold Hills (Miller *et al*. 1988). These species (which were not separated during counting) were present at total abundances up to 1200 g_{dw}^{-1}, and exhibited peaks at the same time as *A. antarcticus* though with different relative abundance. Further peaks occurred between 2500 yr BP and the present. Exuviae, which were present from the earliest appearance of *A. antarcticus* eggs, were not separated during the counts. Maximum abundance of exuviae reached 1000 g_{dw}^{-1}, and the peaks closely matched those of the eggs.

3.4. Lake Reid

The results for Lake Reid (Fig. 2d) were initially presented by Cromer *et al*. (2006). These authors recorded two (and possibly more) unornamented tardigrade egg types in the sediment of Lake Reid that were not identified, but were possibly the same species recorded in Waterfall Lake. The oldest eggs were in sediment deposited more than 20000 yr BP, though abundance was greatest (ca 1000 g_{dw}^{-1} for each of the two species) in recently deposited sediment. Unidentified exuviae were also present (maximum abundance *ca* 700 g_{dw}^{-1}) in sediments deposited less than 5000 yr BP.

3.5. Lake Terrasovoje

The sediment contained eggs of three species (Fig. 2e) - *Macrobiotus blocki* Dastych 1984 (Fig. 3c), *Minibiotus weinerorum* (Dastych 1984) (Fig. 3d) and *A. antarcticus* - but few if any exuviae. Eggs of *M. blocki* were present sporadically and at low abundance until *ca* 7500 yr BP, when a rapid increase in numbers occurred. Peak abundance (> 2500 g_{dw}^{-1}) occurred *ca* 3000 yr BP. Eggs of *M. weinerorum* were less common (<150 g_{dw}^{-1}), and were also present sporadically until about 7500 yr BP. Eggs of *A. antarcticus* occurred intermittently and at low abundance throughout most of the core, though were more common (maximum 100 g_{dw}^{-1}) in sediments deposited in the last 1000 years.

4. DISCUSSION

Preserved tardigrade eggs and in some cases exuviae were found to be abundant in the sediments of Antarctic lakes. The eggs provide important information not only about the identity of tardigrades present throughout the lakes' histories but also changes in tardigrade abundance with time. Preservation can be very good, notably in this study for the eggs of *Dactylobiotus* cf. *ambiguus* and *Macrobiotus furciger* from Lake Boeckella (Fig. 3). The absence of tardigrade eggs in sediment of Lake Reid deposited prior to *ca* 20000 yr BP provides an estimate for the length of time that the eggs can remain in Antarctic lake sediment before decomposition, at least under the chemical and microbiological conditions experienced in the sediment of this lake. Exuviae appear to decompose more rapidly (as evidenced by their distribution in the sediment of Lake Reid), but can remain intact for up to 5000 years.

The distribution and identity of the eggs and exuviae yields information about the colonization of the lakes and the biogeography of the species whose remains were identified. In nearly all cases the species present at the base of a core were the same as those present in the lake today. The exception was the disappearance of *D.* cf. *ambiguus* in Lake Boeckella during a period of eutrophication induced by the input of nutrients from nearby penguin rookeries (Gibson & Zale 2006). This species occurs widely in the maritime Antarctic (Convey & McInnes 2005), and therefore its disappearance from Lake Boeckella was a local, rather than widespread event.

There is little evidence from the distribution of the tardigrade remains recorded in this study for the continued colonization of the lakes by new tardigrade species. The modern-day tardigrade fauna was in place early in the Holocene (or prior to the Holocene), and there is little evidence of significant dispersal of species over the last 10000 years. For example, the faunas of the Vestfold and Larsemann Hills are quite different to that of Lake Terrasovoje, indicating that there has been little if any transport of propagules between these areas. Similarly, the widespread species *Acutuncus antarcticus* does not appear to have reached Limnopolar Lake, even though this species is present on the nearby Antarctic Peninsula. Furthermore, if long-distance dispersal were occurring it would be expected that new species should appear in the sediment record through time as they reached particular lakes. No such step-wise increase in biodiversity was observed.

It is perhaps surprising that in Lakes Waterfall, Terrasovoje and Boeckella tardigrade remains became abundant hundreds if not thousands of years after the onset of biological sedimentation. Scattered occurrences of eggs prior to this increase in abundance suggests that tardigrades were present in the lake basins soon after their formation, but that it took considerable time for the correct ecological conditions to appear to support significant populations of animals. These conditions may have included the formation of extensive lake edge moss beds (for terrestrial species) or lacustrine microbial mats (limnetic species).

The basins of Lake Boeckella, Limnopolar Lake and Waterfall Lake were glaciated at the Last Glacial Maximum, and therefore tardigrades must have colonised the basins after deglaciation. The status of Lake Terrasovoje is uncertain (L. Cromer & J. Gibson, unpublished data), and Lake Reid remained unglaciated

(Hodgson *et al*. 2005). If the conclusions drawn above – that there has been no widespread Holocene colonization of the Antarctic continent - it can be concluded that this was most likely the result of dispersal from local refugia, as the first eggs appeared soon after the onset of sedimentation in the lake. Evidence for local transport of tardigrade propagules was provided by Christner *et al*. (2003), who recorded tardigrades in cryoconite holes on the Canada Glacier (McMurdo Dry Valleys). These are short-lived environments with little physical connectivity between individual holes (Müller & Pollard 2004). Therefore colonisation over a short time period (years) and relatively short distances from more permanent populations nearby must occur (Dastych *et al*. 2003). Identifying the refugia that provided the initial populations of tardigrades for Waterfall Lake and Lake Boeckella is difficult. For Waterfall Lake the source was probably other areas of the Vestfold Hills that had become deglaciated earlier in the Holocene (which in turn may have received its biota from the long-term refugium present in the nearby Larsemann Hills; Hodgson *et al*. 2001), and for Lake Boeckella islands to the north or the possible glacial refugium on Alexander Island (Maslen & Convey 2006) to the south. *Dactylobiotus* cf. *ambiguus* may well have colonized Limnopolar Lake and the remainder of Livingston Island from older ice-free areas on the Antarctic mainland or on nearby King George Island.

After colonization of the lakes the abundances of tardigrades have probably been controlled by the physical environment (temperature and perhaps salinity) and by food supply. In Waterfall Lake the abundances of the two tardigrade species paralleled each other, indicating that both populations were responding to the same ecological pressures. There appeared to be no separation of the species into different ecological niches or marked competition between the species, both of which could have resulted in the breakdown of the positive correlation between the abundances of these species. The situation in Lake Boeckella was different, in that *D.* cf. *ambiguus* became extinct in the lake probably as a result of eutrophication. *Macrobiotus furciger* was relatively scarce prior to the disappearance of *D.* cf. *ambiguus*, but became more abundant just after the other species' disappearance. It appears that *M. furciger* was able to take advantage of the new environmental conditions and perhaps of the absence of the other species, at least in the short term.

5. CONCLUSIONS

This study has shown that tardigrade eggs and exuviae preserved in lake sediments provide insight into tardigrade colonisation processes and dynamics in Antarctic lakes. The species present can in some cases be readily identified; initial times of colonisation can be determined, or at least last possible dates for colonisation can be estimated; and Holocene tardigrade dynamics, including responses to climate, are recorded. The distribution of tardigrade remains in Antarctic lake sediments both in space and time is not consistent with Holocene dispersal, but rather indicates the importance of longer-term, pre-existing distributions. Pugh & McInnes (1998) suggested that tardigrades spread from North America over most of the Arctic quite recently. Study of the sediments of the abundant lakes in the Arctic could provide new clues to test this hypothesis.

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