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Trophic interactions in an austral temperate ephemeral pond inferred using stable isotope analysis

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Abstract Ephemeral ponds are vulnerable aquatic habitats which are difficult to protect given their dynamic nature and sensitivity to degradation during dry periods. Little information is available on these habitats in austral regions, with almost no information on food-web structure and complexity. The study aimed to assess trophic interactions among dominant organisms in an ephemeral pond food web, and investigate the importance of autochthonous and allochthonous carbon, using ^{13}C and ^{15}N isotopes. Results of the investigation suggest that the food web comprised four trophic levels, with the top predators being Notonectids (*Notonecta* sp.) and diving beetles (*Cybister tripunctatus* (Olivier)). Intermediary trophic levels comprised zooplankton (daphniids and copepodids), macroinvertebrates (e.g. micronectids and molluscs) and tadpoles. Generalist feeders dominated the higher trophic levels (>3) with specialists comprising the lower trophic levels (≤3). The consumers preferred autochthonous fine particulate organic matter, epiphyton and submerged macrophyte organic matter sources over allochthonous sources. Autochthonous organic matter was transferred to the food web via zooplankton and select macroinvertebrates including *Micronecta* sp. and *Physa* sp. The food-web structure within the pond appeared to reflect the secondary stage of trophic structural complexity in the evolution of ephemeral ponds over the course of their hydro-period.

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Introduction

Ephemeral ponds are an essential habitat for a variety of amphibians, invertebrates and fish and often represent important foraging ground for wading birds (Heyer et al., 1975; Ferreira et al., 2012; Polačik et al., 2014). Despite their ecological importance, the greater majority of these systems receive little or no protection and are often degraded or destroyed during the dry phase of their hydro-period (Palik et al., 2006).

Ephemeral ponds are among the most difficult freshwater ecosystems to create or restore primarily because of their unique hydrological and ecological properties (Gebo & Brooks, 2012). Since ephemeral ponds are susceptible to degradation and loss, organisms dependent on these water bodies are particularly vulnerable (Gamble & Mitsch, 2009; Gebo & Brooks, 2012). One factor which makes it difficult to conduct ephemeral ponds surveys is that they are often not mapped due to their relatively small size and variable hydro-period. The development of strategies for the successful management of freshwater systems depends on our knowledge of biogeochemical dynamics and their dependence on hydrological regimes (Kopprio et al., 2014).

Ephemeral ponds provide convenient systems for testing ecological theories and represent important habitats for invertebrates and amphibians (Blaustein & Schwartz, 2001; De Meester et al., 2005). The organisms that inhabit these systems typically have characteristics associated with rapid growth and high-reproductive rates reflecting the temporal nature of these systems (Marcus & Weeks, 1997). Among the animal communities, crustaceans, branchiopods, hexapods and amphibians typically predominate, while fish are largely absent (Zacharias et al., 2007).

Many freshwater bodies receive high loadings of organic matter from adjacent environments and/or upland catchments, and consumers are often supported by these externally produced energy sources (Carpenter et al., 2005; Francis et al., 2011). Thus, freshwater ecosystems are exposed to subsidies from the terrestrial environments as a result of the aquatic ecosystems high perimeter to area ratios that characterize the amount of contact between aquatic and terrestrial systems (Francis et al., 2011). This is particularly pertinent for small ephemeral aquatic habitats, and stresses the importance of determining the relative contributions of allochthonous and autochthonous resources in food-web dynamics in these systems. One way to assess food-web dynamics within this context is to employ stable isotopes (Post, 2002; Carpenter et al., 2005; Kopprio et al., 2014; Yang et al., 2014). This method provides time-integrated information on the material assimilated by organisms thereby allowing the understanding of inorganic and organic carbon pools and changes therein (Arcagni et al., 2013). Carbon and nitrogen stable isotope compositions (δ^{13}C and δ^{15}N) are most commonly used in ecological studies (Abrantes et al., 2014). The δ^{13}C changes little from food source to consumer (McCutchan et al., 2003) but can vary between different producers and is generally used as a source indicator (Mao et al., 2012). The trophic fractionation of δ^{15}N, however, is mostly used as an indicator of trophic position (Post, 2002; McCutchan et al., 2003; Layman et al., 2007; Mao et al., 2012). The δ^{15}N can also differ between sources, hence it can give information on diet, especially when combined with δ^{13}C (Fry, 1991; Mao et al., 2012; Abrantes et al., 2014, Hill et al., 2015). As a result, δ^{13}C and δ^{15}N analyses can provide critical information about trophic relationships, food sources and the different energy pathways utilized (Huang et al., 2007; Mao et al., 2012; Arcagni et al., 2013). This approach has, however, been underutilized in African freshwater studies with the majority of published stable isotope food-web studies in the region having been conducted in estuaries or near shore marine environments (see for example, Froneman, 2002; Abrantes et al., 2014; Bergamino et al., 2014; Hill et al., 2015).

There are few studies on the food webs of freshwater environments in southern Africa, although there has been renewed interest in these systems (e.g. Harding & Hart, 2013), the focus has principally been on large freshwater river systems (e.g. Hecky & Hesslein, 1995; O'Reilly et al., 2002; Hill et al., 2015). The absence of food-web studies in small ponds within the temperate zone of South Africa is surprising, given the large number of such systems in the region (Ferreira et al., 2012). The current study, therefore, employed stable isotope analyses to assess the food-web structure and trophic interactions among the dominant faunal groups within a small-sized ephemeral pond located in the temperate Eastern Cape Province of South Africa. The main aim of this study was to assess the importance of autochthonous and allochthonous carbon sources in the food web and to assess trophic interactions among the major components of the zooplankton and macroinvertebrates using δ^{13}C and δ^{15}N isotope analysis in a small ephemeral pond. Given the small size of the pond, we hypothesized that allochthonous sources would be particularly important in the food web as previous research suggests that in oligotrophic freshwater systems, the detrital food web predominates. Moreover, we hypothesized that the detritus in the small system is comprised largely of subsidized material from adjacent terrestrial habitats.

Materials and methods

Study area

The small ephemeral pond (33°15′S, 26°26′E) lies approximately 10 km northwest of Grahamstown in the Eastern Cape Province of South Africa (Fig. 1). The pool is located within a temperate climatic zone, with warm atmospheric summer mean daily temperatures of 20.3°C (January) and mild winter mean daily temperatures of 12.3°C (June; Sinchembe & Ellery, 2010). Rainfall is distributed evenly over the entire catchment, with mean annual rainfall of ∼680 mm, which is concentrated in the summer months from September to March (Sinchembe & Ellery, 2010). The pond, at the time of sampling had a maximum depth of 1.33 m, a length of 61.8 m a maximum width of 27.2 m and an estimated total surface area of 1680 m^2 at full capacity (Suárez-Morales et al., 2015).

The vegetation in the immediate vicinity of the pond comprised grassland (*Sporobolus africanus* (Poir.) Robyns & Tournay), which is dominated by clumps of *Acacia karroo* Hyne shrubs (<1.5 m in total height). The littoral zone of the pond was characterized by extensive beds of *Cyperus marginatus* Thunb., *Potamogeton schweinfurthii* A. Benn., *Lagarosiphon muscoides* Harv. and *Laurembergia repens* P. J. Bergius subsp. *brachypoda* (Welw. ex Hiern) Oberm. The pond is situated on a privately owned farm and is utilized by cattle (*Bos taurus* Linnaeus) and sheep (*Ovis aries aries* var. *merino* Linnaeus) for drinking purposes. While the pond typically dries out during the dry periods, fine-scale hydro-period details are yet to be assessed.

Physical and chemical variables

Portable probes (CyberScan Series 600, Eutech Instruments, Singapore) were employed to measure

Fig. 1 Geographic location and shape of the small ephemeral pond in the Eastern Cape of South Africa
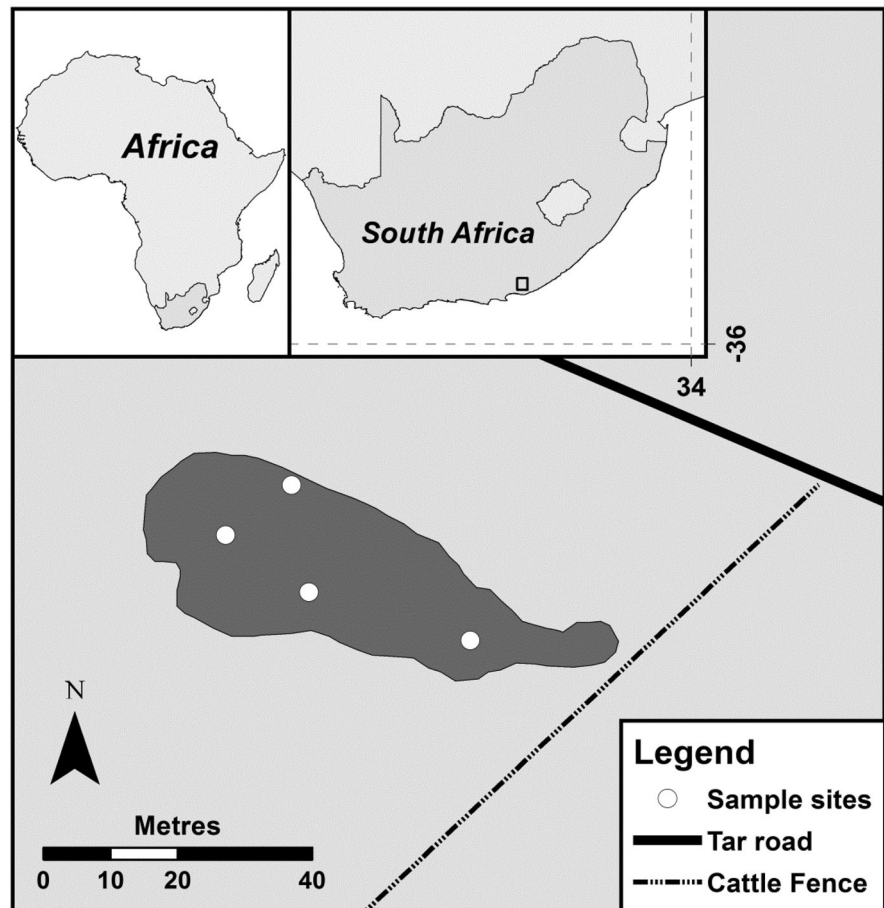
conductivity, dissolved oxygen, pH, salinity, total dissolved solids and water temperature on site from four points in the pond (Fig. 1). Additionally, water samples (500 mL) were collected and placed on ice for the determination of dissolved ammonia, phosphate and nitrate concentrations in the laboratory using a HI 83203 multi-parameter bench photometer (Hanna Instruments Inc., Rhode Island). Concentrations were determined within 1 h of water sample collection.

Chlorophyll-a analysis

Chlorophyll-*a* (chl-*a*) measurements in the water column and macrophytes were analysed to give a proxy of the suspended and epiphyton algal concentrations from the four sampling points. Epiphyton brushed off 10 *Cyperus marginatus* 30 cm stalks using a toothbrush in 500 mL distilled water that were collected from four different sites of the pond together with 500 mL water samples for determination of suspended chl-*a* concentrations. Samples were stored under dark conditions until in the laboratory (within 1 h) whereby 250 mL of each sample was filtered and extracted as described in APHA (1995). Epiphyton chl-*a* concentration was presented as $\mu g \ l^{-1}$ per 30 cm stalk and water column chl-*a* as $\mu g \ l^{-1}$.

Isotope sample collection

Basal food sources and consumer samples were collected in July 2014, when the pond was at approximately half capacity. Four surface water samples, 30–40 cm depth, were collected using 20 l containers for the determination of fine particulate organic matter (FPOM, <500 μm size). Epiphyton samples were collected from submerged stalks of the dominant macrophyte species, *C. marginatus,* and were processed within an hour of collection. This was done in the laboratory, whereby the epiphyton brushed off the stalks, using a toothbrush, into distilled water with replicate samples ($n = 4$) attained from separate stalks. FPOM and epiphyton water were then pre-filtered through a 64 μm mesh to remove zooplankton and coarse particulate organic matter (CPOM). The water was then further filtered through pre-combusted (450°C, 5 h) Whatman GF/F filters. Visible zooplankton which passed through the pre-filtration was removed with forceps under a dissecting Olympus microscope operated at 100× magnification. Each GF/

F filter was then placed in a separate labelled pre-combusted (450°C, 5 h) aluminium foil and stored at −20°C.

Using a van Veen grab, two independent sediment samples were collected (bite depth ≈ 1–2 cm) and placed into sterile plastic bags for laboratory analysis. Green leaves and stems of the macrophytes *C. marginatus, L. muscoides, P. schweinfurthii* and *L. repens* subsp. *brachypoda* were collected by hand, while CPOM was obtained from the surface sediment by hand picking. All samples were placed in separate labelled ziplock bags. Green leaves of the terrestrial shrub *Acacia karroo* Hayne and C_4 grass *S. africanus* were hand collected and placed in labelled ziplock bags. Fresh cow and sheep dung were hand collected around the pond. In the laboratory, the sediment, macrophytes, terrestrial vegetation, cow and sheep dung were placed in pre-combusted foil envelopes and stored at −20°C.

During an exploratory survey to determine the size structuring of the zooplankton community, a 32 cm Ø mouth diameter 63 μm mesh zooplankton net was towed for 10 m through both open water and vegetated zones in the pond. For isotope collection, however, we employed a 200 μm mesh zooplankton net (50 cm Ø), in the same manner as this would collect the vast majority of zooplankton community, while ensuring a sample largely free of particulate organic matter. Zooplankton tows were conducted perpendicular to the littoral zone, while macroinvertebrate and tadpole samples were collected using a nylon hand net (500 μm mesh size, 30 × 30 cm dimension). Benthic invertebrate samples were collected by disturbing the sediment to suspend any organisms and collected using a hand net. All macroinvertebrate and frog species were identified to the lowest taxonomic level (genus or species level) using the field keys of du Preez & Carruthers (2009), Gerber & Gabriel (2002) and Suárez-Morales et al. (2015). The collected samples were sorted in the field and placed in separate containers, and the macroinvertebrate samples were left overnight to clear their guts. The macroinvertebrate samples were then placed in labelled pre-combusted aluminium foil envelopes and stored at −20°C. Tadpole tail muscle tissue samples were used in this study, since these are less variable in $\delta^{13}C$ and $\delta^{15}N$ than other tissue types (Pinnegar & Polunin, 1999; Mao et al., 2012), the whole body mass of invertebrates was used.

Stable isotope sample processing and analysis

All samples were freeze dried using a VirTis Benchtop 2 K freeze drier at −60°C for 36 h. The freeze-dried samples of sediment, macrophytes, macroinvertebrates, muscles, dung and terrestrial vegetation were further ground to a fine homogeneous powder using a mortar and pestle, and about 0.8–2 mg of the sample was placed in tin capsules. However, *Physa* sp., *Cyzicus* sp., *Cypricercus* sp. and *Lynceus* sp. had their shells removed prior to grinding, as these parts are typically carbon enriched. Before placement into tin capsules, dried samples of sediment were acidified by vortexing for 2 min in 2 M hydrochloric acid, centrifugation for 5 min at 3600 rpm, washed twice in deionised water followed again by centrifugation, dried at 50°C and homogenized in a Retsch Mixer Mill. Approximately, 1 mg each of dried epiphyton and FPOM material was collected from the Whatman GF/F filters and placed into tin capsules.

Stable isotope analyses were conducted at the IsoEnvironmental Laboratory at South African Institute for Aquatic Biodiversity (SAIAB) using a Europa Scientific 20–20 Isotope Ratio Mass Spectrometer linked to an ANCA SL Prep Unit. Carbon and nitrogen isotopic signatures were expressed as the relative differences between isotopic ratios in the sample and conventional standards (internal: beet sugar and ammonium sulphate, and certified Casein protein standard), using the standard equation:

$$\delta^{13}C \ or \ \delta^{15}N(\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The trophic positions (TP) of the various consumers in the pond were estimated using the formula of McCutchan et al. (2003) and Huang et al. (2007):

$$TP = \left(^{15}N_{\text{consumer}} - {}^{15}N_{\text{Daphnia magna}} \right) / 3.4 + 2$$

where $\delta^{15}N_{\text{consumer}}$ is the measured consumer $\delta^{15}N$ for which TP needs to be estimated and $\delta^{15}N_{\text{Daphnia magna}}$ is the average $\delta^{15}N$ of the primary consumer, in this case, *Daphnia magna* Straus, and 3.4 is the trophic fractionation for $\delta^{15}N$ (Mao et al., 2012). The level 2 was consequently attributed, empirically, to *D. magna* (Mao et al., 2012).

Data analysis

The $\delta^{13}C$ and $\delta^{15}N$ values of the basal food sources and consumers were compared by means of a one-way ANOVA test using SPSS version16.0 for Windows software (SPSS Inc., 2007). The relative proportions of selected different basal food sources (epiphyton, FPOM, CPOM, *L. repens* subsp. *brachypoda*, *C. marginatus, P. schweinfurthii*, *L. muscoides,* sheep dung) and prey (Acari, *Chironomus* sp., *D. magna*, *Lovenula raynerae* Suárez-Morales, Wasserman & Dalu, *Physa* sp., *Paradiaptomus lamellatus* Sars, *Micronecta* sp., *Xenopus laevis* Loveridge tadpole small) based on the literature (e.g. Fernando & Leong, 1963; Vareschi & Jacobs, 1985; Giller, 1986; Pinder, 1986; Proctor & Pritchard, 1989; Allanson et al., 1990; Measey, 1998; Dillon, 2000; Ohba, 2009) to diets of the potential selected consumers (Acari, *Cybister tripunctatus* (Olivier), *L. raynerae*, *Micronecta* sp., *Notonecta* sp., *Physa* sp. *P. lamellatus, X. laevis* tadpoles) were assessed using a Bayesian mixing model, Stable Isotope Analyses in R (SIAR; Parnell et al., 2010). Following recommendations by Phillips et al. (2005, 2014) and Fry (2006), *P. schweinfurthii* and *L. repens* subsp. *brachypoda* were lumped together to form the group macrophytes (1) as they had very similar isotopic values. The SIAR model was run using data from the basal food sources and prey for the consumers in the pond. The Bayesian SIAR model incorporates uncertainty and variation in parameters (Parnell et al., 2010). Fractionation factors of $\delta^{15}N$ 2.3 ± 0.18 and $\delta^{13}C$ 0.5 ± 0.13 were used for all animals and $\delta^{15}N$ 1.1 ± 0.29 and $\delta^{13}C$ −0.21 ± 0.21 for all acidified samples (McCutchan et al., 2003; Huang et al., 2007; Mao et al., 2012).

The stable isotope Bayesian ellipses in R (SIBER) model in SIAR were employed to analyse the isotopic niche breadth and overlap among select dominant consumers: Acari, *Notonecta* sp., *Micronecta* sp., *P. lamellatus, L. raynerae* and *X. laevis* tadpoles using their $\delta^{13}C$ and $\delta^{15}N$ values (Layman et al., 2007; Jackson et al., 2011). Convex hull areas and ellipses represent the calculated isotopic niche breadths and widths for all individuals as described by Jackson et al. (2011). Standard ellipse area (SEAc), which provides a bivariate measure of mean isotopic niche, was calculated using SIBER (Jackson et al., 2011). The small letter 'c' indicates a small sample size correction for improving SEA values accuracy (Jackson et al., 2011).

The SEAc measures the degree of niche overlap (%), with an absolute limit of 100% indicating complete overlap, which can then be used as a quantitative measure of dietary similarity between populations (see Layman et al., 2007 and Jackson et al., 2011 for detailed methodology). The SEA probability estimates of the relative contribution of dietary resources assimilated by the different consumers were obtained using bivariate, separate single-group mixing models in SIAR, with values >0.6 indicating ecologically significant dietary overlap and potentially direct resource competition (Parnell et al., 2010).

Results

Physical and chemical variables

Table 1 summarizes the physical and chemical characteristics of the ephemeral pond at the time of sample collection. Water column chl-a concentration was low with a mean of 7.33 ($\pm$1.44) µg l^{-1} compared to high concentrations observed for the epiphyton chl-a concentration, with a mean 22.35 ($\pm$7.79) µg l^{-1} per 30 cm. Nitrates and phosphates concentrations ranged from 1.2 to 5.2 mg l^{-1} and 0.3–1.4 mg l^{-1}, respectively (Table 1).

Basal source composition

Macrophytes and terrestrial vegetation, fine- and coarse particulate organic matter (FPOM, CPOM), dung, epiphyton and sediment were considered as potential basal food sources (Table 2). Based on their δ^{13}C values, the basal food sources were distinct from

the consumers, ranging from -32.88 to $-16.43‰$ (Table 2, Fig. 2). All the submerged macrophytes fell within a similar range of 6 for the δ^{15}N values. Allochthonous matter, *A. karroo,* was δ^{13}C and δ^{15}N depleted, while C$_4$ grass *S. africanus* was δ^{13}C enriched. The other basal food sources such as CPOM and epiphyton had similar δ^{13}C and δ^{15}N values (Table 2; Fig. 2). Sheep dung was δ^{13}C depleted ($-25.32 \pm 1.35‰$) and δ^{15}N enriched ($5.7 \pm 0.69‰$), whereas the inverse was observed for cow dung (Table 2; Fig. 2). In the present study, C:N ratios for all consumers ranged from 3.32 to 5.10, with autochthonous organic matter sources having C:N ratios of 9.31 ± 0.26 (epiphyton), 9.57 ± 0.80 (*L. muscoides*) and 7.88 ± 0.15 (FPOM), respectively. In comparison, allochthonous organic matter sources had C:N ratios of 20.52 ± 2.60 (*A. karroo* leaves), 24.69 ± 5.57 (cow dung), 25.05 ± 3.36 (CPOM), 29.69 ± 1.30 (C4 grass *S. africanus*) and 25.64 ± 4.33 (sheep dung), suggesting that the autochthonous organic matter was more readily utilized by most of the primary consumers than allochthonous organic matter.

Consumer composition

During a preliminary survey using a 63 µm mesh zooplankton net, we observed that larger sized crustaceans contributed >95% of the zooplankton community and that small-sized zooplankton (e.g. nauplii and rotifers) were virtually absent (see Appendix Table S1). The δ^{13}C and δ^{15}N values of the consumers were clearly distinguishable from the primary producers (Table 2; Fig. 2). The clam shrimp, *Cyzicus* sp. had the most depleted δ^{13}C values ($-35.26 \pm 0.79‰$),

Table 1 Mean ($\pm$SD) summary of physical and chemical variables in the small temporary pond in July 2014

Variable	Range	Mean
Conductivity (µS cm^{-1})	243–243	243
pH	7.58–8.01	7.81 $\pm$ 0.22
TDS (mg l^{-1})	171.8–172.8	172.13 $\pm$ 0.58
Salinity (ppt)	0.12–0.12	0.12
Temperature (°C)	12–12.4	12.17 $\pm$ 0.21
Ammonium (NH$_4^+$, mg l^{-1})	0.2–0.5	0.41 $\pm$ 0.14
Phosphate (PO$_4^{3-}$, mg l^{-1})	0.3–1.4	1.08 $\pm$ 0.52
Nitrate (NO$_3^-$, mg l^{-1})	1.2–5.2	2.75 $\pm$ 2.54
Chlorophyll-a (epiphyton) (µg l^{-1} 30 cm^{-1})	12.79–29.48	22.35 $\pm$ 7.79
Chlorophyll-a (water column) (µg l^{-1})	5.46–8.66	7.33 $\pm$ 1.44

Table 2 Range and mean (±SD) of the $\delta^{15}N$ and $\delta^{13}C$ ratios for a small temperate ephemeral pond

Species	n	TP	$\delta^{15}N$ Range	$\delta^{15}N$ Mean	$\delta^{13}C$ Range	$\delta^{13}C$ Mean
Basal sources						
Allochthonous						
Acacia karroo Hyne leaves	3		−0.28–0.12	−0.03 ± 0.22	−29.94 to −30.47	−30.22 ± 0.27
Sporobolus africanus (Poir.) Robyns & Tournay) leaves	3		1.67–2.73	2.12 ± 0.55	−16.06 to −17.1	−16.43 ± 0.58
CPOM (0.06–3 cm)	3		4.47–4.85	4.61 ± 0.2	−28.68 to −29.15	−28.96 ± 0.24
Cow (*Bos taurus* Linnaeus) dung	3		4.25–4.42	4.31 ± 0.1	−19.44 to −23.02	−21.58 ± 1.89
Sheep (*Ovis aries aries* var. *merino* Linnaeus) dung	3		5.23–6.5	5.7 ± 0.69	−23.76 to −26.11	−25.32 ± 1.35
Autochthonous						
FPOM (< 500 μm)	4		4.2–4.7	4.39 ± 0.21	−32.05 to −32.38	−32.25 ± 0.16
Epiphyton	4		4.25–4.45	4.35 ± 0.08	−28.48 to −29.02	−28.75 ± 0.22
Sediment	2		4.46–5.2	4.83 ± 0.52	−24.24 to −25.02	−24.63 ± 0.55
Cyperus marginatus Thunb.	3		5.6–10.1	8.15 ± 2.31	−26.71 to −27.96	−27.26 ± 0.64
Lagarosiphon muscoides Harv.	3		6.34–7.38	6.9 ± 0.52	−32.54 to −32.38	−32.88 ± 0.54
Potamogeton schweinfurthii A. Benn.	3		5.36–7.2	6.25 ± 0.92	−21.54 to −28.3	−24.51 ± 3.45
Laurembergia repens P. J. Bergius subsp. *Brachypoda* (Welw. ex Hiern) Oberm.	3		6.15–7.1	6.75 ± 0.52	−23.15 to −25.09	−24.22 ± 0.99
Macroinvertebrates/Zooplankton						
Acari (0.1–0.3 cm)	4	3.39	7.72–10.01	9.09 ± 0.97	−29.11 to −31.51	−30.49 ± 1
Cyzicus sp. (0.7–1.2 cm)	4	2.68	6.68–6.72	6.7 ± 0.02	−34.47 to −36.04	−35.26 ± 0.79
Chironomus sp. (0.8–1.6 cm)	4	2.59	6.53–7.17	6.39 ± 0.34	−26.65 to −28.52	−27.73 ± 0.9
Cybister tripunctatus (Olivier) larvae (1.7–3.5 cm)	4	2.53	6.07–6.42	6.18 ± 0.16	−27.17 to −28.05	−27.65 ± 0.43
Cybister tripunctatus (Olivier) adult (1.4–2.3 cm)	4	3.64	8.76–14.71	9.95 ± 1.68	−21.2 to −25.8	−23.5 ± 3.26
Notonecta sp. (0.8–1.3 cm)	4	4.16	8.12–14.96	11.72 ± 3.26	−23.16 to −29.82	−26.56 ± 3.06
Micronecta sp. (0.2–0.4 cm)	4	2.82	5.72–9.95	7.18 ± 1.89	−25.9 to −31.29	−29.07 ± 2.29
Physa sp. (0.5–1.2 cm)	4	2.18	4.35–5.45	5 ± 0.48	−24.3 to −27.11	−26.06 ± 1.33
Cypricercus sp. (0.4 –0.7 cm)	4	2.69	6.5–7.11	6.74 ± 0.27	−32.05 to −33.76	−32.89 ± 0.7
Daphnia magna Straus (0.3–0.4 cm)	9	2	3.58–4.74	4.38 ± 0.42	−33.41 to −34.55	−34.25 ± 0.45
Lovenula raynerae Suárez-Morales, Wasserman & Dalu (0.3–0.5 cm)	4	3.21	6.8–9.22	8.5 ± 1.14	−30.78 to −31.71	−31.19 ± 0.39
Paradiaptomus lamellatus Sars (0.3–0.4 cm)	4	2.76	6.81–7.1	6.96 ± 0.14	−30.11 to −30.76	−30.48 ± 0.27
Lynceus sp. (0.2–0.5 cm)	4	2.23	4.85–5.38	5.17 ± 0.23	−25.66 to −26.41	−26.09 ± 0.34
Amphibians						
Strongylopus fasciatus (Smith) tadpole (3–5 cm)	2	2.8	7.1–7.11	7.1	−24.85 to −27.36	−26.1 ± 1.78
Xenopus laevis Loveridge tadpole L (3–6 cm)	4	3.03	7.58–8.25	7.89 ± 0.28	−31.1 to −31.46	−31.4 ± 0.25
Xenopus laevis Loveridge tadpole S (1–2 cm)	4	3.44	9.15–9.43	9.29 ± 0.12	−27.58 to −29.42	−28.65 ± 0.79

TP Trophic position, *n* number of samples, *FPOM + CPOM* fine and coarse particulate organic matter

while the water flea, *D. magna*, was the most $\delta^{15}N$ (4.38 ± 0.42‰) depleted. Using one-way ANOVA analysis, significant differences were observed among consumers for both the $\delta^{13}C$ ($P < 0.01$, $F = 22.16$, $df = 64$) and $\delta^{15}N$ ($P < 0.01$, $F = 34.97$, $df = 64$) suggesting that they utilized different food sources.
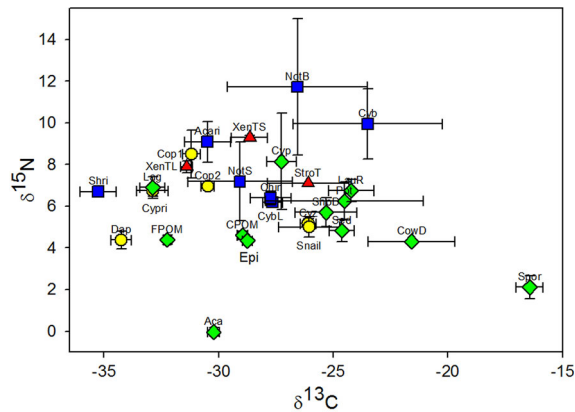
Fig. 2 Mean (±standard deviation) δ^{13}C and δ^{15}N isotope signatures (±standard deviation) for basal food sources (green diamonds), Anuran tadpoles (red triangles), macroinvertebrates (blue squares) and zooplankton (yellow circles) sampled from a small temperate pond. Abbreviations: Aca—*Acacia karroo*, NotB—*Notonecta* sp., Cypri—*Cypricercus* sp., Cop1—*Lovenula raynerae*, Cop2—*Paradiaptomus lamellatus*, Cyz—*Cyzicus* sp., Chir—*Chironomus* sp., CybL—*Cybister tripunctatus* larvae, Cyb—*Cybister tripunctatus* adult, Cyp—*Cyperus marginatus*, CowD—cow dung, CPOM—coarse particulate organic matter, Dap—*Daphnia* sp., Epi—epiphyton, FPOM—fine particulate organic matter, Lag—*Lagarosiphon muscoides*, Pot—*Potamogeton schweinfurthii*, Sed—sediment, ShpD—sheep dung, Shri—*Lynceus* sp., Snail—*Physa* sp., NotS—*Micronecta* sp., Spor—*Sporobolus africanus* (C$_4$ grass), StroT—*Strongylopus fasciatus* tadpole, LauR—*Laurembergia repens* subsp. *brachypoda*, XenTL and XenTS—*Xenopus laevis* tadpole large and small

Trophic positions

The trophic positions of various consumers in the pond were determined using δ^{15}N values. We identified four trophic levels with *Notonecta* sp. at the top of the sampled food web (trophic position 4). The tadpoles and the selected macroinvertebrates occupied trophic position 3, while the majority of the zooplankton had intermediate values, and therefore occupied trophic position 2 (Table 2; Fig. 2). A notable exception in the zooplankton was the predacious copepod *L. raynerae* which occupied trophic level 3 (Table 2; Fig. 2).

Consumer diet proportions, niche breadth and overlaps

SIAR models outputs for the *Notonecta* sp. showed that it fed mostly on *C. marginatus* (mean proportion 19.1%) *Physa* sp. (17.7%), Acari (17.3%), *Micronecta* sp. (17.1%) and *L. raynerae* (16.4%), while *C.*

tripunctatus adult showed a variable diet composition with *D. magna* (21.4%), macrophyte (1) (20.3%) and *Micronecta* sp. (17.4%) being the main food sources (Table 3). Chironomus fed mostly on FPOM (27.4%), CPOM (24.4%) and sediment (21.2%). *Xenopus laevis* tadpoles preferred to feed predominately on allochthonous organic matter; CPOM (27.8%) and sediment (30.5%) but autochthonous organic matter (epiphyton) contributed 41.6% of the individual food sources. *Lagarosiphon muscoides* (52.2%) and FPOM (13.2%) contributed 65.4% of the autochthonous basal food source diet of *X. laevis* tadpoles (Table 3). Of the consumers, Acari, *L. raynerae*, *Micronecta* sp. and *P. lamellatus* showed variable diets of macroinvertebrates, allochthonous and autochthonous, whereas *L. raynerae* and *P. lamellatus* had a slightly greater preference for *P. lamellatus* (23.9%) and *D. magna* (31.3%), respectively (Table 3).

SIBER analysis using convex hull areas and standard ellipses revealed relatively small isotopic niche width among the consumers (Table 4; Fig. 3), as highlighted by the trophic positions of the organisms (Table 2), and large isotopic niche overlap was observed between secondary consumers *Notonecta* sp. and *C. tripunctatus* (Table 4). *L. raynerae* showed the smallest isotopic feeding niche and substantial convex hull overlap with Acari and *Notonecta* sp., respectively (Table 3; Fig. 3). The niche breadth overlap, i.e. the overlap of standard ellipse areas (SEAc) between the *Notonecta* sp. and *C. tripunctatus,* was high (0.87). Niche breadth overlap for the majority of other consumers was low (<0.5), with the exception of Acari, which had moderate niche overlaps with *Notonecta* sp. (0.77), *L. raynerae* (0.68) and *Micronecta* sp. (0.52) (Table 4). The low dietary overlaps observed for *X. laevis* tadpoles and other consumers could be due to the different isotopic niche space positions and feeding at different trophic levels (Tables 2, 4).

Contributions of autochthonous and allochthonous organic matter to consumers

Based on the trophic positions, the consumers were separated into five distinct groups, each serving a different role in transferring organic matter within the small ephemeral pond food web (Fig. 2). The copepods and other invertebrates in the pond gained organic matter directly from the basal food sources,

Table 3 Stable isotope analysis in R (SIAR) results of the food source proportions in the diet of the 5 macroinvertebrate species, 2 copepod species and *X. laevis* tadpole showing the calculated distribution range from low to high 95 % credibility intervals in the small ephemeral pond. Macrophyte (1) represents the average values of *Potamogeton schweinfurthii* and *Laurembergia repens* subsp. *Brachypoda*

Species	Acari		*L. raynerae*		*P. lamellatus*		*Micronecta* sp.		*Notonecta* sp.	
	Mode	Range	Mode	Range	Mode	Range	Mode	Range	Mode	Range
Allochthonous										
CPOM	22.5	0–35.4			26.8	5.2–45.3	24.7	0.2–42.7		
Sheep dung										
Autochthonous										
C. marginatus									21.1	3.2–36.1
Epiphyton										
FPOM	4.2	0–34	21.7	0–37.2			23.6	0–38.1		
L. muscoides	25	5.3–43.8	24.5	0.4–39.6			12.2	0–35.3		
Macrophyte (1)										
Sediment										
Prey										
Acari					2.6	0–30.1			20.6	0–33.5
L. raynerae					3.4	0–31.6			19.9	0–32.8
P. lamellatus			25.3	1.1–42.9						
Daphnia magna	3.6	0–30.4	13.8	0.2–32.3	31	17.8–44.9	19.2	0.0–35.4	2.5	0–27.8
Notonecta sp.										
Micronecta sp.	28.7	6.5–51.9	18.6	1.3–34.3	15.6	0–31.9			20.1	0–33.4
X. laevis small										
Chironomus sp.							24.4	0.5–42.8		
Physa sp.									19.4	0–34

Species	*C. tripunctatus*		*Physa* sp.		*X. laevis* small		*Chironomus* sp.	
	Mode	Range	Mode	Range	Mode	Range	Mode	Range
Allochthonous							25.1	0.2–46.3
CPOM			27.2	0–44.6	3.3	0–40.1		
Sheep dung			26.8	0–47.6			6.9	0.3–40
Autochthonous								
C. marginatus							1.8	0–27.6
Epiphyton			25.9	1.3–46.7				
FPOM					2.6	0–34	26.3	8.3–45.88
L. muscoides					56.9	26.8–75.3		
Macrophyte (1)	22.5	0.2–38.3						
Sediment			26.8	0–47.6	18.9	0.8–33.1	23.3	0.3–40
Prey								
Acari								
L. raynerae	2.9	0–31.1						
P. lamellatus								
Daphnia magna	23.1	3–39.8						
Notonecta sp.	2.3	0–27.6						
Micronecta sp.	18.2	0–34.1						
X. laevis small	3.2	0–30.2						
Chironomus sp.								
Physa sp.								

Table 4 SEA probability (bold values) and niche breadth overlaps, standard ellipse areas with small sample corrections (SEAc) for the selected consumers calculated using SIBER

	Acari	*Notonecta* sp.	*L. raynerae*	*C. tripunctatus*	*Micronecta* sp.	*X. laevis*
Acari		**0.97**	**0.57**	**0.14**	**0.86**	**0.21**
Notonecta sp.	0.77		**0.37**	**0.89**	**0.30**	**0.34**
L. raynerae	0.68	0.22		**0.53**	**0.81**	**0.31**
C. tripunctatus	0.23	0.87	0.42		**0.21**	**0.02**
Micronecta sp.	0.52	0.45	0.72	0.12		**0.03**
X. laevis	0.02	0.05	0.15	0.02	0.04	
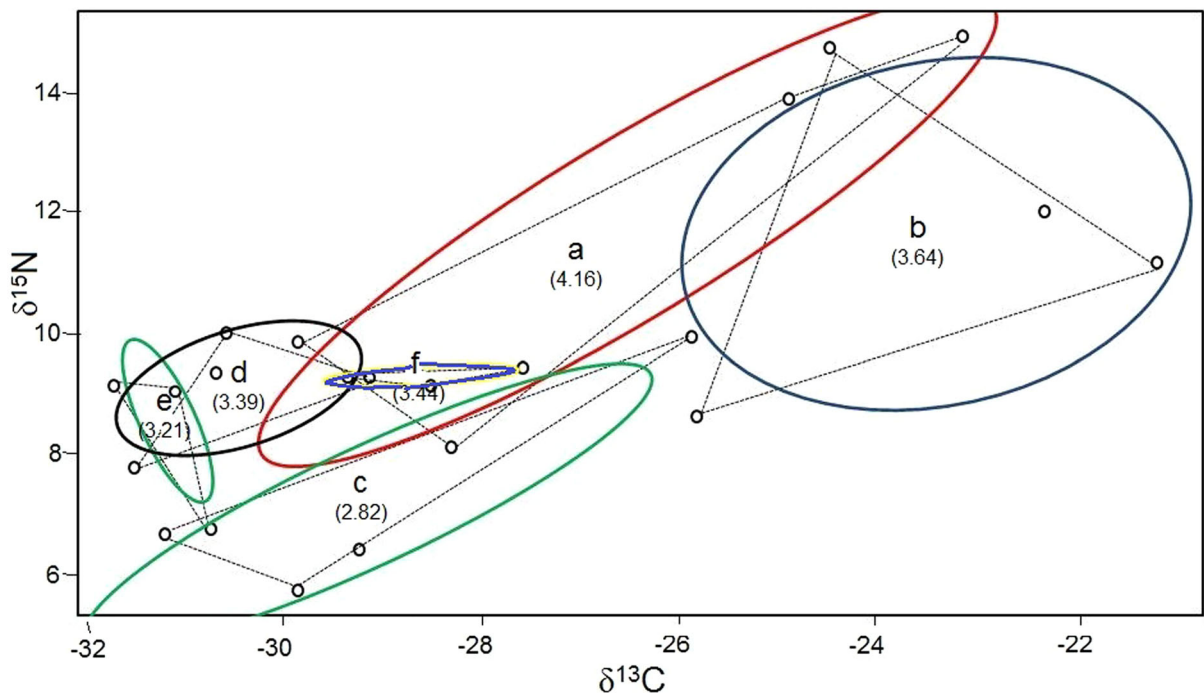
Fig. 3 SIBER output $\delta^{13}C$ and $\delta^{15}N$ biplot of the major predators (**a**) *Notonecta* sp., (**b**) *C. tripunctatus*, (**c**) *Micronecta* sp., (**d**) Acari, (**e**) *Lovenula raynerae* and (**f**) *X. laevis* tadpole (*small*). Convex hulls areas (*dotted lines*) and ellipses (*solid lines*) represent the calculated isotopic feeding niche widths of each species. The *numbers* in parenthesis represent the trophic positions

i.e. autochthonous FPOM, epiphyton, macrophytes and benthic sediment or the allochthonous CPOM at various levels (Table 3). The filter feeders (e.g. *D. magna* and *Cypricercus* sp.), scrapers (e.g. *Physa* sp.), omnivores (e.g. *X. laevis*) and hyperbenthic predators (e.g. *P. lamellatus*) largely obtained their organic matter from autochthonous sources, although the allochthonous sources were represented (Table 3; Fig. 2).

Discussion

The present study highlights the contribution of both autochthonous and allochthonous organic carbon to food webs in ephemeral pond systems. However, autochthonous organic carbon (epiphyton, FPOM, macrophytes and sediment) appeared to be more readily incorporated into the food web than did the allochthonous material (CPOM and sheep dung).

These findings are in agreement with similar studies in permanent aquatic environments (Huang et al., 2007; Mao et al., 2012; Harding & Hart, 2013) and suggest that in freshwater ecosystems, allochthonous processes are particularly important, irrespective of the type of freshwater environment. Numerous studies suggest that in low productive freshwater systems, the detrital food web predominates (Huang et al., 2007), but in our study, the food web was driven by autochthonous organic matter suggesting that it is relatively productive. The isotopic differences in CPOM with macrophytes and terrestrial vegetation suggest that the CPOM was composed of a mixture of all the vegetation fragments. The consumers generally preferred autochthonous FPOM, epiphyton and emerged macrophyte *L. muscoides* organic matter sources over allochthonous CPOM. Kopprio et al. (2014) and Huang et al. (2007) showed that consumers utilized organic matter with high nitrogen and low carbon content as it provided better quality food due to the presence of high proteins and energy levels plus low-fibre levels compared to allochthonous organic matter.

The animal community of the food web incorporated in the present study comprised a mixture of crustaceans (ostracods, water mites, branchiopods and copepods), hexapods, snails and anuran larvae (Table 2). The community composition within the pond was broadly similar to that recorded in other southern African ephemeral ponds (Ferreira et al., 2012; Riato et al., 2014) and indeed, in similar ponds in the northern hemisphere (O'Neil & Thorp, 2014). A notable exception was the poor representation of hexapods which have been shown to represent an important component of the biology within these systems. Insect diversity within temporarily pools is thought largely to be a function of pond size and age, as much of the insect community is a result of immigration from other environments (O'Neil & Thorp, 2014). While the study pond was small in size, we do not have sufficient information on the state of the hydro-period. Insects generally dominate these systems late in the ponds hydro-period, with the initial phase of the hydro-period expected to be depauperate of insects (O'Neil & Thorp, 2014). An additional explanation for the low hexapod diversity in the present study is one of seasonality. Insect activity within temperate regions of the world demonstrates strong seasonal patterns (Lencioni, 2004). It is

therefore, possible that the hexapods would make a more important contribution to the animal community during the warmer summer months. Irrespective of season, however, the immigration of predators from other environments present potential confounds when assessing food-web dynamics within a system that is treated as closed, with regard to the analyses. This may explain the high variability in the isotopic signal observed for the top predators such as *Notonecta* sp. and *Cybister tripunctatus* during the current study. Alp et al. (2013) showed that adult feeding on terrestrial food sources could cause an isotopic shift during the terrestrial stage, which might explain the relatively high variability in isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) observed for adult Notonectids (Fig. 2). This could also explain the difference between *C. tripunctatus* larvae and adult signatures. In addition to the potential isotope fractionation during metamorphosis from larval to adult life stage (Alp et al., 2013), larval *C. tripunctatus* signatures would be reflective of within-pond dynamics, while adults may represent isotopic values inclusive of signatures from other environments.

When assessing niche dynamics across the major groups, SIBER analysis revealed relatively small isotopic (trophic) niche width among the consumers (Fig. 3), with a large isotopic niche overlap being observed between secondary consumers. Acari, *L. raynerae*, *Micronecta* sp. and *X. laevis* tadpoles comprised the intermediate trophic group. Within this group, Acari and *L. raynerae* had the highest degree of overlap, with *X. laevis* tadpole and *Micronecta* sp. exhibiting more distinct niches. *Micronecta* sp. however had a particularly large feeding niche comprising a large C and N ranges. Of the secondary, *Notonecta* sp. and *C. tripunctatus* had a degree of overlap but with a fairly high degree of niche separation. SIBER analysis using convex hull areas and standard ellipses revealed relatively small isotopic niche width among the intermediate consumers of trophic levels 2 and 3 (Fig. 3), suggesting a small degree of resource partitioning between the various primary and secondary consumers. This is in contrast to previous studies, e.g. Pace et al. (1999), Layman et al. (2007) and Baiser et al. (2011) who suggest that freshwater ecosystems are characterized by high levels of "trophic redundancy" as evident from the high degree of overlap in dietary composition and predominance of generalists within these systems. The predominance of generalists

is thought to support the so-called "insurance hypothesis" which predicts that high levels of trophic redundancy may minimize the effects of disturbance (Yachi & Loreau, 1999). In the present study, however, generalist feeders were scant. Even at trophic level 4, where the *Notonecta* sp. had the widest niche width, a degree of separation was observed with other groups.

The complexity of food webs within ephemeral ponds in the northern hemisphere has been strongly linked to diversity within systems, with high insect diversity ponds having more complex food webs than those systems dominated by crustacea (O'Neil & Thorp, 2014). While there was low hexapod diversity in the present study, insects comprised the top of the food web. This was consistent with findings of other studies on small freshwater systems that showed that *Notonecta* spp. were the top predators in these systems (Arnér et al., 1998; Blaustein, 1998; Klecka, 2014). Studies by Mazunder (1994) and Arnér et al. (1998) have highlighted the importance of Notonectids as a top predator within small ephemeral ponds. Notonectids have been demonstrated to be capable of co-existing with, as well as eliminating, zooplankton and other macroinvertebrates under natural conditions (Mazunder, 1994; Arnér et al., 1998). While Notonectids immigrate to ephemeral ponds early in their hydro-period, the zooplankton communities arise from the mass hatching of dormant eggs when ponds first fill up (Pinceel et al., 2013; O'Neil & Thorp, 2014). Within weeks of filling, these ponds are therefore dominated by mostly adult crustaceans that have matured, and in the case of the present pond, the predaceous paradiaptomid copepods (*P. lamellatus* and *L. raynerae*). The absence of small zooplanktonic organisms such as rotifers and crustacean nauplii in the present study (see Appendix Table S1), is likely due to a combination of life-history dynamics of the dominant zooplankton groups as mass hatching is often a once off event (Brendonck & De Meester, 2003), and predation given the large numbers of predators at the time of sampling (Vanni, 1986, 1988; Arnott & Vanni, 1993; Brönmark & Hansson, 2005).

In conclusion, the small ephemeral freshwater pond food web was largely sustained by autochthonous organic matter sources with allochthonous organic matter being of less importance. Autochthonous organic matter in the food web was transferred by zooplankton and other macroinvertebrates (e.g.

Micronecta sp. and *Physa* sp.), with the latter also transferring some allochthonous organic matter. The ephemeral pond animal community comprised both generalist (trophic level ≥ 3) and specialist (trophic level < 3) taxa during this hydro-period stage and was generally less complex than climax northern hemisphere studies (e.g. Klecka, 2014; O'Neil & Thorp, 2014; Riato et al., 2014). The current food-web structure therefore seems to reflect the primary to mid stage of trophic structural complexity in the evolution of ephemeral ponds over the course of their hydro-period (O'Neil & Thorp, 2014). It is likely that there will be more trophic redundancy in the summer months when hexapod diversity in this austral ephemeral pond increases. It is therefore crucial for future studies to assess these environments over the course of their hydro-period for the adequate understanding of their food-web structure and ecosystem functioning.

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