Trophic isotopic carbon variation increases with pond’s hydroperiod: Evidence from an Austral ephemeral ecosystem

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Trophic variation in food web structure occurs among and within ecosystems. The magnitude of variation, however, differs from system to system. In ephemeral pond ecosystems, temporal dynamics are relatively more important than in many systems given that hydroperiod is the ultimate factor determining the presence of an aquatic state. Here, using stable isotopes we tested for changes in trophic chain length and shape over time in these dynamic aquatic ecosystems. We found that lower and intermediate trophic level structure increased over time. We discuss these findings within the context of temporal environmental stability. The dynamic nature of these ephemeral systems seems to be conducive to greater levels of intermediate and lower trophic level diversity, with omnivorous traits likely being advantageous.

The study of trophic structure and community dynamics is a tenet of ecology and is considered central to our understanding of community ecology1,2. Food webs are essentially species networks linked by trophic interactions that describe the ecosystem biodiversity and feeding relations3. These complex interactions are often figuratively depicted by nodes, which represent contributing biota (e.g. species) and connecting lines that represent interaction strengths between nodes. This allows for the characterisation of food webs using shape profiles and associated analyses. As such, food web height and shape have been identified as potentially important characterisations of the trophic structure. Thus, the overall dimensions of a given food web can provide insight into how ecosystems function and change in space and time4,5.

It has been shown that the number of levels in energy transfer from primary producers to top predators is likely to be determined by ecosystem ambient temperature, habitat heterogeneity, productivity, size, and species richness6-8. In addition, since the “height” of a food web is driven by nitrogen and the “width” largely facilitated by carbon, food web shape is also likely influenced by the availability of these elemental compounds5,6. There has been much work done in this regard on permanent water bodies, whereby C and N availability is an aspect of resource accumulation, exploitation and standing biomass, facilitating longer or shorter food webs of various widths5,7-9. Relatively little work has, however, been conducted on ephemeral water bodies.

Much work on the dynamic nature of food webs has been conducted1,2,5,10,11 and, it is now well recognised that trophic variation occurs among and within ecosystems across spatial and temporal scales5. In ephemeral pond ecosystems, temporal dynamics are particularly important given that hydroperiod is the ultimate factor determining whether the aquatic state is even present. Physico-chemical conditions are highly variable over the hydroperiod and likely have implications for succession patterns, particularly for internal community development. With regard to top-down processes in these ecosystems, much of the predation pressure in ephemeral ponds is imported over time through the arrival of organisms such as insects and amphibians that exploit these environments as profitable food patches12,13. Thus, the relative importance of bottom-up and top-down pressures is likely to shift over time, and have significant implications for food web structure and ultimately, trophic shape. Here, we test for the changes in trophic chain length and shape in an ephemeral pond over time.

Ephemeral freshwater systems are widely distributed and include both lentic and lotic habitats14. These environments typically only exist as aquatic environments for limited periods and typically only hold water for a few
days to months\cite{8,15–18}. Given their impermanency as an aquatic ecosystem, processes of basal resource accumulation, community development and food web complexity are fundamentally different to those of permanent water bodies\cite{8,19}. In lentic ephemeral ecosystems, communities are comprised of both internal recruits, in the form of biota emerging from dormant eggs, and external recruits such as insects and amphibians which are mobile and can therefore, invade from other habitats\cite{12,20}. The hydroperiod is a major factor determining community dynamics given that internal and external recruitment is often characterised by varying degrees of temporal separation, linked to hydroperiod state\cite{13,19–23}. For example, many of the internal recruits hatch early in an ephemeral ponds hydroperiod, while immigrating predator arrival occurs later\cite{19,24,25}. The relative contribution and biomass of, and trophic exchange between these biotic components will, therefore, likely change over the course of the hydroperiod\cite{12,26}.

In this study, community food web metrics were used to assess the temporal changes in food web structure in a temperate ephemeral pond. To reveal variations in food web length and shape, we analysed community-wide metrics\cite{27} using stable isotopes ($\delta^{15}N$, $\delta^{13}C$) of invertebrate communities over the course of the pond’s hydroperiod i.e. five months. We were particularly interested in temporal changes in the shape of the isotopic space by conducting food web studies from a pond environment over four discrete points in time over the hydroperiod. We postulated that ephemeral pond food webs would change over time and that internal processes would be less important than the immigration of predators in contributing to changes in shape of the food web. We proposed that these environments would be characterised by: 1) simple, short food webs comprised of lower and intermediate trophic levels early on; 2) little change in the lower and intermediate trophic structure over time as expected based on trophic position i.e. $\delta^{15}N$ values; and 3) an increases in food web length over time based on isotopic nitrogen and organic matter i.e. $\delta^{13}C$ values.

**Results**

The invertebrate taxon richness increased from 9 during the first survey to 19 at survey 3 before decreasing to 13 during the final survey (Fig. 1; Table 1). Macroinvertebrates dominated during surveys 2 and 3 contributing to increased taxa richness, before decreasing at survey 4. During survey 1, the mean $\delta^{13}C$ values of the invertebrates ranged from $-18.9\%$ (Cypricercus sp. 1) to $-23.3\%$ (Daphnia magna), while Lovenula raynerae having the highest trophic position (TP = 2.5) among the invertebrates analysed and Streptocephalus sp. (TP = 1.3) having

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**Figure 1.** Stable isotope-based biplots of trophic position inferred from $\delta^{15}N$ vs $\delta^{13}C$ values over the course of a hydroperiod: (a) survey 1, (b) survey 2, (c) survey 3, and (d) survey 4 showing the convex hull areas encompassing all invertebrate taxa. For each sample point, $n = 2–17$ individuals and error bars represent ± standard deviations, green squares – plants (i.e. macrophytes, POM, detritus), dark blue circles – macroinvertebrates, light blue triangles – zooplankton. Taxa names for invertebrates see Table 1, 10 – *Cyperus marginatus*, 11 – detritus, 12 – filamentous algae, 13 – POM, 14 – *Marsilea* spp., 15 – *Sporobolus africanus*, 16 – sediment, 27 – *Crinium* spp., 28 – Euglenophyta, 29 – *Persicaria* spp., 37 – *Laurembergia repens* subsp. brachypoda, 38 – horse dung.
The lowest. During surveys 2 and 3, the δ¹³C values of the invertebrates ranged from −21.9‰ to −31.1‰ and from −19.1‰ to −30.1‰, respectively, with Chlorolestidae and Baetis sp. being δ¹³C depleted invertebrates during surveys 2 (−31.1‰) and 3 (−30.1‰), respectively (Fig. 1; Table 1). *Lovenula raynerae*, *Enithares sobria* and *Aeshna* sp. were the top predators, with TPs values of 3.1, 3.6 and 4.3 during surveys 2, 3 and 4, respectively. Macroinvertebrates were more enriched than the zooplankton (Fig. 1; Table 1). Trophic chain length (i.e. maximum/highest TP per survey time; TCL) increased over time mostly likely due to an increase in taxa richness vs minimum/highest TP per survey time (TCL) increased over time mostly likely due to an increase in taxa richness.

Table 1. Invertebrate taxa sampled from the ephemeral pond over the course of a hydroperiod used for stable isotope analyses. Abbreviations: Taxa abbr. – taxa abbreviation (i.e. assigned sample number), n – number of analysed samples, TP – trophic position.

| Taxa abbr. | Taxa | Survey 1 | Survey 2 | Survey 3 | Survey 4 |
|-----------|------|----------|----------|----------|----------|
|            |      | n | δ¹³C (%) | n | δ¹³C (%) | n | δ¹³C (%) | n | δ¹³C (%) | TP |
| Macroinvertebrate |  |  |  |  |  |  |  |  |  |  |
| *Aeshna* sp. | 17 | 6 | −24.8 (0.9) | 4 | −27.1 (0.5) | 5 | −26.1 (0.3) | 4.3 (0.4) |  |
| *Anisops* sp. | 18 | 4 | −26.0 (4.1) | 8 | −25.6 (2.6) | 2.7 (0.7) |  |
| *Bulinus tropicus* | 19 | 6 | −13.0 (1.7) | 7 | −19.1 (1.5) | 1.2 (0.1) |  |
| *Baetis* sp. | 30 | 3 | −30.1 (0.02) | 2.2 (0.01) |  |
| *Diaphanosoma caudatum* | 31 | 4 | −25.5 (0.4) | 2.5 (0.2) |  |
| *Chironomus* sp. | 32 | 7 | −24.6 (1.2) | 2.2 (0.1) |  |
| *Chlorolestidae* | 20 | 2 | −31.1 (0.02) | 1.5 (0.01) |  |
| *Cypricercus* sp. 1 | 35 | 2 | −21.1 (0.7) | 1.3 (0.3) |  |
| *Cypricercus* sp. 2 | 1 | 5 | −18.9 (0.3) | 1.6 (0.1) | 3 | −21.4 (1.8) | 1.5 (0.3) |  |
| *Cypricercus* sp. 1 | 2 | 4 | −22.9 (0.4) | 1.7 (0.1) | 6 | −21.9 (3.3) | 1.6 (0.2) | 5 | −19.6 (2.4) | 2.6 (0.4) | 2 | −17.6 (0.1) | 2.4 (0.1) |  |
| *Cypricercus* sp. 2 | 36 | 5 | −26.1 (0.6) | 1.1 (0.3) |  |
| *Enithares sobria* | 21 | 6 | −26.9 (1.1) | 2.8 (0.5) | 3 | −25.6 (0.02) | 3.6 (0.1) |  |
| *Elmidae* | 22 | 3 | −24.0 (1.7) | 2.7 (0.6) | 4 | −23.4 (1.9) | 1.6 (1.0) |  |
| *Hirudinidae* | 23 | 2 | −20.2 (0.3) | 2.7 (0.3) | 3 | −23.3 (1.4) | 2.6 (0.4) | 2 | −21.2 (0.8) | 2.3 (0.1) |  |
| *Hydracarina* | 33 | 2 | −28.4 (0.3) | 2.7 (0.01) |  |
| *Lycus* sp. 1 | 24 | 2 | −24.0 (0.4) | 1.7 (0.1) | 7 | −24.4 (0.7) | 1.3 (0.2) | 5 | −23.7 (0.5) | 1.5 (0.3) |  |
| *Sigara* sp. | 25 | 2 | −22.4 (0.01) | 2.4 (0.02) | 7 | −22.7 (0.4) | 2.5 (0.2) |  |
| *Streptocephalus* sp. | 3 | −23.5 (0.02) | 1.3 (0.01) | 6 | −27.3 (1.2) | 2.0 (0.1) | 2 | −21.2 (0.01) | 1.9 (0.01) |  |
| *Triops granarius* | 4 | 2 | −22.5 (0.03) | 1.8 (0.01) | 5 | −22.6 (1.5) | 2.2 (0.3) |  |
| Zooplankton |  |  |  |  |  |  |  |  |  |  |
| *Daphnia longispina* | 5 | 3 | −23.0 (0.2) | 1.7 (0.03) | 4 |  |
| *Daphnia magna* | 6 | 4 | −23.3 (0.4) | 2.2 (0.7) | 6 | −27.8 (0.5) | 1.8 (0.5) | 6 | −28.1 (0.3) | 1.9 (0.1) |  |
| *D. longispina* + *Karzia spp.* | 26 | 7 | −29.2 (0.5) | 1.6 (0.1) | 4 | −27.7 (0.8) | 1.7 (0.04) | 5 | −26.7 (0.1) | 1.6 (0.2) |  |
| *Lovenula raynerae* | 7 | 8 | −21.4 (0.8) | 2.5 (0.3) | 17 | −23.6 (0.8) | 3.1 (0.1) | 16 | −24.0 (0.6) | 3.1 (0.3) | 17 | −25.2 (1.0) | 2.8 (0.2) |  |
| *Paraspirostomus lamellatus* | 8 | 7 | −21.6 (0.7) | 2.0 (0.3) | 17 | −26.1 (0.9) | 2.0 (0.2) | 8 | −24.7 (0.5) | 1.7 (0.4) | 17 | −25.3 (0.9) | 2.7 (0.5) |  |
| *Mesocyclops* sp. + *nauplii* | 9 | 3 | −23.1 (0.8) | 2.1 (0.2) | 4 | −23.9 (0.5) | 2.9 (0.4) | 4 | −23.9 (0.6) | 2.4 (0.1) | 4 | −23.9 (0.8) | 2.5 (0.2) |  |

Table 2. Community-wide metrics calculated using SIBER analyses based on the invertebrate taxa δ¹³C and trophic position values. Abbreviations: dCr – carbon range, TA – total convex hull area, MNND – mean nearest neighbour distance values, SEAc – standard ellipse areas, TP – trophic position (measure of trophic chain length).

| Survey | dCr | TA | MNND | SEAc | TP |
|--------|-----|----|------|------|----|
| 1      | 5.2 (5.1–5.3) | 8.1 (7.7–8.5) | 0.13 (0.11–0.15) | 3.3 (3.0–3.6) | 2.5 |
| 2      | 19.3 (18.8–20.3) | 25.4 (24.4–26.9) | 0.14 (0.12–0.15) | 7.3 (6.7–7.9) | 3.1 |
| 3      | 12.9 (12.6–13.8) | 27.6 (25.7–29.8) | 0.13 (0.12–0.15) | 5.9 (5.5–6.3) | 3.6 |
| 4      | 9.0 (9.2–9.4) | 19.6 (19.0–21.3) | 0.14 (0.12–0.17) | 4.9 (4.3–5.4) | 4.3 |
The trapezoid shape with a wider TP level 2 of the trophic structure suggests that much characterised by multi-chain omnivory, with one top predator integrating the different carbon sources fuelling increase significantly over time was not observed. Results of the stable isotope study suggest that the system was and overall taxa richness increased over the duration of the investigation; the diversity of predators predicted to pond changes in response to water depth, a correlate of time (hydroperiod). While trophic chain length (TCL) in the trophic complexity in the ephemeral pond is driven by midriff expansion rather than diversification at the top of the food web. This is likely facilitated through specialisation by certain contributors (omnivores) at the intermediary trophic levels. Thus, some of the changes observed in food web shape could be a result of changes in the trophic position of the macroinvertebrates across the surveys.

Our conceptual model (Fig. 3), outlines the patterns of trophic structure change over time in the system. It is likely representative of foodweb dynamics in temporary aquatic ecosystems of a similar climate. However, it is important to highlight that the model might differ from one region to another, particularly with regard to differences in hydroperiod associated with local rainfall and evaporation processes.

The use of $\delta^{15}$N isotopes for TCL estimation, does however, need to be interpreted with caution given that we employed a single average trophic fractionation value (i.e. 2.3‰ used in this study). Trophic fractionation values are unlikely to be constant throughout the entire foodweb. We assumed that there were no trophic enrichment
differences during the different surveys as the main organism groups were found throughout the study, with sampling conducted at the same sites. Thus, we have no reason to suppose that the observed differences were a result of various factors other than the high average number of trophic steps during the different survey times. As such, these aspects do not take away from the main findings of the study within the context of the main hypotheses.

The study provides empirical evidence on the dynamic nature of food webs in an ephemeral ecosystem over a hydroperiod. The realised TCL estimated during this study are, generally lower than those recorded for organisms in permanent freshwater bodies. Predator diversity in ephemeral ponds is heavily reliant on an immigration process from other environments and ultimately results in limited predator diversity levels when compared to permanent systems. Our findings were in agreement with those of O’Neill & Thorp, O’Neill et al., Schalk et al., and Schriever & Williams on ephemeral ecosystems, whereby greater taxa richness contributed to increased trophic complexity over time. Given the temporary nature of the ponds, the largest predators are still small-bodied when compared to those that occupy the tops of food webs in permanent water bodies. Large predacious aquatic organisms such as fish and malacostraca are typically unable to survive in ephemeral wetlands and as such the amplitude of ephemeral pond food webs will never reach the levels observed in permanent bodies as isotopic nitrogen levels are limited by the drying out process. This has implications for food web shape as δ¹⁵N variations are intrinsically limited within these systems, while δ¹³C variations are not.

The dynamic nature of these systems seems to be conducive to greater levels of intermediate and lower trophic level diversity, with omnivorous traits likely being advantageous. When considering these findings within the broader context of aquatic environments, the question of predator diversity accumulation as a function of environmental stability arises. Pelagic marine environments are arguably the most stable of aquatic systems which likely facilitates the high diversity of predators, while ephemeral ponds are arguably the least stable. As such, future investigations should address changes in trophic chain length and shape at large multi spatio-temporal scales and across a range of aquatic ecosystems, from impermanent freshwaters through to the most permanent of large freshwater and marine bodies, representing a gradient of habitat stability.

Materials and Methods

Study site. The study was conducted in an ephemeral pond situated at Burnt Kraal (33°15′S, 26°26′E) in the south–eastern temperate region of South Africa, over the period 24 June to 23 September 2015. When full, the pond had a surface area of 1952 m² (0.48 acres) and mean depth of 0.38 ± 0.02 m (n = 3, measured using a graduated measuring rod). The mean summer and winter daily temperatures and annual rainfall for the area are 20.3 °C, 12.3 °C and 680 mm, respectively. The first sampling event took place two weeks after a high rain-fall (>100 mm) event that rapidly filled the dry pond, with the last sampling occurring two weeks before the pond completely dried out (Fig. 1). Sampling was conducted at four discrete periods over the course of a pond’s hydroperiod: survey 1 (24 June 2015: mean depth at deepest point 0.38 ± 0.02 m), survey 2 (27 July 2015: mean depth 0.24 ± 0.03 m), survey 3 (29 August 2015: mean depth 0.16 ± 0.01 m) and survey 4 (23 September 2015: mean depth 0.09 ± 0.01 m).

Sample collection and processing. Stable isotope samples for invertebrates and plants were collected on each sampling period at midday (Table 1). No vertebrates were encountered while sampling the ephemeral pond. Zooplankton samples (n = 4–17) were collected by towing horizontally a zooplankton net (63 μm mesh size, 32 cm diameter) through the water column. Macroinvertebrate larval samples (n = 2–8) were also collected using a nylon hand net (500 μm mesh size, 30 × 30 cm dimension). All zooplankton and macroinvertebrates were identified to the lowest taxonomic resolution using keys by Day et al., Fernando, Gerber & Gabriel, and others.
Gooderham & Tsyrlin42 and Suárez–Morales et al.43. All samples were then stored in toluene-cleaned labelled Eppendorf tubes for later processing in the laboratory.

Detritus (n = 4), horse dung (n = 4), macrophyte leaves (n = 4) and terrestrial grass (n = 3–5) were collected on each sampling trip by hand and placed in labelled zip-lock bags. Four surface water samples ~20 cm depth were collected in 2L carboys for the determination of particulate organic matter (POM). The POM was then further filtered through pre–combusted (450 °C, 5 h) Whatman GF/F filters after initial filtering through a 63 µm mesh to remove zooplankton. Any visible zooplankton on the filters, that might have escaped pre-screening were removed with forceps under a dissecting Olympus microscope operated at 100 × magnification before being placed in separate labelled pre–combusted (450 °C, 5 h) aluminium foil envelopes. Sediment (n = 4) was collected using a van Veen grab (bite depth ~1–5 cm) and placed into sterile plastic bags. In the laboratory, all samples in zip-lock were placed into separate, labelled pre–combusted aluminium foil envelopes.

All plant material (detritus, macrophytes, grass, POM), dung, sediment and invertebrate samples were oven dried at 60 °C for 72 hrs before being further ground to a fine homogeneous powder using a mortar and pestle. To obtain sufficient amounts of material to conduct the stable isotope analysis, some zooplankton taxa were pooled together i.e. Cladocera (Daphnia longispina and Kurzia spp.) and Copepoda (Mesocyclops spp. and nauplii). Before placement into toluene cleaned tin capsules, dried sediment samples were acidified by vortexing for 2 min in 2M hydrochloric acid, centrifuged at 3600 rpm for 5 min, washed in deionised water twice followed by re-centrifugation, drying at 50 °C and homogenising in a Retsch Mixer Mill46. Aliquots of approximately 0.6–0.7 mg (animals) and 1–1.2 mg (plant material, dung, sediment) were weighed into aluminium tin capsules that were pre–cleaned in toluene.

Stable isotope sample analysis. Stable isotope analysis was carried out using a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (Thermo Fischer, Bremen, Germany), housed at the Stable Isotope Laboratory, University of Pretoria. Merck Gel ($\delta^{13}$C = −20.57‰, $\delta^{15}$N = 6.8‰, Cw = 43.83, Nw = 14.64) standards and blank samples were run after every 12 unknown samples. All results were referenced to Vienna Pee–Dee Belemnite and to air for carbon and nitrogen isotope values, respectively. Results were expressed in delta notation using a per mille scale from the standard equation 1:

$$\delta X (\‰) = \frac{R_{Sample} - R_{Standard}}{R_{Standard}} \times \frac{1000}{1}$$

where $X = {^{15}}$N or $^{13}$C and R represents $^{15}$N/$^{14}$N or $^{13}$C/$^{12}$C, respectively. Average analytical precision was <0.15‰ for $\delta^{14}$C and <0.1‰ for $\delta^{15}$N.

The trophic positions of all invertebrates in the pond were estimated using the formula of Vander Zanden44 (equation 2):

$$\text{Trophic position (TP)} = 2 + \frac{15N_{\text{consumer}} - 15N_{\text{zooplankton}}}{2.3}$$

where: $\delta^{15}$Nconsumer is the measured consumer $\delta^{15}$N for which TP needs to be estimated and $\delta^{15}$Nzooplankton is the average $\delta^{15}$N of the primary consumer (mean of Cladocerans) at a particular hydroperiod and 2.3 is the trophic fractionation for $\delta^{15}$N.45 The level 2 was consequently attributed, empirically, to zooplankton i.e. Cladocera46.

Data analysis. To investigate the ephemeral pond trophic structure, quantitative population metrics47 were derived for each species using the Stable Isotope Bayesian Ellipses (SIBER)46 model in R. Despite the fact that the estimation of these metrics is usually made using raw $\delta^{15}$N49, they have also been estimated by standardising $\delta^{15}$N to trophic chain length (i.e. trophic position). We used the latter method in our study as it shows reduced variability in $\delta^{15}$N due to factors other than trophic fractionation47. Layman metrics included the mean distance to the centroid (CD), which provides a description of trophic diversity (niche width and species spacing); dCr ($\delta^{15}$C range), providing a univariate estimate as a measure of the diversity of basal resources; mean nearest neighbour distance (MNN), providing a measure of density and clustering of species within the community and standard ellipse area (SEAc), which provides a bivariate measure of mean core isotopic niche width (see Jackson et al.46 for detailed methodology). To allow comparisons between species populations with varying sample sizes, all metrics were bootstrapped (n = 9999) and a small sample size correction for improving accuracy to SEA values is indicated by the subscript ‘c’46. Pearson correlations were then carried out to assess the relationships between trophic chain length (TCL; i.e. maximum/highest TP value per survey time) vs invertebrate taxa richness, dCr, TA and water depth (as a proxy of hydroperiod).

Data Accessibility. Once the paper has been accepted for publication, a data manuscript will be finalised and submitted to Scientific Data.

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Author Contributions
Conceived and designed the experiments: T.D., R.J.W. Performed the experiments: T.D., R.J.W. Analysed the data: T.D., R.J.W. Contributed reagents/materials/analysis tools: T.D., R.J.W., O.L.F.W. Wrote the paper: T.D., R.J.W., P.W.F., O.L.F.W.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

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