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The effects of supra-seasonal drought on stream macroinvertebrates

**Variability in the response of amphipods and macroinvertebrate
assemblage structure to prolonged drought in forested upland streams**

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Acknowledgements

This work was supported in part by the Melbourne Waterway Research-Practice Partnership
and the Australian Research Council (ARC) Linkage Program (LP0883610 and
LP130100295). We thank Chris Szota, Joe Greet and two anonymous referees for their
constructive comments.

Summary

With climate change set to increase the frequency and severity of drought in many parts of the world, there is a need to better understand the effects of drying on stream ecosystems. We investigated the long-term effects of drought on two amphipod taxa *Paramoera fontana* (Pontogeneiidae) and *Austrogammarus australis* (Paramelitidae) and macroinvertebrate assemblage structure through an analysis of 13 years of data collected from four forested stream reaches (Victoria, Australia). Abundances of *A. australis* and *P. fontana* in the lower reach of Lyrebird Creek declined to zero following surficial streambed drying. Similar declines in abundances were not observed in Sassafras Creek or the two headwater springs, which continued to flow throughout the drought. *P. fontana* was detected again in the lower reach of Lyrebird Creek 12 months after the final cease-to-flow, however *A. australis* remained undetected 5 years later, despite an upstream source population within 2 km. Both the entire and shredder macroinvertebrate assemblage structure in Sassafras Creek and the lower reach of Lyrebird Creek shifted significantly pre- and post-surficial streambed drying in the lower reach of Lyrebird Creek. Despite signs of recovery following a return to more average flows, assemblage composition remained considerably different. The substantial differences in the recovery of the two species indicates varying resistance and resilience traits. The failure of *A. australis* to recolonize after 5 years indicates an absence of any significant resistance or resilience traits. In contrast, the rapid re-colonization of *P. fontana* may indicate poor resistance traits, but strong resilience traits. The sensitivity of *A. australis* to cease-to-flow events points to the need to carefully manage water extraction to protect this threatened species. The effective management of macroinvertebrate assemblages in the face of drought requires a clear understanding of their response to drying, the conservation of refugia and the minimization of additional stressors which reduce ecosystem resilience.

Keywords

Resistance, Resilience, Threatened species, Long-term recovery lag, Freshwater, Lotic.

1 **Introduction**

2 Drought alters aquatic ecosystem structure and function (Bond et al. 2008). While no unified
3 definition of drought exists across disciplines and regions (Humphries and Baldwin 2003,
4 Wood and Armitage 2004), we follow Gibbs and Maher (1967) who defined meteorological
5 drought as a rainfall deficiency where the three monthly rainfall total lies in the lowest 10%
6 of the long-term average. Supra-seasonal drought is an unpredictable and infrequent natural
7 phenomenon (cf. seasonal drought; Boulton and Lake 2008). However climate change and
8 other anthropogenic stressors (e.g. water extraction, landscape fragmentation) are increasing
9 both the frequency and severity of drought and the magnitude of its impacts on aquatic
10 ecosystems (Bond et al. 2008, Boulton and Lake 2008, Robson et al. 2011). While drought
11 can be defined as a ‘ramp’ disturbance (described by its intensity, duration and spatial
12 extent), the ecosystem responses are often stepped as a function of critical biological
13 thresholds (Suren and Jowett 2006, Boulton and Lake 2008).

15 Hydrology and hydraulics are crucial to structuring lotic biological assemblages (Poff and
16 Ward 1989). Hydrological drought reduces stream volumes and velocity and this in turn
17 decreases lateral connectivity with backwaters, emergent macrophytes, fringing riparian
18 vegetation and floodplain wetlands, and reduces longitudinal connectivity at the catchment
19 scale as streams dry to isolated pools (Bond et al. 2008). This results in the sequential loss of
20 in-stream habitat; firstly of shallow riffle environments, then of runs and deep pools and
21 eventually the complete surficial channel (Boulton and Lake 2008). In addition to this habitat
22 loss there are also alterations to predator-prey interactions and declines in water quality (e.g.
23 increased temperature and electrical conductivity and decreased dissolved oxygen and pH)
24 and lateral (e.g. riparian litter) and longitudinal (e.g. drifting prey, organic matter and
25 nutrients) resource subsidies (Bond et al. 2008, Boulton and Lake 2008). Drought
26 consequently not only effects the survival of individual species and overall community
27 structure, but also ecosystem function, given the critical role of stream insects in processing
28 organic matter and transporting nutrients and energy (Stubbington and Wood 2013).

30 Recovery of aquatic macroinvertebrate assemblages is influenced by the resistance and
31 resilience traits of individual species, but also by the intensity and spatial extent of the
32 drought, habitat heterogeneity and catchment condition (Wood and Armitage 2004, Robson
33 et al. 2013). Resistance traits include those that allow taxa to ‘sit out’ a drought, such as the

1 use of physical refugia (e.g. crayfish burrows, deep pools, hyporheic sediments, and
2 headwater springs) and other physiological adaptations to drying (e.g. desiccation resistant
3 eggs, aerial adults and cohort splitting) (Humphries and Baldwin 2003, Robson et al. 2011,
4 Stubbington and Wood 2013). Conversely, resilience traits include those which allow a
5 species to rapidly disperse from adjacent habitats and thus recolonize areas upon the
6 resumption of flow (e.g. drift and efficient aerial or aquatic migration) (Robson et al. 2011).
7 However despite clear recognition of the critical role macroinvertebrates play in food-web
8 structure and ecosystem function, the long-term impacts of drought on macroinvertebrates
9 (especially in perennial systems) and the individual species traits and environmental
10 requirements which influence them remain little studied (Wood and Armitage 2004, Bond et
11 al. 2008, Boulton and Lake 2008).

12
13 Many amphipod species inhabit the land-water interface and are thus often expected to be
14 well adapted to drying and, more broadly, the effects of drought (Poznańska et al. 2013).
15 However there are very few published studies investigating the effects of drying and drought
16 on amphipods and those which do show inconsistent patterns and variable recovery times
17 (Wood and Armitage 2004, Poznańska et al. 2013). Most studies have focused on the family
18 Gammaridae and specifically *Gammarus pulex* (e.g. Wood and Armitage 2004). Despite
19 evidence that gammarids have adaptations that suggest resistance (Wood et al. 2010,
20 Poznańska et al. 2013, Stubbington and Wood 2013) and resilience to drought (Hughes 1970,
21 Elliott 2002, Meyer et al. 2004), recovery times following supra-seasonal drought are highly
22 variable (Wood and Petts 1994, Wood and Armitage 2004, Stubbington et al. 2009). The
23 effects of drought on other amphipod taxa are even less clear, representing a significant
24 knowledge gap that limits the effective management of threatened species (e.g. Department
25 of Sustainability and Environment 2000a, Department of Sustainability and Environment
26 2000b) and stream ecosystems as a whole.

27
28 This study aimed to investigate the long-term effects of supra-seasonal drought on two
29 amphipod taxa, *Paramoera fontana* (Pontogeneiidae) and *Austrogammarus australis*
30 (Paramelitidae) and macroinvertebrate assemblage structure. We analysed 13 years of
31 macroinvertebrate monitoring data from four reaches (two headwater spring-fed reaches and
32 two upland perennial reaches) located on three small first-order streams in the Dandenong
33 ranges in south-eastern Australia. More specifically, we aimed to investigate the recovery
34 times of these two amphipod taxa and the resultant macroinvertebrate assemblage to supra-

seasonal drought and channel drying. We expected recovery lags would provide useful information on species resistance and resilience traits and facilitate effective management of these important species and stream ecosystems more generally in the face of increasing drought frequency and severity.

Methods

We used data collected from four reaches (two headwater spring-fed reaches and two upland perennial reaches) on three small first-order streams located in the Dandenong ranges, Victoria, Australia (Figure 1). Catchments were largely forested, however the Sassafras Creek catchment also contained some urban development, albeit with little formal stormwater drainage infrastructure (Walsh et al. 2012). We sampled the four reaches intermittently from (austral) Spring 2001 to Spring 2014. The lower reaches of Lyrebird and Sassafras Creek were sampled biannually 2001–2005 and 2007–2014. The upper reaches of Lyrebird Creek and Wallaby Creek were sampled on six dates between Spring 2002 and Summer 2003, and then again in Autumn 2013, and Wallaby Creek was sampled again on five dates between Summer 2004 and Autumn 2005.

While droughts are common in Australia, the millennium drought impacted south-eastern Australia between 1997 and 2009 (inclusive) and represented the driest 13 years since reliable records began (SEACI2010, Dijk et al. 2013). This drought had unprecedented impacts on stream flows, with a 13 % decline in annual rainfall (with greatest declines in autumn) resulting in a 44 % decline in annual streamflows in the southern region of Australia's largest river system, the Murray-Darling Basin (SEACI2010, Dijk et al. 2013). This resulted in reduced flows in all study reaches from 2001 to 2009; and culminated in the lower reach of Lyrebird Creek (~1 km in length) experiencing several cease-to-flow events between 2007 and 2009, and complete surficial streambed drying in the early autumns of 2007, 2008 and 2009. The millennium drought was prolonged and severe, however, its impacts were spatially variable across our study sites; with Sassafras Creek and the upper spring fed reaches of Lyrebird and Wallaby Creeks exhibiting reduced discharge, but continuous flow throughout the drought.

We undertook semi-quantitative sampling of macroinvertebrates at Sassafras Creek and the lower reach of Lyrebird Creek following standard rapid biological assessment (RBA) methods (EPA2003). In short, we sampled 10 m of riffle habitat and 10 m of edge habitat

1 using a 250- μ m mesh net with a 30 x 30-cm opening. Samples were preserved in the field
2 using 70% ethanol and sorted in the laboratory (a minimum random subsample of 10% was
3 sorted, with larger subsamples taken until 300 individual macroinvertebrates had been
4 collected (see Walsh 1997)). Total abundances in each sample were estimated by dividing
5 the number sorted by the proportional subsample size, and reported as count per unit effort.
6 Specimens were primarily identified to family, but Acarina were not identified further, and
7 Chironomidae were identified to subfamily. To assess shifts in macroinvertebrate assemblage
8 composition, families were assigned to function feeding groups; where families containing
9 any known shredder genera were considered shredders (Imberger et al. 2008 and references
10 therein, Hawking JH et al. 2013 and references therein). In Wallaby Creek and the upper
11 reach of Lyrebird Creek we undertook quantitative sampling of riffle and emergent/riparian
12 habitats using 3-5 replicate quadrats with an area of 0.11m². Following haphazard placement
13 of the quadrat in either habitat type, all organic material and loose substrate was collected and
14 washed free of invertebrates over a 250 μ m sieve. Amphipods were identified in the field
15 using illuminated magnifying lenses (Jaycar, 10 diopetre) to genus and reported as mean
16 count/m² for each site on each date.

17
18 Our analysis combines samples collected from several studies that used different collection
19 methods and taxonomic resolution for amphipod identification. While collection methods
20 differed between sites, our primary analysis is to contrast trends in RBA samples from
21 Sassafras Creek, which maintained flow throughout the study period and those from the
22 lower reach of Lyrebird Creek, which dried on several occasions. We additionally report
23 amphipod abundances from Wallaby Creek and upper Lyrebird Creek to demonstrate
24 persistence of amphipod populations in perennial spring-fed streams, including a site
25 upstream of the focal lower reach of Lyrebird Creek.

26
27 We used information from these and previous studies (Doeg et al. 1996, Papas et al. 1999) to
28 infer the identity of amphipod taxa at each site. A subset of amphipod taxa in multiple
29 samples across the study period from each site were identified to species. All specimens
30 identified to species from Wallaby and Lyrebird Creek were either *A. australis* or *P. fontana*.
31 As *A. australis* is the only recorded Paramelitidae species in Lyrebird Creek in this or
32 previous studies (Doeg et al. 1996, Papas et al. 1999), we report all Paramelitidae from these
33 streams as *A. australis*. In Sassafras Creek, all Pontogeneiidae were *P. fontana*, while

Paramelitidae were both *A. haasei* and *A. australis*. We therefore refer to Paramelitidae collected from Sassafras Creek as *Austrogammarus* spp.

Clear patterns of individual taxon abundance were evident through time and our assessments of persistence were based on visual analysis of temporal plots. Alterations to macroinvertebrate assemblage composition were assessed using non-metric multidimensional scaling (MDS) based on Bray-Curtis dissimilarity in R (R Development Core Team 2009). Three dimensional Kruskal stress values were < 0.10 , however, the third dimension provided no significant additional exploratory power beyond the first two dimensions. We consequently presented two-dimensional ordinations with Kruskal stress values ranging between 0.132 and 0.142. We used permutational analysis of variance (PERMANOVA) and similarity percentages (SIMPER) using Bray-Curtis values in PRIMER, to further investigate the statistical significance of the changes in assemblage composition and identify the individual families driving dissimilarity through time. In the case of PERMANOVA, both site (lower reach of Lyrebird Creek and Sassafras Creek) and drying phase (categorical: pre- and post-initial surficial streambed drying in the lower reach of Lyrebird Creek (i.e. Autumn 2007)) were treated as fixed factors. Throughout the multivariate analyses, count data were $\log_{10}(x+1)$ transformed, which downweighted the contribution of abundant taxa to dissimilarity (the inference from the analysis was, however, unchanged if data were untransformed).

Results

In the upper spring fed reaches of Lyrebird and Wallaby creeks, both *A. australis* and *P. fontana* were present on every sampling occasion (Figure 2). Mean abundance of *A. australis* was 20–210 m⁻² in the upper reach of Lyrebird Creek and 69–490 m⁻² in Wallaby Creek. Mean abundance of *P. fontana* was 88–740 m⁻² in the upper reach of Lyrebird Creek and 5–460 m⁻² in Wallaby Creek. In Sassafras Creek, which also flowed continuously during the study period, *P. fontana* was abundant in all samples (5–11000 per unit effort). *Austrogammarus* spp. were less abundant (0–160 per unit effort), but persisted at similar densities throughout the study period, although they were not detected on two occasions (Spring 2007 and Autumn 2010, Figure 3b).

A. australis and *P. fontana* were consistently abundant in the lower reach of Lyrebird Creek before the first cease-to-flow and surficial stream bed drying in 2007 (20–490 and 240–1600

per unit effort, respectively: Fig. 3a). However, neither species was detected in samples from this reach in the following two years. *A. australis* remained undetected in the lower reach of Lyrebird Creek until sampling concluded in Spring 2014 (Figure 3a). A single *P. fontana* individual was collected from the reach in Autumn 2010, twelve months after the final cease-to-flow event and a return to more average flow conditions, with abundances tending to increase in subsequent samples to a maximum of 41 per unit effort in Spring 2014 (Figure 3a).

Other shredder families in the lower reach of Lyrebird Creek did not show similar losses following cease-to-flows and surficial stream drying, with Elmidae and Leptophlebiidae occurring consistently at densities of 42–5100 and 9–920 per unit effort respectively (Figure 4). Compositional similarity of the broader macroinvertebrate assemblage changed significantly following initial stream cease-to-flow and surficial streambed drying in 2007 in the lower reach of Lyrebird Creek (PERMANOVA $t_{1,2} = 2.752$, $p < 0.001$; Table 1, Figure 5). This shift in composition was driven primarily by a reduced abundance of both *P. fontana* and *A. australis*, with these taxa contributing to 8.68 % and 6.42 % of dissimilarity through time (Table 2). There was also a lesser, but still significant, shift in macroinvertebrate assemblage composition pre- and post-Autumn 2007 at Sassafras Creek, despite continuous flows throughout the study period (PERMANOVA $t_{1,2} = 1.925$, $p = 0.001$; Table 2, Figure 5). However there was a greater degree of similarity between pre- and post-Autumn 2007 assemblages than in Lyrebird Creek (Figure 5). The dissimilarity between assemblages pre- and post-Autumn 2007 in Sassafras Creek were driven by a broad range families, with *P. fontana* and *Austrogammarus* spp. contributing only 4.62 % and 2.78 % respectively (Table 2).

Compositional similarity of the shredder (functional feeding group) assemblage in both the lower reach of Lyrebird Creek and Sassafras Creek also changed significantly as a function of drying phase (i.e. pre- and post-initial surficial streambed drying in the lower reach of Lyrebird Creek in Autumn 2007). However MDS ordinations were visually similar to the equivalent plots based on the entire assemblage, and are thus not presented here. In the lower reach of Lyrebird Creek, the shift in composition was driven primarily by a reduced abundance of both *P. fontana* and *A. australis*, with these taxa contributing to 36.23 % (20.82 % and 15.42 % respectively) of dissimilarity between pre- and post-2007 samples (Table 3). By comparison, dissimilarity in shredder assemblage composition between pre- and post

2007 samples from Sassafras Creek were influenced by a larger number of families, with *P. fontana* and *Austrogammarus* spp. contributing only 11.36 % and 6.96 % respectively (Table 3).

Discussion

This study has demonstrated that freshwater amphipod species, despite adaptations to life at the land-water interface (Poznańska et al. 2013), are highly susceptible to the impacts of drought and that their capacity to recover varies between species. Despite a source of potential colonizers < 2 km upstream (i.e. the upper reach of Lyrebird Creek: Fig. 1) and a return to low perennial flows in Spring 2009 and more average flows from 2010 onwards, *A. australis* remained undetected in the lower reach of Lyrebird Creek. Conversely, individuals of *P. fontana* were detected again, albeit in reduced numbers, in the lower reach of Lyrebird Creek 12 months after the final cease-to-flow event and immediately upon the return of more average flow conditions.

The failure of *A. australis* to be detected 5 years after the resumption of more average flow conditions suggests a sensitivity to drought substantially greater than reported for any other amphipod species, while the recovery of *P. fontana* suggests a similar sensitivity to drought and capacity to recolonize to that reported for *G. pulex*. Several studies have demonstrated the loss of *G. pulex* following cease-to-flow events, with recovery times ranging from 5 days in intermittent and ephemeral headwater streams (Stubbington et al. 2009) to 12–24 months in drought-affected perennial streams (Ladle and Bass 1981, Wood and Petts 1994, Wood and Armitage 2004). While *P. fontana* re-colonized the lower reach of Lyrebird Creek over a comparable period, it is noteworthy that abundances have remained an order of magnitude lower than in the early phases of the drought and prior to complete surficial stream drying (Fig. 2a).

The loss of both *A. australis* and *P. fontana* following cease-to-flow events and surficial streambed drying in the lower reach of Lyrebird Creek suggests these species exhibit poor resistance traits (Lancaster and Belyea 1997). This may indicate an inability to effectively utilize refuges (e.g. crayfish burrows, deep pools, hyporheic sediments or headwater springs, see Bond et al. 2008, Boulton and Lake 2008) or an absence of morphological/physiological adaptations to physical stress (e.g. aestivation, desiccation tolerant life history stages, positive rheotaxis, aerial adults or cohort splitting, see Lancaster and Belyea 1997, Bond et al. 2008,

1 Robson et al. 2013). In contrast, Elmidae and Leptophlebiidae persisted in the lower reach of
2 Lyrebird Creek throughout the drought and subsequently exhibited strong resistance traits.
3 This observation is consistent with other published research indicating some coleopterans can
4 utilize positive rheotaxis and the hyporheic zone in response to drought (Wood et al. 2010,
5 Robson et al. 2011), while some Leptophlebiidae have drought resistant eggs and exhibit
6 aerial adults (Gooderham and Tsyrlin 2002, Robson et al. 2011). However differences in the
7 recovery of amphipod taxa suggest that *A. australis* exhibit poor resilience traits (e.g.
8 recruitment or recolonization via drift or active migration), while *P. fontana* are far more
9 resilient and thus capable of a relatively rapid recolonization.

10
11 While amphipods have been reported using several refugia (including pools, pholeteros,
12 hyporheic zone and aestivation; Robson et al. 2011); few studies have investigated the
13 resistance and resilience traits of amphipod taxa and those which do, show variable results
14 (Wood and Armitage 2004, Poznańska et al. 2013). In a laboratory study looking at the
15 effects of drying on several gammarids, Poznańska et al. (2013) showed some species
16 migrated laterally following receding water lines and also horizontally into saturated
17 sediments, while others desiccated and died. Wood et al. (2010) observed *G. pulex* using the
18 hyporheic zone during supra-seasonal drought in a UK chalk stream. However Stubbington et
19 al. (2009) found *G. pulex* was unable to use the hyporheic zone of their relatively fine grained
20 karst stream during drought. Stubbington and Wood (2013) similarly showed *Crangonyx*
21 *pseudogracilis* was unable to use hyporheic sediments; however Harris et al. (2002) showed
22 that *C. pseudogracilis* could use shallow groundwater as both a temporary refugia and
23 dispersal corridor. Stubbington and Wood (2013) also observed both *G. pulex* and *C.*
24 *pseudogracilis* migrating towards spring-fed reaches in response to reduced flows and
25 elevated stream temperatures; while Myer et al. (2004) also noted *G. pulex* can retreat to and
26 survive in remnant pools.

27
28 These previous studies and ours serve to highlight the variability in individual species
29 resistance traits and the need for further investigation. Reported resilience traits of amphipod
30 taxa are however less variable, with numerous studies noting rapid recolonization via drift
31 and active migration (Meyer et al. 2004, Robson et al. 2011, Poznańska et al. 2013). Hughes
32 (1970) found that *G. pulex* passively drifted at both low stream velocities and immediately
33 following rapid increases in velocity. Similarly, Elliott (2002) observed both active upstream
34 swimming and passive downstream drifting by *G. pulex* across a range of stream velocities.

Elliott (2002) also noted that passive drift lasted an average of 8 seconds and the mean distance travelled ranged from 0.28 - 7.52 m with stream velocities varying between 0.03 and 0.96 m s⁻¹. The response of *P. fontana* following rewetting of the lower reach of Lyrebird Creek is consistent with such travel speeds if the recolonizers were sourced from the persisting headwater population 2 km upstream. However, the lack of recolonization by *A. australis* 5 years after the resumption of flow, suggests this species lacks the resilience traits exhibited by other amphipod taxa. Using the data of Elliott (2002) and an average stream velocity of 7 cm s⁻¹ in the lower reach of Lyrebird Creek (Imberger et al. 2011), we calculated that *G. pulex* could travel between our upstream source population and our downstream sample site in as little as 7 h. While this recolonization rate assumes a continuous rate of drift (which is unlikely), it does serve to highlight how little time could be required to travel between our sites under summer low flow conditions.

We are unable to explain the differential response of *A. australis* and *P. fontana* to drought and complete surficial streambed drying in the lower reach of Lyrebird Creek. *A. australis* is listed as a threatened species under the Australian Flora and Fauna Guarantee Act (DEPI 2015) and a clear understanding of factors influencing its abundance during drought are paramount. Walsh et al. (2004) showed that stormwater drainage from roofs, paving and sealed roads was the strongest predictor of *A. australis* distribution during wetter years (rather than septic tank or unsealed road densities). However we observed no significant changes in stormwater connection or more proximate shifts in sediment particle size or long-term water quality, (including nutrient and suspended solid concentrations, temperature, dissolved oxygen saturation, electrical conductivity or pH) in the lower reach of Lyrebird Creek over the 13 years the site has been monitored as part of this and other studies (data not reported here, but see the reference site data of Walsh et al. 2015). Our data indicates that alterations to habitat quality, independent of drought, are not restricting the recovery of *A. australis* in the lower reach of Lyrebird Creek. This study demonstrates a strong association between *A. australis* abundance and stream drying, however to date there is no published research on the species individual traits, habitat requirements, or life history strategy that can explain our observations. We thus seek to highlight the need for greater research into *A. australis*; particularly given its threatened conservation status (DEPI 2015).

Data from the lower reach of Lyrebird Creek clearly shows a strong correlation between *A. australis* persistence and complete surficial streambed drying. Our study indicates that water

1 extraction, particularly during drought should be considered as an additional threatening
2 process requiring active management for the conservation of *A. australis*. We contend that
3 conservation of *A. australis* habitat requires careful regulation of water abstraction to prevent
4 anthropogenic induced cease-to-flows and surficial streambed drying.

5
6 Drought induced alterations to amphipod abundance can have significant and sustained
7 impacts on both macroinvertebrate assemblage structure and more specifically, shredder
8 assemblage composition. Our analyses showed that reduced abundances of *P. fontana* and *A.*
9 *australis* were the strongest drivers of significant changes to both the entire assemblage and
10 shredder assemblage composition following surficial streambed drying in the lower reach of
11 Lyrebird Creek in Autumn 2007. We also found a significant difference in assemblage
12 composition pre- and post-Autumn 2007 in Sassafras Creek, which continued to flow during
13 the drought. This compositional shift was, however, driven less by changes in abundance of
14 *Austogammarus* spp. and more so by other families/genera including increases in
15 Chironominae and Ptilodactylidae abundance and decreased *P. fontana* abundance. While not
16 included here, temporal plots of individual shredder families in the lower reach of Lyrebird
17 Creek also showed that reduced abundances of *P. fontana* and *A. australis* were not
18 accompanied by any significant concomitant increases in other shredder families.

19
20 Our findings are similar to several previous studies of drought in UK chalk streams which
21 showed significant changes to assemblage structure, driven by alterations to taxon abundance
22 alone (Wright and Symes 1999) and both taxon richness and abundance (Wood and Petts
23 1994, Wright et al. 2002). For example Wright and Symes (1999) observed a significant
24 decrease in the density of Simuliidae and an increase in the densities of Chironomidae and
25 Ceratopogonidae during drought. Similarly, Woods and Petts (1994) observed the loss of
26 Elmidae, Simuliidae and Ephydriidae during drought, but also a significant decline in
27 Lymnaeidae and *G. pulex*. In the case of two intermittent Australian streams, Boulton and
28 Lake (1992) also showed shifts in faunal composition following drought. Acuña et al. (2005)
29 also showed significant compositional shifts with increases in the densities of Chironomidae
30 and Ceratopogonidae following seasonal drought in an intermittent Spanish stream. While the
31 drought induced shifts in assemblage composition shown in this and other studies are
32 significant unto themselves, they can also have cascading trophic impacts and furthermore
33 effect organic matter processing/transport, nutrient cycling and ultimately energy transfer
34 through aquatic ecosystems (Lake 2003, Bond et al. 2008, Boulton and Lake 2008). This is

1 particularly true in our study where increases in other shredder families were not observed in
2 response to the sudden absence of *A. australis* and the reduced long-term abundance of *P.*
3 *fontana* in samples from the lower reach of Lyrebird Creek.

4
5 The extended duration of our study also provides a rare opportunity to assess the recovery,
6 both trajectories and associated lag periods, of macroinvertebrate assemblages to drought
7 (Wood and Armitage 2004, Bond et al. 2008). Our whole macroinvertebrate and shredder
8 assemblage composition ordination plots showed signs of recovery from Spring 2010
9 onwards in both the lower reach of Lyrebird Creek and Sassafras Creek. However even upon
10 completion of the study, the assemblage composition at both sites remained considerably
11 different to that seen prior to initial surficial streambed drying in the lower reach Lyrebird
12 Creek (i.e. Autumn 2007). In the case of the lower reach of Lyrebird Creek, this lag period
13 was likely driven by the continued absence of *A. australis* from samples and to a lesser
14 extent, the reduced abundance of *P. fontana* and increased abundances of Hyrdobiosidae and
15 Ptilodactylidae. However, in the case of Sassafras Creek, recovery lags were potentially
16 driven by a reduced abundance of *P. fontana* and increased abundance of other families
17 including Chironominae and Ptilodactylidae. Increased abundance of such pioneer taxa (e.g.
18 Chironomidae) following the resumption of flows are often noted in the literature, however
19 these are usually followed by relatively rapid declines as species with longer life cycles
20 increase in numbers (Wood and Petts 1994, Wright and Symes 1999, Lake 2003, Acuña et al.
21 2005).

22
23 The recovery lag periods seen in this study are significantly longer than any others reported
24 in the literature (see Ladle and Bass 1981, Wood and Petts 1994, Wood and Armitage 2004,
25 Stubbington et al. 2009). Recovery periods can be influenced by numerous site-specific (e.g.
26 habitat heterogeneity and fragmentation, refuge availability and ecosystem stability and
27 resilience) and drought-specific factors (e.g. intensity, duration, extent and timing) and their
28 interaction with individual species traits, biological requirements and life history strategy
29 (Wood and Petts 1994, Boulton 2003, Robson et al. 2013). While the millennium drought
30 assessed in this study was particularly long and intense, our streams were minimally impacted
31 by anthropogenic disturbance and in very good condition; thus likely exhibiting high
32 resilience and a diversity of connected refugia and in turn conducive to rapid recovery. The
33 extended recovery lag observed in the current study was thus unexpected and highlights the

1 potential for severe long-term impacts of drought on individual species persistence and both
2 ecosystem structure and function (Boulton and Lake 2008), even in healthy, intact systems.
3
4 Drought is a natural reoccurring disturbance to Australian ecosystems; however its frequency
5 and severity is expected to increase over Southern Australian in response to climate change
6 (CSIRO & BOM2015). Many freshwater ecosystems are being threatened by
7 overexploitation, water pollution, flow modification, habitat degradation and invasive species
8 (Dudgeon et al. 2006); leaving them increasingly vulnerable to the effects of drought and its
9 increasing frequency and severity. This study has shown that amphipod populations and
10 shredder assemblage composition more broadly are strongly affected by drought and more
11 specifically the thresholds associated with cease-to-flow events and surficial streambed
12 drying; but that these impacts are not consistent across species. Our findings suggest *A.*
13 *australis* displays a sensitivity to drought substantially greater than reported for any other
14 amphipod species. Our results reinforce the need for further study into individual traits,
15 habitat requirements and life histories of individual species; but especially those of the
16 threatened *A. Australis*. Only when armed with such information can we hope to effectively
17 manage both individual species and entire communities in the face of drought and other
18 anthropogenic stressors.

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Figure Captions

Figure 1. Study sites and their catchments (dashed dark grey lines) in Victoria, Australia. W, Wallaby Creek; uL, upper Lyrebird Creek; L, Lyrebird Creek (lower); Sassafras Creek. Impervious surfaces are shown as light grey polygons. The primary land cover in the study catchments is forest.

Figure 2. Abundance of *P. fontana* and *A. australis* in (a) the upper reach of Lyrebird Creek and (b) Wallaby Creek through time. Vertical lines in the corner of the plot indicate maximum and minimum standard error. Note: Abundance data was not collected between 2006 and 2012.

Figure 3. Abundance of *P. fontana* and *Austrogammarus* spp. in (a) the lower reach of Lyrebird Creek and (b) Sassafras Creek through time. Arrows indicate times of both cease-to-flow and complete surficial streambed drying.

Figure 4. Abundance of Elmidae and Leptophlebiidae in the lower reach of Lyrebird Creek through time. Arrows indicate times of both cease-to-flow and complete surficial streambed drying.

Figure 5. Non-metric MDS ordination of the macroinvertebrate assemblage data at the lower reach of Lyrebird Creek and Sassafras Creek from 2001 – 2014. Lines and arrows trace sequential shifts in the ordination space through time.

1	Figures
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3	Figure 1.
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5	Figure 2.
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7	Figure 3.
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13	Figure 5.

Table 1. Comparing macroinvertebrate assemblage composition between sites (Sassafras Creek and the Lower reach of Lyrebird Creek) and drying phase (pre- and post-initial surficial streambed drying in the lower reach of Lyrebird Creek; i.e. pre- and post-Autumn 2007) using PERMANOVA. Note: Site and drying phase were treated as fixed factors.

Main Effects					
Source	df	SS	MS	Pseudo-F	P(perm)
Site	1	3978	3978	6.490	< 0.001
Drying	1	5400	5400	8.811	< 0.001
Res	37	22678	613		
Total	40	34205			

Pairwise tests			
Lower reach of Lyrebird Creek			
Drying		t	P(perm)
		2.752	< 0.001

Sassafras Creek			
Drying		t	P(perm)
		1.925	0.001

1 **Table 2.** SIMPER output showing the relative contribution of individual families to
2 differences in macroinvertebrate assemblage composition as a function of drying phase (pre-
3 and post-initial surficial streambed drying in the lower reach of Lyrebird Creek). Only
4 families contributing to the first 50% of similarity between times are shown here. Note that
5 Pontogeneiidae and Paramelitidae are represented here only by *P. fontana* and
6 *Austrogamarus* spp. respectively.

Family	Post-Autumn 2007 Average Abundance (log10(x+1))	Pre-Autumn 2007 Average Abundance (log10(x+1))	Diss/SD	Contribution (%)
Lower reach of Lyrebird Creek				
Pontogeneiidae	0.99	6.61	3.05	8.69
Paramelitidae	0.00	4.21	3.77	6.42
Hydrobiosidae	3.37	1.16	1.68	3.61
Ptilodactylidae	2.97	0.85	1.71	3.60
Chironominae	5.14	3.25	1.60	2.96
Phreodrilidae	2.03	0.12	1.32	2.87
Simuliidae	2.80	1.23	1.36	2.84
Psephenidae	2.72	1.51	1.45	2.76
Naididae	2.11	1.24	1.41	2.74
Empididae	2.13	0.53	1.73	2.73
Scirtidae	2.61	2.51	1.35	2.54
Acarina	2.62	2.48	1.37	2.50
Ceratopogonidae	3.63	2.08	1.41	2.49
Elmidae	6.58	5.29	1.37	2.47
Sassafras Creek				
Pontogeneiidae	4.55	7.42	1.72	4.62
Chironominae	3.68	1.21	2.04	3.78
Ptilodactylidae	3.54	2.26	1.27	3.60
Hydrobiidae	4.09	2.58	1.19	3.43
Tanypodinae	2.75	1.11	1.76	3.26
Elmidae	6.04	4.83	1.07	3.10
Tipulidae	2.95	1.49	1.40	3.04
Hydropsychidae	3.45	2.71	1.33	2.81
Austroperlidae	2.18	2.36	1.32	2.81
Paramelitidae	2.83	3.39	1.49	2.78
Acarina	2.49	1.22	1.26	2.78
Simuliidae	1.95	2.12	1.37	2.73
Dixidae	2.13	1.27	1.37	2.72
Ceratopogonidae	2.30	1.29	1.29	2.67
Athericidae	2.47	2.31	1.36	2.56
Hydrobiosidae	1.73	0.00	1.45	2.52

Table 3. SIMPER output showing the relative contribution of individual families to differences in shredder assemblage composition as a function of drying phase (pre- and post-initial surficial streambed drying in the lower reach of Lyrebird Creek i.e. pre- and post-Autumn 2007). Only families contributing to the first 50% of similarity between times are shown here. Note that Pontogeneiidae and Paramelitidae are represented here only by *P. fontana* and *Austrogammarus* spp. respectively.

Family	Post-Autumn 2007 Average Abundance (log10(x+1))	Pre-Autumn 2007 Average Abundance (log10(x+1))	Diss/SD	Contribution (%)
Lower reach of Lyrebird Creek				
Pontogeneiidae	0.99	6.61	3.31	20.82
Paramelitidae	0.00	4.21	3.95	15.42
Ptilodactylidae	2.97	0.85	1.80	8.51
Sassafras Creek				
Pontogeneiidae	4.55	7.42	1.78	11.36
Chironominae	3.68	1.21	2.01	9.53
Ptilodactylidae	3.54	2.26	1.25	9.10
Elmidae	6.04	4.83	1.03	7.88
Tipulidae	2.95	1.49	1.36	7.73