

1 Abstract

- 2
- 3 1. Invertebrate assemblages of tropical freshwater rock pools (FRP) are poorly
4 known compared with temperate regions, where FRP contain specialised, endemic
5 taxa of high conservation value. We sampled invertebrates from pristine FRP in
6 the Morgan River catchment in the Australian wet-dry tropics in the 2015 and
7 2016 wet-seasons. Taxa were classified as either active or passive dispersers and
8 by functional feeding groups. A range of physical variables were examined to
9 identify associations with patterns in invertebrate assemblages.
- 10 2. Forty-seven taxa were identified from FRP; most were actively-dispersing
11 predatory insects, primarily Coleoptera and Hemiptera. Five adult insect species
12 were found in most pools; other taxa were rare and scattered across FRP. Two
13 previously undescribed species of Crustacea: Spinicaudata (*Eulimnadia*
14 *kimberleyensis* and *Ozestheria pellucida*) were collected, but few crustacean taxa
15 were recorded, and passively-dispersing taxa were rare. Species-accumulation
16 curves indicated that had more pools been sampled, more taxa would have been
17 collected in 2015, but in 2016 most species were collected.
- 18 3. There was no pattern in FRP assemblages related to any physical variable, but
19 assemblages in pools were correlated between sampling times. The opportunistic
20 and mobile nature of this assemblage composed mostly of actively-dispersing
21 predatory insects may explain the absence of relationships with measured
22 variables. Furthermore, torrential tropical rainfall causes surface flow between
23 FRP that may homogenise the fauna.
- 24 4. The potential rarity of crustaceans in these assemblages from pristine FRP in the
25 wet-dry tropics, contrasts with assemblages recorded from FRP in more temperate
26 and arid regions. Additional studies of a wider range of FRP from tropical regions
27 are required to determine whether the invertebrate fauna of these Kimberley rock
28 pools are typical of FRP in the wet-dry tropics. The discovery of two new species
29 of Spinicaudata, from a relatively small survey, demonstrates the importance of
30 conserving FRP in tropical landscapes.
- 31

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1 **Introduction**

2 Freshwater rock pools (FRP) are precipitation-filled eroded depressions formed on exposed
3 rock, and are both biologically and hydrologically isolated from the surrounding landscape
4 matrix (McLachlan & Ladle, 2001; Cross *et al.*, 2015a; Brendonck, Lanfranco, Timms
5 & Vanschoenwinkel, 2016). Hydroperiod, inundation regime (hydroregime) and water
6 volume are the major environmental drivers in FRP (Bayly, Halse & Timms 2011; Timms,
7 2012a; Cross *et al.*, 2014; 2015a), and are determined by seasonal rainfall patterns, basin
8 morphology, and catchment size (Hulsmans, Vanschoenwinkel, Pyke, Riddoch & Brendonck
9 2008; Jocqué, Vanschoenwinkel & Brendonck, 2010; Cross *et al.*, 2015a). Hydroperiod in
10 FRP ranges from several days to months, and in rare cases some pools hold permanent water
11 (Cross *et al.*, 2015a; Timms, 2013). These factors regulate the composition of aquatic
12 invertebrate assemblages, because organisms must contend with the ever-present risk of
13 drying before life cycles can be completed (McLachlan & Ladle, 2001; Williams, 2006;
14 Hulsmans *et al.*, 2008; Vanschoenwinkel *et al.*, 2009). FRP represent a harsh and dynamic
15 habitat of environmental extremes for freshwater organisms (McLachlan & Ladle 2001;
16 Cross *et al.*, 2015a), as these often shallow and exposed pools have limited buffering capacity
17 for pH, temperature, and dissolved oxygen (Brendonck *et al.*, 2016).

18

19 Invertebrate fauna adapted to FRP have been characterised as having either active or passive
20 dispersal abilities, and these are associated with different responses to drying (McLachlan &
21 Ladle 2001; Jocqué *et al.*, 2010). Active dispersers (commonly winged insects) migrate away
22 from drying pools, re-colonising them following inundation. Passive dispersers, commonly
23 crustaceans including ostracods and branchiopod shrimp, have at least one life stage that is
24 resistant to desiccation and commonly survive dry periods as eggs or embryonic cysts in a
25 dormant egg bank in the sediment (Jocqué *et al.*, 2010; Brendonck *et al.*, 2016). Only a
26 portion of the egg bank hatches in response to each inundation event, avoiding abortive
27 hatching should the hydroperiod be too short for organisms to reach reproductive maturity
28 (Williams, 2006; Strachan, Chester & Robson, 2015).

29

30 Under suitable hydrological conditions, FRP can harbour aquatic macrophyte communities
31 that are often comprised of FRP-endemic, highly specialised, and range-restricted species
32 (Tuckett, Merritt, Hay, Hopper & Dixon, 2010; Cross *et al.*, 2014, 2015a,b). Macrophytes
33 usually influence invertebrate assemblages directly through provision of habitat and refuges

1 from predation, mediated by the structural complexity of the vegetation (McAbendroth *et al.*,
2 2005; Warfe & Barmuta, 2006) and interactions with algal epiphytes, an important food
3 source for invertebrate grazers (Ferreiro, Giorgi & Feijoó, 2011). Typically, macrophytes
4 with high structural complexity and habitat heterogeneity support more diverse and abundant
5 assemblages of invertebrates than macrophytes with simple stems, or habitats where
6 macrophytes are absent (McAbendroth *et al.*, 2005; Walker, Wijnhoven & van der Velde,
7 2013). Despite their importance in other freshwater bodies, including tropical rivers and
8 wetlands (e.g. Kay *et al.*, 1999; Warfe *et al.*, 2011), the influence of macrophytes on
9 invertebrate assemblages in FRP has received little research attention (but see Bayly *et al.*,
10 2011).

11
12 The fauna of FRP in Mediterranean and semi-arid climatic regions around the world are
13 relatively well studied (e.g. Jocqué, Riddoch & Brendonck, 2007; Jocqué, Graham &
14 Brendonck, 2007; Vanschoenwinkel *et al.*, 2009, 2010; Anusa, Ndagurwa & Magadza, 2012;
15 review in Jocqué *et al.*, 2010). Particularly high species diversity and endemism have been
16 recorded from FRP in southwest Western Australia (Bayly, 1997; Pinder, Halse, Shiel &
17 McRae, 2000; Jocqué, Timms & Brendonck, 2007; Bayly *et al.*, 2011; Timms, 2012a,b,
18 2013). High levels of endemism are also reported for the plant communities of these habitats
19 (Tuckett *et al.*, 2010), and this biodiversity is likely a reflection of the region's long history of
20 climatic stability and isolation (Pinder *et al.*, 2000). In contrast, there are few studies of FRP
21 in tropical regions of South America, Asia, and Australia (Deil, 2005; Jocqué *et al.*, 2010;
22 Brendonck *et al.*, 2016), including the wet-dry tropics that show strongly seasonal rainfall
23 patterns (Warfe *et al.*, 2011), aside from research in Africa (e.g. McLachlan & Ladle, 2001).
24 Endemic dipterans are to be found adapted to the challenging conditions in tropical African
25 rain-pools (McLachlan, 1981, 1988; McLachlan & Ladle, 2001). Such endemism is possible
26 because although FRP are temporally unpredictable, they are spatially stable, existing for
27 thousands of years (or longer) (McLachlan & Ladle, 2001).

28
29 The Kimberley region of Western Australia lies in the wet-dry tropics north of 18 °S. Despite
30 having an area > 400 000 square kilometres (approximately three times the size of England),
31 it has a permanent population of fewer than 40 000 people (2011 data) and contains some of
32 the most pristine waterbodies in the world (Stein, Stien & Nix, 2002; Warfe *et al.*, 2011).
33 However, due to its remote location, there are few studies of the composition, structure and
34 ecology of the Kimberley freshwater invertebrate fauna (Storey & Lynas, 2007). Samples

1 from six Kimberley river systems showed the fauna was dominated by insects (74%) and taxa
2 richness was higher amongst macrophytes than in other habitats (Kay *et al.*, 1999). Storey &
3 Lynas (2007) studied the regulated lower Ord River and showed that the fauna was insect-
4 dominated (80%, taxa identified to species level). Both studies also observed marked
5 associations between invertebrate assemblages and habitats (e.g. macrophytes, riffles, pools).
6 Recent research shows that north Kimberley FRP are among the most dynamic and
7 ephemeral freshwater habitats on the planet, harbouring highly specialised freshwater plant
8 communities characterised by high levels of local endemism (Cross *et al.*, 2014, 2015a,b).
9
10 The aim of this research was to describe the invertebrate fauna of north Kimberley FRP and
11 determine whether assemblage composition was related to selected environmental conditions.
12 As desiccation-tolerant, passively dispersing crustaceans are typical FRP fauna (Brendonck *et*
13 *al.*, 2016), and have been recorded from FRP in Western Australia's Pilbara region (Pinder,
14 Halse, Shiel & McRae, 2010), we expected that these taxa would be abundant in Kimberley
15 FRP in contrast to Kimberley river systems which are insect-dominated (Kay *et al.*, 1999;
16 Storey & Lynas 2007). Intermittent streams flowed in declivities between pavements
17 containing FRP, providing the opportunity to compare invertebrate assemblage composition
18 in FRP and adjacent streams. As macrophytes influence invertebrate assemblages through
19 provision of food, habitat and refuges from predation, we hypothesized that invertebrate
20 assemblage composition would differ between FRP with and without macrophytes. Physical
21 variables such as pool depth, hydroperiod length, number of inundations per season and mean
22 sediment depth are common drivers of invertebrate assemblage composition in FRP. We
23 hypothesized that these variables would be associated with patterns in invertebrate
24 assemblage composition. Lastly, as geographic distance, dispersal ability and desiccation-
25 resistance may also influence invertebrate assemblage composition in FRP, we hypothesized
26 that dissimilarities in invertebrate assemblages between pools and pavements would increase
27 as geographic distance between pools increased.

28

29 **Methods**

30 *Study site*

31 The study site is located in the catchment of the Morgan River, on the northwest tip of the
32 Gardner Plateau in the monsoon tropical north Kimberley region of Western Australia (14°
33 47' 46" S, 126° 31' 27" E; Fig. 1). FRP are distributed patchily across approximately 10 km²
34 of discontinuous sandstone pavement and are separated by maximum distances of < 10 km.

1 (Cross *et al.*, 2015a,b). They are geologically weathered depressions of varying size and
2 depth in the sandstone bedrock, distributed almost exclusively on flat or gently sloping
3 sandstone pavement (Fig. 1). FRP are mostly shallow (27.9 ± 1.9 mm), small (surface area <
4 1 m^2), of small volume (100–500 L), and may contain a shallow deposit of nutrient-poor
5 acidic (pH 4.2–4.9) loam or loamy sand sediment (Cross, 2014; Cross *et al.*, 2014; Table S1).
6 Approximately 10% of FRP harbour submerged macrophyte communities, usually species-
7 poor assemblages (generally 1–2 species) dominated by the annual FRP endemics *Eriocaulon*
8 sp. Morgan River (A.T. Cross ATC 62), *Myriophyllum striatocarpum* M.D. Barrett, M.L.
9 Moody & R.L. Barrett or *Myriophyllum foveicola* M.D. Barrett, M.L. Moody & R.L. Barrett
10 (Cross *et al.*, 2015b; Barrett, Moody & Barrett, 2016; Fig. 1, Table S1). Although all were
11 structurally simple macrophytes with rapid growth (Cross *et al.*, 2015b), the habitat provided
12 was markedly more complex than pools lacking macrophytes.

13

14 Rainfall in the north Kimberley is highly seasonal, with around 95% falling in the wet season
15 between November and April (ca. 1200 mm annually; McKenzie, Start, Burbidge, Kenneally
16 & Burrows, 2009). However, the timing of onset and intensity of the wet season is
17 unpredictable (Garnett & Williamson, 2010), and the amount of rainfall is consistently
18 exceeded by evapotranspiration rates (> 2000 mm annually; Luke, Burke & O'Brien, 1997).
19 Nevertheless, rivers in the Kimberley have low pH and conductivity: average conductivity
20 $220 \mu\text{S cm}^{-1}$ ($\pm 29 \mu\text{S cm}^{-1}$), pH 7.6 (± 0.1) and turbidity 6 (± 1.5) NTU (Kay *et al.*, 1999).
21 The study site has an average of 80 rainfall days per year, ranging from 36–130 days (based
22 on 35 years of data; Australian Bureau of Meteorology (BOM),
23 <http://www.bom.gov.au/climate/data/>). FRP hydroperiod (length of inundation) ranges from
24 1.9–121.0 days; pools with vegetation have a mean hydroperiod of 25 days (± 1 day) and
25 unvegetated pools a mean of 15.5 days (± 0.3 day) (Cross *et al.*, 2015a,b). However, the
26 mean number of inundations per year does not differ significantly between vegetated and
27 unvegetated pools (5.0, 5.6 d respectively) (Cross *et al.*, 2015a,b). Even small rainfall events
28 (5 mm) were sufficient to fill most pools, and on average pools were inundated between 5
29 and 6 times annually (Cross *et al.*, 2015a,b). Surface water temperature in rock pools during
30 periods of inundation is generally between 40–45 °C (mean 42.3 °C), but can exceed 50 °C in
31 shallow rock pools on days where ambient temperature exceeds 40 °C (Cross *et al.*, 2015a).

32

33 As FRP fill with unpolluted rainwater, water quality varies little across the study site or
34 between years (Table S2); conductivities remain low even during drying, and shallowness

1 means that oxygen concentrations remain high (Cross, 2014; Cross *et al.*, 2014). Similarly,
2 McLachlan & Ladle (2001) noted that water quality remained good in tropical African rain-
3 pools, and that aside from drying, temperature fluctuations were the main challenge for pool
4 fauna. Elsewhere in Australia, conductivities have also been found to be very low ($< 100 \mu\text{S}$
5 cm^{-1}) even in drying FRP (e.g. Timms, 2017). Wet season rainfall at the nearest gauge (BOM
6 station 1010, located approximately 1 km from the study area, <http://www.bom.gov.au>) was
7 higher in 2015 (1050 mm) than in 2016 (982 mm).

8

9 Streams flowing between pavements were separated from FRP on each respective pavement
10 by distances of approximately 10–100 m. These streams are intermittently-flowing sandstone
11 channels mostly < 1 m wide. Streamflow arises from rainfall runoff from the sandstone
12 pavements, and channels are endorheic, delivering water into low-lying areas. Consequently,
13 streamflows are highly flashy, dependent on wet-season rainfall, and stream-pools are also
14 highly temporary.

15

16 *Study design*

17 FRP were grouped on discrete sandstone pavements, separated by streams and/or areas of
18 lowland and/or vegetation and separated by distances ranging from tens to hundreds of
19 metres (to nearest adjacent pavement, Fig. 2). Separation of pavements by landscapes hostile
20 to freshwater invertebrates might affect their dispersal and therefore assemblage structure and
21 composition. Thus, FRP were sampled from multiple pavements to represent the natural
22 arrangement of pools across the study area. Within pavements, sampled pools were chosen
23 haphazardly from among a larger number studied previously (Cross 2014; Cross *et al.*,
24 2015a), to provide a representative sample of the range of physical, hydrological and
25 biological characteristics (e.g. depth, hydroperiod, presence of submerged macrophytes)
26 found across the study area.

27

28 Invertebrates were sampled in the late wet season (March) in 2015 and 2016. Forty-three FRP
29 on seven discrete sandstone pavements were sampled in 2015, with additional collections
30 made from four streams adjacent to pavements. Due to lower rainfall during the 2016 wet
31 season a number of FRP were dry at the time of collection, resulting in the sampling of 35
32 FRP from six areas of sandstone pavement and two streams; including some pools not
33 sampled in 2015. Also, three additional FRP and one stream on basalt were sampled in 2016
34 following the discovery of an area of basalt pavement in the study area. Geographic

1 coordinates (latitude, longitude) and the presence or absence of macrophytes (by visual
2 observation) were recorded for all studied FRP. Macrophyte communities display little or no
3 species turnover between seasons, with the same species consistently occurring in the same
4 pools each year (Cross *et al* 2015a). Maximum pool depth and mean sediment depth were
5 measured using digital callipers and a metal rod during sampling in 2015 and 2016 (see Cross
6 *et al.* 2015a).

7
8 The late wet season is the period of highest productivity in freshwaters in the wet-dry tropics
9 (Warfe *et al.*, 2011). However, it is important to note that rock pools in the study site often
10 experience one or more cycles of inundation and drying within one wet season (Cross *et al.*
11 2015a), as also occurs in tropical rain-fed pools in Africa (McLachlan & Ladle, 2001). The
12 periodicity and duration of individual inundation events is strongly influenced by the size and
13 catchment area of each rock pool (Cross *et al.*, 2015a), and seasonal rainfall in the North
14 Kimberley is not only unpredictable in its timing and intensity but can also be extremely
15 localised (McKenzie *et al.*, 2009). Sampled FRP were therefore at a range of temporal stages
16 when sampled, from recently inundated through to late hydroperiod. A hydrological model
17 was used to estimate the number and duration of inundation periods for all studied FRP,
18 based on 42 years of rainfall records (1966-2007, Australian Bureau of Meteorology),
19 average annual evaporation data for the region, and measurements of total catchment area,
20 maximum depth, basal area and maximum surface area of pools (detailed description of
21 model equations and parameters in Cross *et al.*, 2015a).

22
23 The study site is located in one of the most remote and rugged regions of northern Australia
24 (McKenzie *et al.*, 2009), is approximately 500 km from the nearest major town, and served
25 by a single unsealed road. During the wet season, access by road is frequently restricted or
26 impossible due to widespread waterlogging and flood damage (e.g. Brann, 2011), and the
27 study area can be accessed only by light aircraft. Access to sandstone pavement areas is on
28 foot only. This created logistic difficulties for sampling: strict remote aircraft weight limits
29 meant that only small samples could be transported to the laboratory (\approx 2000 km to the south)
30 for identification, necessitating presence/absence sampling. Additionally, the cost and
31 difficulty of access restricted fieldwork to only one visit per wet season (in mid-season).
32 These limitations mean that we have not been able to estimate intraseasonal variation in
33 assemblage composition, and may not have captured the very early or late stages of
34 inundation in FRP with long-hydroperiods. However, the advantage of sampling these remote

1 sites is that they are pristine, remaining undisturbed even from rangeland cattle which
2 typically avoid the sandstone plateaux (McKenzie *et al.*, 2009).

3 4 *Invertebrate sampling*

5 Invertebrate sampling followed the methods of Vanschoenwinkel *et al.* (2010), and aimed to
6 provide a representative sample of invertebrate assemblages in individual FRP (see also
7 Spencer, Blaustien, Schwartz & Cohen, 1999; Anusa *et al.*, 2012). All invertebrate samples
8 were taken with a funnel-shaped dip net (500 μm mesh) with a maximum diameter of 85 mm,
9 with all microhabitats traversed to obtain a representative composite sample for each FRP
10 and stream pool. The net was scraped along the pool bottom as well as amongst macrophytes
11 (if present). Samples were then live-picked and up to five of each morpho-taxon collected
12 and preserved in 70 % ethanol. All remaining individuals were returned alive to their
13 respective FRP to prevent potentially damaging oversampling from these small and sensitive
14 habitats. All specimens were identified to the lowest possible taxonomic level: to species in
15 most cases, although in some cases identification was only possible to genus (Chironomidae)
16 or family (larval Tabanidae, Scirtidae, for which taxonomic keys do not exist, and Culicidae).
17 Many species have not yet been named, so those species were allocated museum codes or, for
18 species absent from any keys, we allocated our own species numbers (e.g. Ostracoda).

19
20 In 2016, sediment samples were collected and examined for the presence of crustaceans and
21 their artefacts (shells and ephippia). Such features can persist and survive in sediments from
22 years to centuries (Strachan *et al.*, 2015), and retrieval of these artefacts would confirm the
23 occurrence of common FRP Crustacea (e.g. Ostracoda, Cladocera and Copepoda) in the
24 absence of live specimens in netted samples. Approximately 50 g of sediment, comprising
25 five randomly collected samples using a metal spoon (pooled), were taken from each FRP
26 sampled for invertebrates in 2016. Sediments were dried in the field for transport. Sediment
27 depth in studied FRP is shallow (mean 23.6 mm in vegetated pools, and 8.1 mm in non-
28 vegetated; see Cross *et al.* 2015a), thus 50 g provided a representative sample for each pool.
29 In the laboratory, each sediment sample was examined using a dissecting microscope
30 mounted on a moveable arm at 10x magnification. All specimens were removed and
31 identified using 40x magnification.

32 33 *Data analysis for FRP fauna*

1 All multivariate analyses were calculated using PRIMER-E version 6 (Clarke & Gorley,
2 2006). Sample-based species accumulation plots were drawn for each sampling time based on
3 999 permutations of samples (i.e. rockpools), using the same set of 31 pools sampled in both
4 years. The number and identity of pools were held constant in this analysis so that estimated
5 taxa richness (i.e. taxa density) could be compared between years (Gotelli & Colwell, 2011).
6 The non-parametric Chao2 index for presence/absence data was used. It uses information on
7 the rare species in assemblages to adjust for the number of species present, but not detected
8 (Gotelli & Colwell, 2011). When the accumulation curve reaches an asymptote (i.e. is flat),
9 additional sampling will not record new species (Gotelli & Colwell, 2011). If the number of
10 samples at which the asymptote is reached is equal to or less than the number of samples
11 taken, then the sampling adequately describes the richness of the assemblage.

12

13 To identify patterns in invertebrate assemblage composition, Bray-Curtis similarities were
14 calculated for the taxon by pool matrix using data from both years (following Anderson &
15 Walsh, 2013). This dataset comprised pool data only, and included only pavements sampled
16 in both years. A two factor PERMANOVA (sample time, 2 levels; pavement 6-7 levels) was
17 used to test for differences in assemblage composition between sample times and pavements
18 and to estimate the proportion of variation in the assemblages due to these factors. Similarity
19 percentages (SIMPER) were used to identify distinguishing species between times and
20 pavements. Although assemblages differed between the two times, potentially assemblages
21 from a single pool might be correlated between years, especially if many species hatched
22 from the sediment egg bank. Mantel tests were used to determine whether assemblages in
23 each pool were correlated between sampling times, by comparing the Bray-Curtis matrices
24 for pools at each sampling time. To investigate the role of dispersal in faunal distribution,
25 pool fauna were classified as either active or passive dispersers using the list in Brendonck *et*
26 *al.* (2016). Then, a Bray-Curtis matrix was calculated among taxa (rather than among
27 samples), based on their distribution among pools and an nMDS ordination of taxa was used
28 to visualise whether taxa were grouped spatially across pools based on dispersal ability.

29

30 Associations between FRP assemblages and physical variables were tested using multivariate
31 analyses. Prior research has showed that rock-pool surface area was positively correlated with
32 maximum pool depth, sediment depth and pool volume (Cross *et al.*, 2015a). Similarly,
33 catchment area was positively correlated with sediment depth, rock-pool surface area and
34 volume (Cross *et al.*, 2015a). Consequently, catchment area and rock-pool surface area were

1 omitted from our data analyses, because they were adequately represented by other variables
2 (sediment depth, maximum depth). To test the hypothesis that physical variables (maximum
3 pool depth, hydroperiod length, number of inundations per season, mean sediment depth)
4 would be associated with patterns in invertebrate assemblages, a sample matrix for these
5 physical variables was created using Euclidean distance. Variables were normalised prior to
6 calculating Euclidean distance. Mantel tests (RELATE) were then used to compare this
7 Euclidean distance matrix to the Bray-Curtis matrix of the faunal assemblage of pools for
8 each sampling time separately.

9

10 To test the hypothesis that dissimilarities among invertebrate assemblages increased with
11 geographic distance, pool locations (latitude – longitude) were converted into degree
12 decimals which were then used to create a Euclidean distance matrix. Mantel tests were then
13 used to determine whether geographic distances were associated with assemblage distances
14 (using the Bray-Curtis distance matrices for pool data only, above) for each sampling time
15 separately. The hypothesis that the presence of macrophytes would alter invertebrate
16 assemblage composition was tested using single-factor ANOSIM (2 levels, plants or no
17 plants) for the 2015 data only (too few pools with plants were sampled in 2016).

18

19 *Data analysis comparing fauna between FRP and adjacent streams*

20 Although individually the streams were under-sampled, overall there were seven sets of pools
21 with adjacent stream data. To determine whether streams could potentially have provided
22 sources of colonisation for pools, the overlap in assemblage composition was assessed using
23 single-factor ANOSIM; SIMPER was used to identify species that distinguished pools and
24 streams and NMDS ordination was used to display dissimilarities between pools and adjacent
25 streams.

26

27 **Results**

28 *Taxonomic composition of FRP and streams*

29 A total of 79 invertebrate taxa were identified in this study, 47 from FRP and 48 from
30 streams. FRP had an average of 4.8 species per pool, while stream sites had 9.6 species per
31 pool. Thirty-four taxa were recorded in the 2015 FRP samples. There were 30 taxa in the
32 subset of 31 pools used to calculate the accumulation curve; taxa richness reached an
33 asymptote after 28 pools were sampled giving a predicted 68 species (Fig. 3). In 2016, thirty
34 taxa were recorded in the FRP. There were 27 taxa in the subset of 31 pools used in the

1 accumulation curve, where an asymptote was reached after 12 FRP were sampled giving a
2 predicted 36 species (Fig. 3). These results indicate that the sampling effort in 2016 was
3 sufficient to get close (27) to the predicted diversity, thereby providing a representative
4 description of the FRP fauna. However in 2015, the fauna was considerably more diverse
5 (Fig. 2, 3, 4) and less than half (30) of the predicted 68 species were sampled, suggesting that
6 at that time there were likely to be more species collected had more pools been sampled.

7
8 Insects dominated the invertebrate fauna (Table S3, Fig. 2, 4): dytiscid beetle adults (and
9 larvae of some species) were present in all pools and streams, and adult Hydrophilidae
10 beetles were also present in pools on all pavements and in streams (Fig. 2, 4, Table S3).
11 Hemipteran adults were also widespread: Notonectidae and Micronectidae were the most
12 common. The damselfly families Isostictidae and Coenagrionidae and the dragonfly family
13 Libellulidae were also collected in FRP (Table S3), although frequency of occurrence was
14 low. Crustaceans were rare in FRP, comprising two ostracod species and three species of
15 clam shrimp (Cyzicidae *Ozestheria pellucida* Timms, Limnadiidae *Eulimnadia*
16 *kimberleyensis* Timms and *Limnadopsis multilineata* Timms.), of which the first two were
17 previously undiscovered species (Timms 2018 a,b).

18
19 Only 10 of the 32 sediment samples from FRP contained invertebrates and their artefacts,
20 comprising 6 taxa (Table S4). Low numbers of larval Chironomidae (Chironominae
21 *Conochironomus* sp., Orthocladiinae *Cardiocladius* sp., Tanypodinae *Procladius* sp.) and
22 hydrophilid beetle larvae (*Berosus* sp.) were recovered from sediments (Table S4).
23 Surprisingly, crustaceans (and their artefacts) were almost absent: only the two ostracod taxa
24 also collected in netted samples were recorded, each from a single FRP.

25
26 Predators dominated rock pool assemblages (approximately 60% of species found) mainly
27 dytiscid beetles, notonectids and micronectids, and occasionally larval Coleoptera and
28 Odonata (Tables 1, S3). Gathering collectors comprised mainly adult Hydrophilidae and
29 scattered occurrences of adult *Hydrochus* (Hydrochidae) and *Hydraena* spp. beetles
30 (Hydraenidae). Predators also dominated stream samples (63% of species found), but too few
31 samples were taken to reliably characterise trophic structure.

32
33 *Spatial and temporal invertebrate biodiversity patterns in FRP*

1 PERMANOVA analysis showed that pavements accounted for a somewhat higher proportion
2 of the variation in assemblages (9.8%, $F_{5,62} = 2.4$, $P = 0.001$) than did sampling times (7.5%,
3 $F_{1,62} = 4.0$, $P = 0.006$), with no significant interaction between these factors. However, a large
4 amount of variation among FRP remained unexplained by these factors (residual variation =
5 80%). Among pavements in 2015, Tugboat Creek (TB) differed from other pavements
6 because *Conochironomus* sp. occurred frequently there (it was rare or absent on other
7 pavements) and there were fewer taxa present than at other pavements (Fig. 2). In 2016,
8 differences among pavements were few (Fig. 2): Theda Pillar differed from Tugboat and
9 Loonjool because *Conochironomus* sp., *Berosus reardonii* and *Berosus* larvae were rare at
10 Theda Pillar and *Hydrochus australis* and *Pantala flavescens* were present at Theda Pillar but
11 rare at Tugboat and Loonjool.

12

13 The two sampling times differed only slightly because the dominant taxa changed little
14 between years. The beetles *Allodessus bistrigatus* (Clark), *Copelatus bakewelli* Balfour-
15 Browne and *Berosus gibbae* Watts were the most frequently occurring taxa and were present
16 in almost all pools at both sampling times. In 2015, the beetles *Berosus reardonii* Watts and
17 *Hydroglyphus basalis* (Macleay) also occurred in > 30% of samples, whereas in 2016, adults
18 and nymphs of the waterbug *Anisops douglasi* Lansbury and the larvae of *Conochironomus*
19 sp. occurred in > 20% of samples. The hemipteran *Micronecta adelaidae* Chen and the
20 hydrophilid *Sternolophus (Neosternolophus) australis* Watts were also commonly recorded in
21 both years. The remaining taxa recorded were each found scattered across only a few pools
22 (Table S3). There was a significant but modest correlation in assemblage composition
23 between times ($Rho = 0.264$, $P = 0.001$) for the subset of pools sampled twice, probably
24 caused by the presence of common species in both years.

25

26 *Dispersal traits of FRP invertebrates*

27 Of the 47 taxa found in pools, 5 were passive dispersing crustaceans (Spinicaudata,
28 Ostracoda) and 42 were actively dispersing insects (Coleoptera, Hemiptera, Odonata, Diptera,
29 Table S3). The ordination plot showed no discernible clustering of taxa according to dispersal
30 ability (Fig. 5). The only apparent clustering was of the common taxa found across the study
31 area, grouped on the right-hand side of the plot (Fig. 5).

32

33 *Association between FRP assemblages and physical variables, geographic location and* 34 *presence/absence of macrophytes*

1 Physical variables were not associated with patterns in invertebrate assemblages (Rho =
2 0.094, $P = 0.189$). Furthermore, examination of scatterplots (not presented) showed that there
3 was no correlation between taxa richness in pools (regardless of pavement) and any of these
4 variables, nor with pool length or width. Dissimilarities in invertebrate assemblages were not
5 associated with geographic distance. There was no correlation between assemblage
6 composition and geographic distance among rock pools at either sampling time (2015: Rho =
7 0.107, $P = 0.073$; 2016: Rho = 0.008, $P = 0.457$). Surprisingly, invertebrate assemblages did
8 not differ between pools with and without macrophytes in 2015 (Global $R = 0.014$, $P =$
9 0.308). Mean invertebrate taxa richness in pools also did not differ: (mean richness: no
10 macrophytes = 4.2 ± 2.5 (SD), $n = 18$; macrophytes present = 4.8 ± 2.9 , $n = 24$).

11

12 *Comparison of taxonomic composition in FRP and adjacent streams*

13 Although sampling was unbalanced, assemblage composition of FRP and streams differed
14 markedly (Global $R = 0.80$, $P < 0.001$) (Fig. 4, 6). Across all pools and times, FRP were more
15 similar to each other (42% similarity) than streams (18% similarity); when compared, pools
16 and streams had a dissimilarity of 86%. Even when each stream sample was compared to its
17 closest rock pool, assemblage composition was dissimilar (Fig. 6). The difference was caused
18 by the presence of 31 taxa in stream samples that were not collected from nearby rock pools,
19 including species of trichopterans, hemipterans, coleopterans, ephemeropterans, oribatid
20 mites, freshwater crabs and amphipods (Table S3). Sixteen species were found in both
21 streams and FRP. The most common taxon found only in pools was the chironomid
22 *Conochironomus* sp., which was found in seven vegetated FRP. In total, 30 species were
23 found only in FRP, including 19 beetle species (Dytiscidae, Hydrophilidae, Hydraenidae) and
24 all three Spinicaudata species. However, all of these species had low frequency of occurrence
25 in pools, so their absence from stream samples may be due to under-sampling. Consequently,
26 we were not confident that the assemblages recorded in the streams were representative of
27 that fauna, so these data were not analysed further.

28

29 **Discussion**

30 *Fauna of Kimberley FRP*

31 Due to difficult and costly access to these FRPs they were only sampled once during each wet
32 season and sample numbers were constrained by the need to transport them in light aircraft.
33 Consequently, species present only at other times will have been missed. Although most of
34 the species likely present in 2016 were collected, in 2015 additional taxa may have been

1 collected had we sampled more FRP. These FRPs show a wide variety of hydroperiods and
2 many fill for short periods and then dry, repeatedly, in a single wet season (Cross *et al.*,
3 2015a). So, when sampling occurred in the late wet-season, some pools would have filled
4 recently, while others would have been inundated for some weeks (Cross *et al.*, 2015b). This
5 is unavoidable in the tropics where rainfall occurs as intense downpours separated by periods
6 without rain (McLachlan & Ladle, 2001), but it increases the likelihood that pools across a
7 range of successional stages were sampled at a single time. Consequently, although our
8 sampling probably underestimates taxa richness in 2015, common species will have been
9 representatively sampled, and uncollected species are likely to be rare. In 2016, the samples
10 adequately represent both taxa richness and assemblage composition. Species richness was
11 higher in 2015 than 2016, which was a drier year. As this is the first study of the invertebrate
12 fauna of Kimberley FRP, we had no prior information on which to base our sampling design.
13 Instead, we took the maximum practicable number of samples, and more intensive sampling
14 is needed to fully describe this fauna.

15

16 Kimberley headwater streams likely have a different fauna to FRP. Richness may be higher
17 in streams, which may differ markedly from each other, showing the high turnover typical of
18 headwater streams (Finn, Bonada, Murria & Hughes, 2011). Kay *et al.* (1999) recorded 68
19 invertebrate families from Kimberley rivers, of which 18 were also recorded from streams
20 and 19 from FRP in the present study. However, our study recorded two additional stream
21 taxa: an ostracod and the phreatoicid isopod *Eremisopus beei* Wilson & Keable. The large
22 number of families in common between streams (this study and Kay *et al.* 1999) and FRP
23 shows that many species are from families that are not FRP specialists.

24

25 Most species in Kimberley FRP were actively-dispersing insect taxa; taxa richness was low
26 and there were very few crustaceans or dipterans. This composition is unusual compared with
27 most FRP in Australia (Pinder *et al.*, 2000; Jocqué *et al.*, 2010; Bayly *et al.*, 2011; Timms,
28 2012b) and elsewhere (Brendonck *et al.*, 2016). For example, FRP fauna of south-western
29 Australia comprises mostly crustacean and dipteran species, with exceptionally high
30 endemism and diversity (Pinder *et al.*, 2000; Jocqué *et al.*, 2007c; Timms, 2012a,b).
31 However, rain-pools in some parts of tropical Africa (Malawi, Nigeria) contained few
32 invertebrate species (mostly Chironomidae or Ceratopogonidae) and no microcrustacea
33 (McLachlan & Ladle, 2001), with a few predatory Hemiptera and freshwater crabs
34 (McLachlan, 1981). Pinder *et al.*, (2010) included four FRP in their study of Pilbara

1 waterbodies, and found twelve families and five species in common with our study. However,
2 these 5 species are widespread across Australia, rather than FRP specialists. In their
3 synthesis, Jocqué *et al.* (2010) showed that not only was the Australian FRP invertebrate
4 fauna more species rich than other continents, there was a larger proportion of actively-
5 dispersing insects in the fauna ($\geq 50\%$) than on other continents (north America, Europe,
6 Africa which are dominated by passively-dispersing Crustacea). Invertebrate assemblages in
7 northern Australian tropical FRP may be less diverse than those in southern Australia, but
8 they may also be insect-dominated. Tropical FRP have been poorly studied (Brendonck *et al.*,
9 2016), and further studies are needed to determine whether the assemblage observed here is
10 typical.

11
12 Assemblages in Kimberley FRP showed very low evenness. Usually there are a few common
13 species at a locality (usually crustaceans), and most species are found at several sites (e.g.
14 Bayly, 1997; Timms, 2014). In contrast, Kimberley FRP contained a few widespread species
15 (of adult Dytiscidae, Hydrophilidae, Notonectidae, Micronectidae) that occurred in all pools;
16 remaining species were rare and scattered among pools. These common taxa occurred in both
17 years and were responsible for the correlation between sampling times and for the small
18 differences detected between pavements. Coleoptera and Hemiptera are species rich in FRP
19 globally and often dominate the predatory component of trophic structure; but Cladocera,
20 Ostracoda, Anostraca and Diptera are also usually common, and are typically key primary
21 consumers in FRP food webs (e.g. Jocqué *et al.*, 2007c; Bayly *et al.*, 2011; Timms, 2012b;
22 Castillo-Escrivà, Aguilar-Alberola & Mequita-Joanes, 2017). The detritivorous component of
23 the Kimberley FRP was predominantly adult Hydrophilidae beetles, with few other
24 detritivores or algal grazers present. Our collection method likely missed smaller taxa such as
25 rotifers ($< 500 \mu\text{m}$, Williams, 1980) and Copepoda ($< 4000 \mu\text{m}$, Williams, 1980). Cladocera,
26 which are often larger (250 – 10 000 μm , Williams, 1980), were not observed swimming in
27 pools. Furthermore, the absence of ephippia from pool sediment samples (ephippia in
28 sediments may remain viable for more than 200 years (Strachan *et al.*, 2015)) indicated that
29 they were absent. Ostracods (250 – 6000 μm , Williams, 1980) were collected, but were rare,
30 and ostracod carapaces (last for thousands of years in sediments, readily forming fossils (De
31 Deckker, 1981)) were not recorded in sediment samples. It therefore appears likely that the
32 Kimberley FRP sampled here, at least, were dominated by active-dispersing predatory insects
33 and that other groups were absent.

34

1 Total numbers of taxa found in FRP from one or more pavements or outcrops are difficult to
2 compare among studies, because they have sampled different numbers of pools of different
3 sizes, depths, shapes and geological origins (e.g. Bayly *et al.*, 2011; Timms, 2013, 2017).
4 Taxa richness per rock pool is more comparable but varies greatly among studies. Kimberley
5 FRP averaged 4.8 taxa per pool, within the broad range of values from other FRP studies. For
6 example, FRP in Mediterranean Spain averaged 4.1 species per pool on isolated pavements
7 and 4.9 species per pool in ravine rock pools (Castillo-Escrivà *et al.*, 2017). Desert FRP in
8 Utah also showed low richness per pool (5.9, Jocqué *et al.*, 2007b). Southeastern Australian
9 pan-shaped FRP averaged 2.7 – 9.3 taxa per pool (Timms, 2017). Australian desert FRP also
10 had a low average number of species per pool (4.6 from a single sampling event, Bayly *et al.*,
11 2011) but the fauna was dominated by crustaceans (clam shrimp, Cladocera, Ostracoda) with
12 few insects (Diptera, Zygoptera) and evenness was higher than in the Kimberley FRP.

13

14 The Kimberley FRP contained three species of endemic freshwater plants (Cross *et al.*,
15 2015b; Barrett *et al.*, 2016), and endemic plants and invertebrates are common in FRP
16 elsewhere in Western Australia (Timms, 2006; Jocqué *et al.*, 2007c; Tuckett *et al.*, 2010). In
17 the present study, the only (presumably) locally-endemic species found were two new species
18 of clam shrimp *Ozestheria pellucida* and *Eulimnadia kimberleyensis*. The third species, *L.*
19 *multilineata*, occurs in FRP and other temporary pools elsewhere (Timms, 2009), and in more
20 permanent riverine environments in the Kimberley (e.g. Morgan River, this study). Large
21 branchiopods such as clam shrimp are typical FRP animals (Timms, 2006), but their low
22 frequency of occurrence in these Kimberley pools is unusual. Timms (2006) commented that
23 clam shrimp rarely co-occur in FRP, and the three species found here did not co-occur in any
24 rock pool at either sampling time. Potentially, if further pools were sampled, more new
25 species of clam shrimp may be found at the study site.

26

27 *Potential explanatory variables*

28 We explored the physical variables hydroperiod, maximum pool depth, number of
29 inundations per season and sediment depth, but none were associated with invertebrate
30 assemblage composition. Hydroperiod constrains development time for invertebrates and
31 facilitates succession and biotic interactions in FRP (Bayly, 1997; Jocqué *et al.*, 2007a,b;
32 Timms, 2012b). It is usually positively associated with higher diversity (Vanschoenwinkel,
33 Buschke & Brendonck, 2013), but we did not observe this pattern despite Kimberley FRP
34 showing a similar range of hydroperiod and depth to rock pools in south-western Australia

1 (Timms, 2012a,b) and elsewhere (Brendonck *et al.*, 2016). The absence of these relationships
2 may partly result from the infrequent occurrence, or absence, of many of the typical FRP
3 specialist species from Kimberley FRP, in particular, the rarity of crustaceans. While
4 crustaceans must complete their life cycle during the hydroperiod, mobile adult insects can
5 leave drying pools when necessary, reducing their association with pools of differing
6 hydroperiod. Hydroperiods in Kimberley FRP ranged from 17 to 237 days, yet species
7 regarded as being part of the second stage of succession in FRP (adult Dytiscidae,
8 Notonectidae), occurred in every pool regardless of hydroperiod. In contrast, patterns of
9 succession in FRP typically see a shift from specialist crustacean species toward more
10 generalist species in pools with hydroperiods of more than one month (Jocqué *et al.*, 2010;
11 Brendonck *et al.*, 2016). By only sampling once per wet season, we could not describe
12 successional patterns. However, given the comprehensive range of hydroperiods sampled
13 (and by extension, successional phases) these data should have reflected differences among
14 pools with different hydroperiods, had they occurred. Physical variables are unlikely to limit
15 distributions of invertebrates inhabiting the Kimberley FRP. Conductivities were low (< 400
16 $\mu\text{S cm}^{-1}$) and they had a narrow, slightly acidic pH range, which did not differ across the
17 study area (Cross *et al.*, 2014). Several studies describe similar water quality (e.g. Timms,
18 2017) and show that it had little influence on invertebrate assemblages, probably because
19 most FRP are rainwater filled. Indeed, Pinder *et al.*, (2000) and Timms (2017) attributed the
20 very high richness of south-western Australian FRP (compared to elsewhere in southern
21 Australia) to speciation occurring across large numbers of pools spread across large
22 distances, compared to other areas with FRP. McLachlan & Ladle (2001) point out that
23 although FRP may be quite ephemeral, over geological times scales they are very stable
24 habitats and through their spatial isolation, they are ideal for speciation via genetic drift and
25 natural selection. Timms (2012b) found that insects in FRP were less influenced by
26 environmental factors than crustaceans, attributing this to their active dispersal and lack of
27 response to pool hydrology. However, larval Hydrophilidae and Notonectidae were recorded
28 in some Kimberley FRP, showing that some mobile insect taxa were breeding there. These
29 life stages cannot fly, but although included as separate taxa in our analyses, no correlation
30 with pool hydroperiod was observed.
31
32 No differences in assemblage composition were observed between pools with or without
33 macrophytes. Macrophytes were rare in pools with volumes < 50 L and all pools with
34 volumes > 1000 L contained macrophytes (Cross *et al.*, 2015a). Although macrophyte

1 presence and volume were correlated, still there was no relationship with invertebrates. Other
2 studies have found increased taxa richness in FRP containing plants (e.g. Bayly *et al.*, 2011),
3 and this is commonly reported in freshwaters more generally (e.g. McAbendroth *et al.*, 2005;
4 Warfe & Barmuta, 2006). Overall then, we identified no relationships between invertebrate
5 assemblages and any of the measured variables, including variables known to influence FRP
6 (Brendonck *et al.*, 2016). Although some studies have shown that species sorting (i.e. local
7 environmental factors) is the dominant process structuring invertebrate assemblages in FRP
8 (e.g. Vanschoenwinkel, De Vries, Seaman & Brendonck, 2007), this does not appear to be the
9 case in Kimberley FRP.

10
11 It is difficult to determine why the fauna of these FRP was insect-dominated. A wider range
12 of crustacean species were observed in adjacent streams, including isopods and decapods, but
13 these species were absent from rock pools potentially because they could not complete their
14 life cycles there, or because of predation of larger invertebrates by birds. The presence of
15 passively dispersing fauna in FRP worldwide suggests that dispersal barriers cannot explain
16 this pattern. The sandstone geology that weathers to form Kimberley FRPs has also existed
17 for sufficient time to have developed endemic species of plants and clam shrimps, so this also
18 fails to explain the pattern observed.

20 *Dispersal and connectivity for FRP fauna*

21 Many studies have recognized that FRP fauna fall into two groups: active and passive
22 dispersers. FRP taxa in these two groups are consistent globally (see list in Brendonck *et al.*,
23 2016) and these two groups may respond differently to the spatial arrangement of rock pools
24 in landscapes (Castillo-Escrivà *et al.*, 2017). Most active dispersers in FRP are insects found
25 in a range of temporary freshwater habitats and they are dominated by particular families
26 (Chironomidae, Culicidae, Ceratopogonidae, Hydrophilidae, Dytiscidae, Corixidae,
27 Notonectidae, Jocqué *et al.*, 2010; Brendonck *et al.*, 2016), that were also found in Kimberley
28 FRP.

29
30 The spatial arrangement of pools and pavements was not reflected in the distribution pattern
31 of taxa, and although assemblages differed between pavements, the effect was small (< 10%
32 of assemblage variation) and driven by small differences in pool occupancy by common
33 species. Most other studies of multiple clusters of FRP have shown differences between their
34 invertebrate assemblages, but the outcrops or pavements have often been separated by much

1 larger distances (tens or hundreds of kilometres e.g. Timms, 2012a,b), than those studied here
2 (< 10 km apart). However, one Spanish study over a very small area (100's of m) observed
3 spatial structure in the invertebrate assemblage (Castillo-Escrivà *et al.*, 2017). While active-
4 dispersers were structured by species sorting regardless of landscape structure, passive
5 dispersers showed spatial structure (i.e. autocorrelation or isolation-by-distance) where pools
6 were not hydrologically connected (Castillo-Escrivà *et al.*, 2017). The rarity of passive
7 dispersers in the Kimberley fauna may thus explain the absence of differences in assemblage
8 composition among pavements and therefore the absence of any correlation with
9 geographical distance.

10

11 Spatial and especially hydrological connectivity of rock pools may therefore be an important
12 factor shaping FRP assemblages (Vanschoenwinkel *et al.*, 2007; Castillo-Escrivà *et al.*, 2017)
13 and may explain the absence of spatial patterns in the Kimberley FRP fauna. Firstly, passive
14 dispersal by wind (Vanschoenwinkel, Gielen, Seaman & Brendonck, 2008) is probably not
15 limiting the movement of the few passively-dispersing species across the relatively small
16 distances among pools and pavements. Similarly, for the many actively-dispersing, flying
17 adult insects, these small distances are likely to be within their dispersal range (*cf.* Chester,
18 Miller, Valenzuela, Wickson & Robson, 2015; Razeng *et al.*, 2016). Secondly, heavy wet
19 season rainfall causes overland flow across pavements and flow along ephemeral channels
20 connecting rock pools. These events may occur more than once in a wet season, and may
21 permit dispersal of all types of invertebrates (McLachlan & Ladle, 2001; Vanschoenwinkel,
22 Gielen, Vanderwaerde, Seaman & Brendonck, 2008; Razeng *et al.*, 2016), potentially
23 homogenising the fauna of pools within pavements. Hydrological connectivity within
24 pavements may also homogenise water quality among pools and transport sediment and
25 organic matter among them (McLachlan & Ladle, 2001), potentially transferring dormant
26 invertebrate propagules between pools. Such mixing may explain the absence of relationships
27 between invertebrate assemblages and variables such as sediment depth, water depth, pool
28 volume, hydroperiod or the presence of macrophytes. It may also be typical of FRP in the
29 tropics, where intense rainfall is common, so rainfall-driven hydrological connectivity should
30 be included as a variable in future studies of tropical FRP. It does not however, explain the
31 dominance by actively-dispersing insects.

32

1 Vanschoenwinkel *et al.*, (2013) showed that highly isolated pools had low diversity of
2 passive dispersers because of frequent local extinctions. However, highly connected pools
3 also had low diversity of passive dispersers due to high abundances of generalist predators.
4 The actively-dispersing insects that dominated the Kimberley FRP were overwhelmingly
5 predators, which may explain the rarity of smaller prey taxa such as chironomid larvae and
6 ostracods. Adult and juvenile beetles and waterbugs may also have preyed on clam shrimp
7 (Calabrese, McCullough, Knott & Weeks, 2016). Timms (2012a) found that predator
8 populations were stable during the hydroperiod in gnammas, and thought that most species
9 developed independently in FRP, with patterns driven by species' individual phenology
10 rather than by inter-specific interactions. Arrivals of active-dispersing predators have been
11 found to eliminate populations of large branchiopods (Anostraca, Jocque *et al.*, 2010b), and
12 competition from large numbers of actively-dispersing detritivores is possibly a further
13 limitation to the ability of crustaceans to maintain viable populations in Kimberley FRP.
14 Without more information on species diet, and more regular sampling of pools, it is not
15 possible to explain the dominance of Kimberley FRP by insect predators.

16
17 Brendonck, Joqcúe, Tuytens, Timms & Vanschoenwinkel (2014) found hydrologic stability
18 (defined as frequency and length of hydroperiod) strongly determined FRP invertebrate
19 richness, both locally and regionally. Richness declined along an 800 km south to north
20 transect in south-western Australia, corresponding with increased aridity (and thus hydrologic
21 instability; similar findings in Kneitel (2016)); the authors predicted this trend would reverse
22 moving from the arid zone further northward to the tropical Kimberley region (Brendonck *et*
23 *al.*, 2014). Although high species richness was not observed in this study, study of FRP from
24 the arid southern margin toward the wetter northern parts of the Kimberley may reveal an
25 increasing trend of richness. Climate and connectivity are two key differences between
26 Kimberley FRP in the wet-dry tropics and mediterranean to semi-arid south-western
27 Australia, which are rarely hydrologically connected and often many kilometres apart. The
28 extremes of the Kimberley regions' wet-dry monsoonal climate may preclude local
29 structuring of FRP assemblages. Either way, the presence of locally endemic species of
30 freshwater macrophytes and clam shrimp demonstrates the importance of conserving these
31 FRP. Similarly, in their study of southwest WA, Pinder *et al.* (2000) concluded that the
32 primary conservation value of FRPs was as habitat for endemic FRP specialists. Many more
33 studies of tropical FRP are needed globally before trends in biodiversity will be apparent and

1 comparable to studies in more temperate regions. The value of tropical FRP for vertebrate
2 conservation has already been identified (McLachlan, 1988).

3

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12

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1 TABLES (1)

2 **Table 1.** Number of macroinvertebrate species from north Kimberley rock pools and streams
 3 assigned to the functional feeding group (FFG) classifications of Merritt & Cummins (1978)
 4 and the Murray- Darling Freshwater Research Centre (www.mdfrc.org.au). Species data are
 5 combined for sampling in 2015 and 2016, $n = 45$ for rock pools and 6 for streams.

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7

Rock pools			Streams		
FFG	No. species	Macroinvertebrate groups	FFG	No. species	Macroinvertebrate groups
Predator	28	Adult & larval Dytiscidae, adult & larval Coleoptera; adult & larval Hemiptera; Odonata nymphs; Tabanidae larvae	Predator	31	Adult & larval Dytiscidae, adult & larval Coleoptera; adult Hemiptera; Odonata nymphs
Gathering collector	19	Adult Hydrophilidae, adult <i>Hydrochus</i> , adult <i>Hydraena</i> , Spinicaudata, Ostracoda; Chironomidae larvae	Gathering collector	12	Adult Hydrophilidae, adult <i>Hydrochus</i> ; <i>Cloeon</i> (Baetidae) nymphs; Oribatida; larval Scirtidae; Isopoda, Ostracoda, Decapoda
			Shredder	2	Larval Leptoceridae; adult <i>Haliphus</i>
			Scraper	3	Mollusca
			Collector	1	Culicidae larvae
Total	47		Total	49	

1 FIGURE CAPTIONS (5)

2 **Figure 1.** Studied FRP are located on the northwest tip of the Gardner Plateau, in the north
3 Kimberley region of far north-western Australia. The pools are weathered depressions on flat
4 or gently sloping areas of elevated sandstone platforms (A) and sandstone pavement (B). The
5 pools are commonly shallow and pan shaped (B), although larger, deeply weathered pools
6 with vertical sides often occur on isolated platforms (C, location indicated by arrow in A).
7 Many FRP harbour communities of the FRP-specialist macrophyte species *Myriophyllum*
8 *striatocarpum* (B), *M. foveicola* (C) and *Eriocaulon* sp. Morgan River (C). Images by T.M.
9 Buters (A) and A.T. Cross (B,C). Scale bar indicates 1 m in all images.

10

11 **Figure 2.** Similarity in invertebrate species composition and diversity at pavement level in
12 the study area in 2015 (top) and 2016 (bottom); and between streams and rock pools using
13 data pooled for both years (bottom right). Pie charts display the species (grouped by family)
14 cumulatively contributing >90% assemblage similarity between pools within pavements
15 (determined by SIMPER), with each major family contribution labelled with the number of
16 contributing species. ‘Other’ comprises all species accounting for the remaining <10%
17 assemblage similarity. Pie chart size is indicative of total species richness, with larger pie
18 charts representing greater richness. Arrows indicate significant differences in assemblage
19 composition between pavement areas within each year (pairwise ANOSIM $P < 0.05$). Grey
20 shaded areas indicate areas of exposed sandstone. Pavement abbreviations are: LJ = Loonjool
21 Outcrop, BF = Billyfire Flats, SS = Sandstone Flats, TP = Theda Pillar, TB = Tugboat Creek
22 Flats, OC = Ondinea Creek Flats.

23 **Figure 3.** Number of taxa (richness S) and sample-based species accumulation curves
24 (Chao2) calculated for invertebrate presence/absence data from samples collected at each
25 sampling time.

26 **Figure 4.** Total invertebrate taxa richness for eight sandstone pavements and adjacent
27 streams in the north Kimberley, from sampling conducted in 2015 and 2016. Common
28 species were grouped by family, while families with few species and/or occurrences were
29 grouped by order. The category “rare” encompasses families with only one species with a
30 single occurrence. P = FRP, S = stream, *- location not sampled at that time point. Pavement

1 abbreviations are as for Figure 2, with the addition of BP = Basalt Panel, MR = Morgan
2 River.

3 **Figure 5.** NMDS ordination of sandstone FRP invertebrate species by the sites in which they
4 occurred. Each species was assigned active or passive dispersal traits (dispersal mode
5 following the classifications of Brendonck *et al.* 2016). Data were pooled from 2015 and
6 2016 sampling, and excludes stream samples and basalt FRP. 2D stress = 0.11.

7 **Figure 6.** NMDS ordination of total invertebrate species composition of rockpools and their
8 adjacent streams. Arrows indicate the rock pool in closest geographic proximity to each
9 stream sample in each respective year, highlighting the large dissimilarity (86%) in
10 invertebrate assemblages between adjacent rock pools and streams. Years plotted separately
11 for each pair of adjacent pool and stream samples, except for Sandstone sampled 2015 only
12 and Basalt 2016 only). 2D stress = 0.19.

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