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1 Identifying conservation priorities for aquatic refugia in an arid zone,
2 ephemeral catchment: a hydrological approach

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8 **ABSTRACT**

9 The conservation priorities for protecting aquatic habitat of arid zone rivers requires an understanding
10 of the drivers of distribution, characteristics and persistence of aquatic refugia in ephemeral to
11 intermittent arid zone rivers. We describe and analyse the aquatic refugia that persist during drought
12 periods in a large, arid, ephemeral river, the Neales River of central Australia, concentrating on refugia
13 for fish and the hydrological processes that influence their location, characteristics and persistence.
14 Field data were collected on flow events, water-level fluctuations in waterbodies during periods of no
15 flow, waterbody dimensions and salinity. Modelling of open-water evaporation rates, analysis of salinity
16 changes and frequency of flow events were used to investigate the main processes determining the
17 persistence of waterbodies. The catchment contained both fluvial waterholes and artesian springs
18 providing aquatic refugia. In addition, anthropogenic refugia, in the form of small dams situated on the
19 floodplain and uncontrolled flow from artesian bores, also provided potential refuge. The catchment
20 only contained one waterhole that had a cease-to-flow depth sufficient to persist over a no-flow period
21 of 24 months, but this would also dry within 24–30 months. The capacity of the artesian springs to act as
22 refugia is limited by their shallow depths and limited connectivity with the main river system. These data
23 illustrate the vulnerability of the fluvial ecology of ephemeral, arid zone rivers to long-term droughts and
24 water resource use and assist in identifying specific refugia that require conservation management.

25 **KEY WORDS**

26 Neales River; Lake Eyre Basin; salinity; refugia; springs; evaporation modelling

27 **INTRODUCTION**

28 ***Conservation priorities in arid zone rivers***

29 The conservation priorities of arid zone rivers can be masked by a paucity of data on the hydrological
30 and geomorphological framework supporting habitat requirements of aquatic fauna. Refugial
31 waterbodies are critical for sustaining populations of many aquatic fauna during periods of no flow
32 (Arthington et al., 2005; Sheldon et al., 2010), including endemic species (Fensham et al., 2011), and
33 their distribution can have a large influence on the recolonization movement of aquatic fauna during
34 flow events (Magoulick and Kobza, 2003; Sheldon et al., 2010). In arid zone catchments, the existence of
35 long-term aquatic refuges influences the persistence of much of the fish assemblage at a catchment or
36 even regional scale (Fensham et al., 2011). However, knowledge of the drivers of distribution,

37 characteristics and persistence of aquatic refugia at the catchment scale is commonly unavailable
38 (Magoulick and Kobza, 2003; Bunn et al., 2006; Larned et al., 2010).

39 Conservation priorities for wetlands typically focus on high biodiversity (Mittermeier et al., 1998; Junk et
40 al., 2006) to identify regionally important sites and with a particular focus on endemism for arid zone
41 wetlands (Fensham et al., 2011). However, arid zone rivers are commonly typified by only moderate
42 diversity in their aquatic fauna and favour generalist species with high dispersal capacity (Hoagstrom
43 and Berry, 2006; Maltchik and Medeiros, 2006; Balcombe et al., 2007). As a result, an approach
44 concentrating on biodiversity and endemism can ignore conservation requirements of large catchments
45 (i.e. >10 000 km²) with moderate and non-endemic biodiversity but which are vulnerable to
46 anthropogenic pressures on key refugia. The vulnerability of such catchments is heightened if
47 recolonization pathways from neighbouring catchments for aquatic fauna are poorly understood or
48 unlikely under current climatic conditions (Faulks et al., 2010; Fensham et al., 2011). Long drought
49 periods with no streamflow can result in the available aquatic habitats contracting to a small handful
50 (e.g. Bunn et al., 2006), or even a single waterbody. Even though the aquatic fauna have evolved under
51 these variable conditions, during drought periods, these aquatic refugia then become highly vulnerable
52 to anthropogenic disturbances; ranging from broad-scale changes in the natural flow regime through
53 regulation (Bunn and Arthington, 2002), to more local scale effects that influence individual refuges,
54 such as water extraction, local diversion of flow altering the frequency of inundation, pollution and over-
55 fishing (Sheldon et al., 2010). Therefore, the identification and protection of these refugia is an
56 important conservation priority at the catchment scale and potentially at a larger regional scale.

57 ***Types of aquatic refugia and persistence characteristics***

58 The characteristics that define aquatic refugia are taxon-dependent and so display considerable spatial
59 and temporal variability (Lancaster and Belyea, 1997). In the context of the requirements of fish and
60 fully aquatic invertebrates in intermittent to ephemeral rivers, this simplifies to the provision of water in
61 the form of persistent waterbodies (Magoulick and Kobza, 2003; Sheldon et al., 2010). For the fish
62 assemblage, the refuge requirements will differ between species, potentially as a result of predator–
63 prey interactions within isolated waterbodies. As a result, the identification of aquatic refugia needs to
64 consider all waterbodies within a catchment, such as fluvial pools (e.g. Arthington et al., 2005; Bunn et
65 al., 2006; Carini et al., 2006), natural springs (Perez et al., 2005; Kodric-Brown and Brown, 2009) and also
66 any anthropogenic refugia (e.g. small floodplain dams and flowing bore drains, Lutton et al., 2010). In
67 addition, the lateral and longitudinal connectivity of flow patterns (Larned et al., 2010) and temporal
68 water quality variations (Hudson et al., 2003; Costelloe et al., 2005b) can influence the utility of a
69 persistent waterbody as an aquatic refuge.

70 We contend that an evaluation of the conservation value of aquatic refugia in an ephemeral arid zone
71 catchment requires the following steps:

- 72 1. Identify aquatic habitats that persist for periods of > 9–12 months (i.e. between annual wet
73 season streamflow events).
- 74 2. Determine the criteria that explain the persistence of the aquatic refugia, e.g. groundwater
75 connectivity, large maximum depths, more frequent inflow.

76 3. Evaluate the importance of any aquatic refugia in terms of its capacity to link with other refugia
77 during periods of streamflow.

78 4. Identify temporal and/or spatial water quality variations that may impede the capacity of the
79 aquatic habitat to act as refugia for aquatic fauna.

80 We illustrate these steps with field data and analysis of the distribution and persistence of aquatic
81 refugia in a large, ephemeral, arid zone river in central Australia.

82 **STUDY AREA**

83 The Neales River is an unregulated ephemeral river system in the arid Lake Eyre Basin of central
84 Australia. The catchment has an area of 34 000 km² before flowing into the endoreic Lake Eyre North
85 (Figure 1), with a median annual rainfall of 140 mm and a mean annual pan evaporation loss of 3577
86 mm (Australian Bureau of Meteorology). The majority of the rainfall occurs during the summer season
87 (December to March), but significant falls can occur during winter.

88 The Neales River is ungauged, but streamflow events have been monitored by a number of water-level
89 loggers since 2000 (Costelloe et al., 2005a, 2005b; Costelloe et al., 2007). The catchment contains
90 mapped fluvial waterholes and artesian springs sourced from groundwater discharging from the Great
91 Artesian Basin (GAB). The age of GAB springs could be in the order of >100 000 years (Prescott and
92 Habermehl, 2008), and these refugia are known to provide habitat for endemic fish (Wager and Unmack,
93 2000; Fensham et al. 2011) and invertebrate species (Perez et al., 2005; Murphy et al., 2009). No
94 endemic fish are reported for the springs in the Neales catchment but endemic invertebrates are
95 reported (Murphy et al., 2009).

96 Eleven fish species have been recorded in the fluvial waterholes and springs of the Neales catchment
97 (Table I, data from Costelloe et al., 2004; McNeil and Schmarr, 2009). These range from large predator
98 species, such as *Macquaria ambigua* (golden perch), to small saline-tolerant species, such as
99 *Craterocephalus eyresii* (Lake Eyre hardyheads). All species are native to the Lake Eyre Basin except for
100 the exotic species *Gambusia holbrooki* (mosquito fish). The previous studies report generally healthy,
101 abundant and diverse fish populations during wet periods (Costelloe et al., 2004) but much lower
102 diversity following drought periods (McNeil and Schmarr, 2009). The fish species that typically occur in
103 the artesian spring habitats are the small-bodied *Chlamydogobius eremius* (desert gobies) and *G.*
104 *holbrooki*, although medium-sized fish, such as *Leiopotherapon unicolour* (spangled perch) and
105 *Melanotaenia splendidatatei* (desert rainbowfish), can colonize these habitats but only persist for
106 limited periods of time, except in rare deeper pools (Fensham et al., 2011).

107 **METHODS**

108 The evaluation of the conservation value of aquatic refugia requires both field data and analysis of
109 available datasets.

110 ***Location of aquatic refugia and characterisation***

111 The locations of the aquatic refugia within the catchment were identified from 1:250 000 scale
112 topographical maps, and additional investigation used a combination of local knowledge, field
113 inspections and satellite images. The GAB artesian springs located in close proximity of the Neales River
114 (i.e. within 1 km of the floodplain) were visited in the field to evaluate their connectivity with river flow.

115 In addition, a selection of anthropogenic aquatic habitats (e.g. small floodplain dams and free-flowing
116 artesian bores) were also visited and evaluated.

117 The dimensions of fluvial waterholes were measured using simple 'wet survey' techniques and surveying
118 using a total station. The wet survey data were collected over a series of field trips between 2000 and
119 2010 and involved measuring between 1 and 13 (mean 6) transects across the waterhole, depending on
120 its length, with a surveying tape to measure length and a weighted tape to measure depth along the
121 transect. Surveys by total station were used to characterize the out-of-water morphology for the
122 remainder of the waterhole survey (i.e. from water level to top of bank and onto the surrounding
123 floodplain). The surveys identified the maximum depth of the waterhole when it first became isolated
124 from other pools in the channel system when flow ceased, termed the 'cease-to-flow depth' (CTFD), and
125 this measure is significantly less than the more common geomorphological descriptor of channels, the
126 bankfull depth. Waterholes surveyed within 2–4 weeks of a flow event were considered to be at the
127 CTFD. Field observations found that the CTFD typically coincided with riparian vegetation zonation (i.e.
128 the base of the lignum (*Muehlenbeckia florulenta*) zone, Costelloe et al., 2004). For waterholes surveyed
129 some months after flow cessation, this vegetation zonation and downstream river bed sill levels were
130 used to estimate the CTFD. The error for the CTFD was considered as ± 0.2 m, incorporating uncertainty
131 in depth measurements, repeat positioning of transects and measuring vegetation zonation.

132 The occurrence of flow events was evaluated using data from water-level loggers that recorded water-
133 level variations each hour at six locations since 2000 (Dataflow conductance loggers, Odyssey Data
134 Recording Systems) and at a further eight locations (Odyssey temperature-pressure loggers, Odyssey
135 Data Recording Systems) since 2009 (Figure 1). At four of the sites, the loggers were installed in
136 waterholes, and the logger data provided information on waterhole loss rates during no-flow periods.

137 Conductivity and temperature data were collected from all aquatic refugia at 0.1–0.2 m below the
138 surface of the waterbody using water quality instruments (WP-84, TPS Pty Ltd; U-50, Horiba; YSI 6920).

139 ***Characterisation of aquatic refugia persistence, connectivity and water quality***

140 The probability of groundwater discharge contributing to the persistence of fluvial aquatic refugia was
141 investigated using (1) electrical conductivity (EC) data, (2) comparison of observed water-level loss rates
142 (from logger data) to modelled evaporative loss rates and (3) frequency of flow determined from water-
143 level time-series data.

144 An EC threshold of $>3000 \mu\text{S cm}^{-1}$ in fluvial waterholes (Table III) is used to identify waterholes receiving
145 some saline groundwater discharge. This is based on the assumptions that the conductivity of peak
146 magnitude streamflow was $<300 \mu\text{S cm}^{-1}$ (see minimum EC values measured for waterholes in Table III)
147 and of conservative evapo-concentration behaviour (reasonable for Na-Cl dominated surface water
148 systems of Australia) in a waterhole acting like a sealed basin. The evapo-concentration during isolated
149 drawdown would result in an order of magnitude increase in EC ($3000 \mu\text{S cm}^{-1}$) at 10% of its initial
150 starting volume. The temporal salinity behaviour was evaluated to determine if this could be a limiting
151 factor in the value of that waterbody to act as an aquatic refuge for a wide range of biota.

152 Water-level time-series data have also been used to determine persistence using two methods. First,
153 the water-level data were used to determine if particular reaches had higher frequencies of flow events
154 that could enhance the refugial value of a waterhole. Second, the waterhole drawdown rates in the

155 absence of flow were compared with expected open-water evaporation rates. The open-water
156 evaporation rate was calculated using the Penman combination equation and climate data from the
157 closest weather station in the catchment (methods described by Costelloe et al., 2007). For this study,
158 an approximate error bound for the modelled evaporation was estimated by plotting a data envelope of
159 monthly time-series of modelled evaporation and adjusted pan evaporation for the closest three climate
160 stations to the field area; Oodnadatta (Station 017043, 27.56° S, 135.45° E), Moomba (Station 017096,
161 28.11° S, 140.21° E) and Birdsville (Station 038002, 25.90° S, 139.35° E). The modelled time-series used
162 both wind speed data and wind run data from each climate station, as uncertainty in the wind data was
163 found to be the largest contributor to the variation in modelled open-water evaporation. In addition,
164 the data envelope included adjusted pan evaporation data from Moomba and Birdsville (using pan
165 factors derived by linear regression with Penman modelled evaporation). Approximate error bounds on
166 the modelled evaporation data at Oodnadatta were determined by comparing them to the data
167 envelope formed by all modelled open-water evaporation time-series.

168 Surveying using differential global positional systems (Leica VIVA GNSS in rover and base mode) and
169 total stations (Sokkia 30R Series) was used to determine water-level thresholds that resulted in
170 connectivity between fluvial waterholes and other refugia (e.g. springs, flowing bores drains and
171 floodplain dams).

172 **RESULTS**

173 *Distribution and characteristics of aquatic refugia*

174 A total of 20 significant fluvial waterholes were identified and surveyed, and their distribution is shown
175 in Figure 1, along with the location of all known GAB springs, the inspected small floodplain dams and
176 flowing artesian bores. The hydrological characteristics of these refugia are shown in Table II and salinity
177 characteristics in Table III. The CTFD of the fluvial waterholes ranged from 1.2 to 4.7 m. The deepest
178 waterhole, Algebuckina Waterhole, also had the largest width and length and was typically deeper by
179 0.7–0.9 m than the next deepest waterbodies (Figure 2). However, measurements of the CTFD of
180 Algebuckina during 2000–2010 showed that aggradation had occurred, and this measure decreased
181 from 4.5 to 3.5 m between 2003 and 2009 and then recovered to 4.7 m following a flood in February
182 2011 with an approximate recurrence period of one in 10 years. Multiple measurements of CTFD for
183 other waterholes were limited but, where available, did not show any evidence of significant
184 aggradation within the error limits of the surveying techniques. The distribution of the deeper fluvial
185 waterholes is restricted to the middle to lower reaches of the catchment.

186 Small dams (or water storages) with areas of 2500–5000 m² are occasionally situated on the outer
187 floodplain but do not impede or capture flows along primary channels. Two of these were surveyed
188 (Slate Hole, Eaglehawk Dam, Figure 1) and have depths comparable to many of the natural waterholes
189 (Table II). Their position on outer floodplain channels may result in lower frequencies of inflow and
190 shorter periods of connectivity compared with the natural waterholes, but no field data are available to
191 test this.

192 The artesian springs occur on both the floodplain and in association with smaller tributaries (Figure 1).
193 The springs range from minor seeps (<10 m² in area) to free-flowing springs that create shallow pools
194 (typically <0.2 m in Figure 2. Cease-to-flow depths for waterholes in the Neales River catchment. The
195 range in values measured for Algebuckina is shown by the error bars. Codes for waterholes are shown in

196 Table I. Horizontal bars show modelled open-water evaporation losses with error range for 1 year (black
197 solid and dashed lines) and 2 years (grey solid and dashed lines) without inflow.

198 depth) and outflow tails that create extensive wetlands. The immediate pool area is usually <1600 m²,
199 but the flowing tails can create wetlands up to 1000 m long, and both the pool and tail are typically
200 defined by the occurrence of reeds (Phragmites and Typha) and patches of shallow open water. The EC
201 of the spring pools vary with flow rate but are most commonly in the range of 3000–9000 μS cm⁻¹. The
202 largest spring-fed, open-water body occurs at North Freeling pool (<0.3-m maximum depth, 10 000 m² in
203 area) on the outer edge of the floodplain.

204 Analogous to GAB springs, drains from free-flowing artesian bores can maintain persistent wetlands that
205 can potentially connect with the river system. Most GAB bores are capped, but historically, they were
206 uncapped and free-flowing. Two wetlands currently fed by uncapped bores were identified in the
207 catchment – One Mile and Big Blythe (capped in 2012) bores (Figure 1). Both form large wetland areas
208 (up to 420 000 m²) with considerable macrophyte vegetation (Phragmites and Typha), but some open
209 pools of limited depth (usually <0.2 m). These wetlands have very similar hydrological characteristics as
210 the springs, i.e. stable but shallow water depths, with wetland areas similar to those of the larger
211 natural springs and occur close to the outer edge of the floodplain (Big Blythe) or on a tributary (One
212 Mile).

213 ***Causes of refugia persistence***

214 According to the maximum conductivity criterion, eight of the 20 waterholes received likely saline
215 groundwater discharge (Table III), and all but one (Stewart) occurred in the lower reaches. The effects of
216 unconfined groundwater discharge on the persistence of the saline pools are uncertain, but they were
217 observed reaching very shallow depths (e.g. Peake Crossing <0.2-m depth) indicating that groundwater
218 discharge does not lead to greatly increased waterbody persistence.

219 The modelled mean annual open-water evaporation rate was 2372 mm with an estimated uncertainty of
220 +14% to -34%. Where available, time-series of water-level data were used to test the assumption of no
221 groundwater connectivity, and this is illustrated with drawdown rate data for the period 2000–2008
222 from two instrumented waterholes in the Neales catchment (Algebuckina and South Stewart). This
223 analysis (Figure 3) shows that neither waterhole received observable groundwater inflow despite the
224 high conductivity observed in Algebuckina Waterhole (Table III). For instance, Algebuckina Waterhole
225 largely loses water within the uncertainty bounds of the modelled evaporation rate but that some
226 recharge to groundwater (i.e. leakage) could be occurring. South Stewart experiences consistent
227 drawdown rates higher than the uncertainty bounds of the modelled evaporation rates (1.4 m per
228 annum greater than the modelled mean annual evaporation rate), indicating that it is a ‘leaky’
229 waterhole and experiences significant loss to the unconfined groundwater. The South Stewart waterhole
230 is within a few hundred metres of Cramps Camp waterhole, the second deepest waterhole in the
231 catchment (Table I), and leakiness within this reach may reduce the refugial value of this cluster of
232 waterholes in the upper reaches of the Neales.

233 No-flow conditions within a reach were observed to extend for periods greater than 1 year. For instance,
234 Algebuckina Waterhole experienced four periods of no flow between 311 and 427 days in length during
235 the monitoring period. The longest measured period of no flow at any of the monitoring sites in the
236 catchment (without data gaps) was 591 days at Arckaringa Creek. In conjunction with the modelled

237 evaporation rate data, these no-flow periods indicate that waterholes with CTFD less than the annual
238 evaporation loss (i.e. 2.4 m) are unlikely to play significant roles as long-term refugia.

239 The logger data (Table IV) indicate that the frequency of flow was similar in the upper and lower reaches
240 of the Neales. Variations in frequency of flow events in the upper and lower reaches of the Peake were
241 more difficult to identify because of data gaps. The lower Peake probably has a similar frequency of flow
242 as the Neales; however, the lower frequency of flows in the upper Peake appears significant but also
243 coincides with a lack of any deep waterholes (i.e. CTFD > 2.4 m). Therefore, the persistence of the refugia
244 in this catchment is not significantly enhanced by catchment variations in the frequency of flow events.

245 ***Flow and connectivity patterns***

246 The waterbodies with the greatest long-term persistence in the catchment are shallow GAB spring pools,
247 and their conservation importance for aquatic fauna (particularly fish) at the catchment scale depends
248 partly on their connectivity with the river. The connection between springs and the river system can be
249 direct at high river stages for springs occurring on the floodplain of the river (i.e. North Freeling (NO),
250 Figure 1) or indirect for springs that experience periodic connection from tributary flow (e.g. Ockenden
251 Spring (OS), Figure 1) that can connect with river flow, as illustrated in Figure 4. These connectivity
252 patterns indicate that at least some springs could act as refugia for small-bodied fish and be capable of
253 repopulating the river following drought periods.

254 ***Salinity variations in refugia***

255 The fluvial refugia of the Neales River showed an extraordinarily large range in conductivities, both
256 spatially and temporally (Table III). Minimum observed conductivities were typically fresh (<300 $\mu\text{S cm}^{-1}$)
257 and were considered to be representative of larger flow events. In the upper reaches, the waterholes
258 remain relatively fresh even at low water levels; however, in the middle to lower reaches, the
259 conductivity of the waterholes ranges from fresh to hypersaline (>50 000 $\mu\text{S cm}^{-1}$). During periods of no
260 flow, shallow residual pools in this reach were observed with conductivities > 200 000 $\mu\text{S cm}^{-1}$. These
261 lower reaches contain a number of important waterholes, including the deepest waterhole in the
262 catchment, Algebuckina Waterhole. The latter is generally fresh, but after larger flows, its conductivity
263 can be > 20 000 $\mu\text{S cm}^{-1}$ (Table III).

264 The conductivity of the saline to hypersaline waterholes can vary temporally over several orders of
265 magnitude, and this is illustrated for the Peake Crossing waterhole (Figure 5). Flow events are quite
266 fresh, and the waterhole typically has moderate conductivity (4000–9000 $\mu\text{S cm}^{-1}$) soon after most flow
267 events. However, EC > 150 000 $\mu\text{S cm}^{-1}$ has been observed forming within 195–285 days from the peak
268 of the most recent flow event.

269 ***Persistence patterns of fluvial refugia***

270 The modelled evaporation rate and CTFD were used to estimate the persistence time of the surface-fed
271 refugial waterholes (excluding the saline waterholes of Peake, Tardetakarinna, Baltacoodna) and
272 identified the sequence of waterholes drying over the catchment (Figure 6). Allowing for the higher loss
273 rates from the 'leaky' waterholes, the evaporation modelling clearly demonstrates that Algebuckina has
274 the longest persistence of the fluvial waterholes of approximately 23–24 months without surface inflow.
275 The uncertainty in this modelling is in the order of 1–2 months. Water loss rates in the saline waterholes

276 are likely to be less because of some contribution from groundwater discharge, but the refugial value of
277 these waterholes is limited by hypersaline conditions forming within 10 months of a flow event.

278 **DISCUSSION AND CONCLUSIONS**

279 ***Conservation priorities at the catchment scale***

280 The Neales catchment contains a wide array of aquatic refugia that include waterholes, small floodplain
281 dams, artesian springs and wetlands fed by free-flowing artesian bores. This mix of natural and
282 anthropogenic, surface-fed and groundwater-fed aquatic refugia are not commonly considered when
283 determining critical refugia of an arid zone catchment, where the previous attention has often been on
284 fluvial refugia (e.g. Arthington et al. 2005; Hamilton et al. 2005; Bunn et al. 2006; Sheldon et al. 2010).
285 The mixture of fluvial and groundwater-fed refugia is not that unusual for an arid zone catchment (Box
286 et al. 2008; Keleher and Rader 2008; Fensham et al. 2011) and has important conservation implications,
287 as the different waterbody types provide refuge to different components of the fish assemblage. The
288 most persistent aquatic refugia in the catchment are the artesian springs (Prescott and Habermehl,
289 2008), but their suitability for the complete range of the fish assemblage is likely to be limited by their
290 limited depth and habitat type (Fensham et al. 2011), being most suitable for aquatic fauna adapted to
291 very shallow water depths (i.e. <0.2 m) and dense macrophytes. Notwithstanding these limitations, the
292 artesian springs showing connectivity with the river system and having larger open pools (e.g. North
293 Freeling) clearly have high conservation value for components of the fish assemblage.

294 In contrast to the springs, the fluvial waterholes have a maximum persistence of approximately 24
295 months without inflow and likely have a much lesser long-term persistence but provide a greater range
296 of habitats for a wider variety of fish (Arthington et al. 2005). For waterholes that normally experience at
297 least one flow event per year, a useful definition of a fluvial refuge in an ephemeral river is one that can
298 persist in the event of there being no flow for an entire 'wet' season. Such drought (no-flow) periods
299 would be in the order of 18–24 months, depending on the timing of flow cessation in the previous wet
300 season and the onset of flow in the proceeding wet season. Given the expected evaporation rates in the
301 Neales catchment, refuge waterholes would require a maximum CTFD of >4m to sustain aquatic biota
302 during such drought periods. As shown in Figure 2, in the Neales catchment, only Algebuckina
303 Waterhole has sufficient depth (albeit subject to aggradation) to fulfil this criterion for an aquatic
304 refuge. It clearly has the largest CTFD (despite some temporal variation) and does not show any
305 evidence of receiving groundwater discharge (Figure 3, see also Costelloe et al. 2005b). The
306 conservation importance of other waterholes was lessened by leakiness (e.g. South Stewart) or high
307 salinity (e.g. Baltucoodna) limiting their effective persistence. In addition to its persistence, the size
308 (Table I) and connection potential of Algebuckina Waterhole enhance its conservation importance.
309 Unlike the small dams located on the edges of the floodplain that would only be connected to the river
310 during larger flow events, Algebuckina is located on the primary channel of the Neales River. The
311 significant variation in the CTFD of Algebuckina Waterhole emphasizes the importance of larger floods
312 (i.e. with approximate recurrence intervals of one in 10 years or greater) in re-setting the geomorphic
313 conditions that provide the large waterholes with the capacity to act as refugia. This also provides a note
314 of caution on how even larger floods are likely to change the refugial characteristics of the catchment
315 over the long term.

316 The catchment positions of any of the refugia do not seem to have any significant bearing on their
317 conservation importance. There was no consistent longitudinal pattern in refugia size that may relate to

318 stream power, and hence, the drying pattern of the waterholes did not show any consistent spatial
319 distribution pattern. Rather, the patterns of waterhole persistence and water quality were shaped by
320 the local geology. It might be expected that catchments with more consistently decreasing gradients
321 between highland upper reaches and lowland lower reaches may be more influenced by longitudinal
322 gradients in the distribution of aquatic refugia. However, the pattern in the Neales River emphasizes the
323 need to collect data and carefully consider the refugia capacity at the catchment scale.

324 The effects of salinity variations on refugia are not commonly considered. For instance, Magoulick and
325 Kobza (2003) identified temperature, pH, dissolved oxygen and nutrients as the dominant water quality
326 influences on refugia. However, variability of the salinity of waterbodies during the no-flow phases in
327 arid zone rivers has been recognised as a driver of biotic assemblages, particularly for invertebrates and
328 algae (Costelloe et al. 2005c; Jolly et al. 2008; Sheldon and Fellows 2010). The spatial and temporal
329 variability of saline pools in the Neales catchment represent an extreme example of salinity variations
330 (i.e. ranging from fresh to hypersaline), which exert considerable influence on the capacity of a
331 waterhole to act as an aquatic refuge for fish, as well as for invertebrates and algae (Costelloe et al.
332 2005c; Shiel et al. 2006) and also may present barriers to the migration of aquatic fauna during low
333 flows. Their salinity trajectory is governed by the degree of mixing between fresh surface water and
334 saline unconfined groundwater, and further evapo-concentration of this mixture. The rapidly rising
335 salinity of the saline pools during the low recession flow and during disconnection will have dramatic
336 implications for the aquatic fauna that can utilise these pools (Jolly et al. 2008). For instance, the saline
337 waterholes only provide medium-term (i.e. <12 months) refuges for salt-tolerant fish. For instance, the
338 highest salinity tolerance reported for any of the fish species of the Lake Eyre Basin was 110 000 mg l⁻¹
339 for Lake Eyre hardyheads (*Craterocephalus eyresii*, Glover and Sims 1978), and only a few algal species
340 can survive in these hypersaline pools (Costelloe et al. 2005c). As a result, probably all of the recognised
341 saline refuges would not function as long-term refuges, even for salt-tolerant fish species, and this
342 significantly decreases their conservation value.

343 In terms of the fish fauna of the catchment, the complete drying of fluvial refugia (other than the
344 artesian springs on the floodplain) would see catchment-wide extinction of a number of larger species,
345 and reintroduction would require the migration of fish from larger connecting catchments, through Lake
346 Eyre North. Such an occurrence would probably only occur during significant interannual wet periods, as
347 the migration requires Lake Eyre North to hold sufficient water with only moderate salinity and then
348 flows from the Neales to reach Lake Eyre North while it is inundated (Fensham et al., 2011). Genetic
349 studies of the largest fish in the catchment, *M. ambigua*, have demonstrated close relationships
350 between the Neales and the Diamantina catchment (large catchment to the northwest of the Neales
351 also flowing into Lake Eyre North), implying relatively recent migration between the two catchments
352 (Faulks et al. 2010). So it is probable that complete drying of fluvial refugia and then re-connection with
353 other catchments has occurred in the Holocene.

354 ***Conservation implications***

355 This study emphasizes the value in collecting field data to identify aquatic refugia with the highest
356 conservation value based on hydrological criteria. The distribution of refugia can likely be determined
357 from existing datasets (e.g. topographic maps), but depth and water quality data for individual
358 waterbodies, and the occurrence of flow events, require the collection of field data.

359 In addition to identifying the key aquatic refugia, the field data provide insights into dominant
360 ecohydrological processes, such as the vulnerability of the fluvial refugia to dessication and why the fish
361 assemblage at the catchment scale shows no endemism. In the case of the Neales catchment, only one
362 fluvial waterhole (Algebuckina) would retain water after a period of 24 months without streamflow, and
363 other waterholes connected to local unconfined groundwater are likely to have reached salinity levels
364 toxic to most aquatic species. Thus, despite a lack of endemism or high biodiversity in the fish
365 assemblage of the Neales catchment, this hydrological assessment of aquatic refugia clearly identifies
366 Algebuckina Waterhole as having the highest conservation importance of the fluvial waterholes and
367 would benefit from management plans that protect this site from local scale, deleterious effects, such as
368 water extraction or over-fishing by recreational fishers. The springs fed by the regional GAB
369 groundwater system form the only long-term aquatic refugia in the catchment but their hydrological
370 characteristics are only suitable for a small range of fish species (Fensham et al., 2011). Even so, the
371 springs with the best connection with the river system would also benefit from management plans that
372 protect these habitats from over-grazing and trampling by cattle. The results clearly demonstrate
373 catchment-scale conservation priorities and the need to identify and protect the hydrological and
374 ecological values of the key fluvial waterholes and springs and illustrate their vulnerability to long-term
375 droughts, climate change and water resource use.

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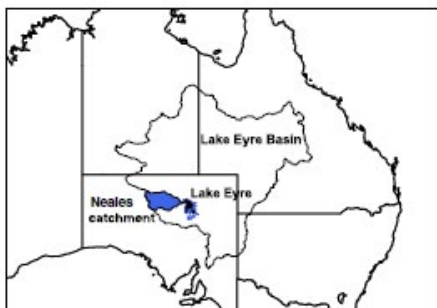
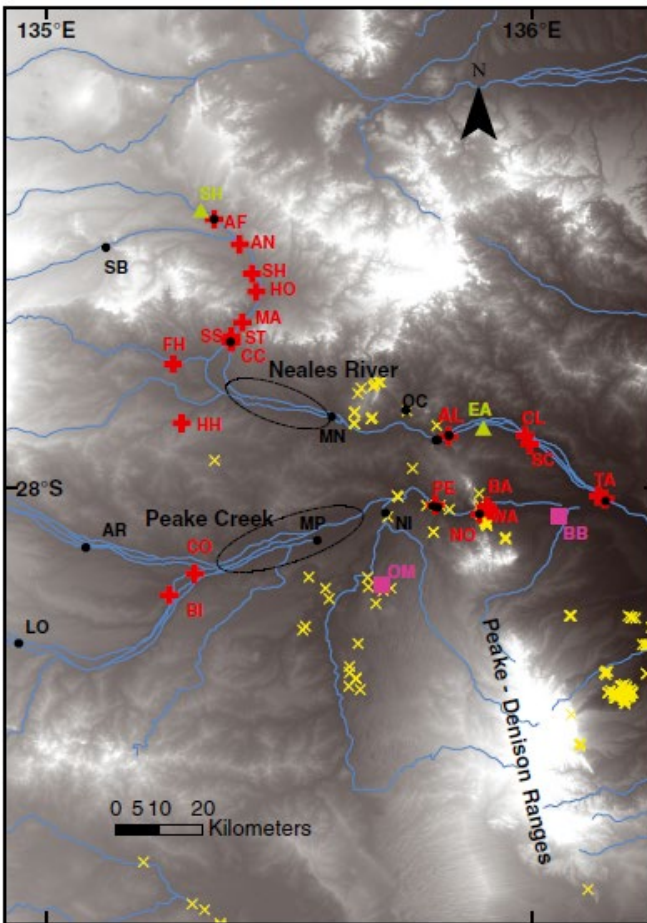
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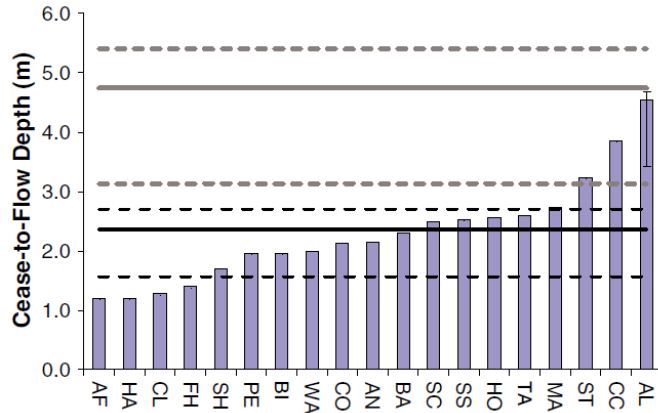
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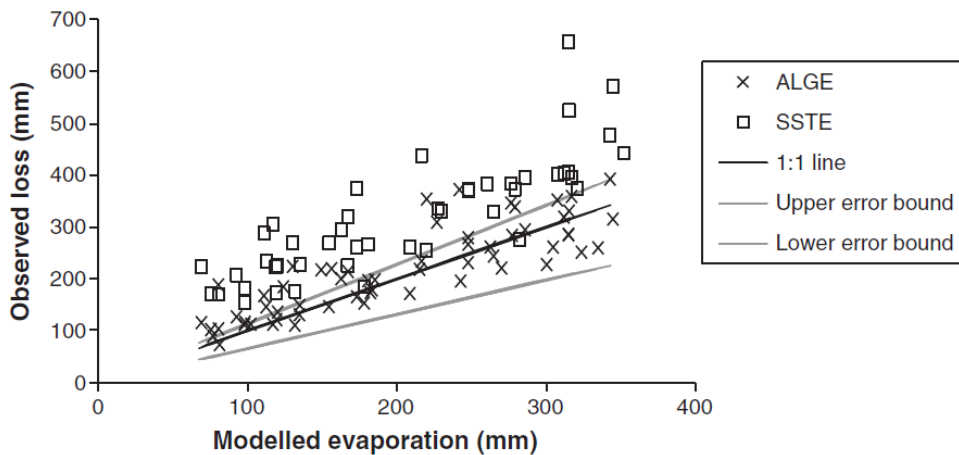
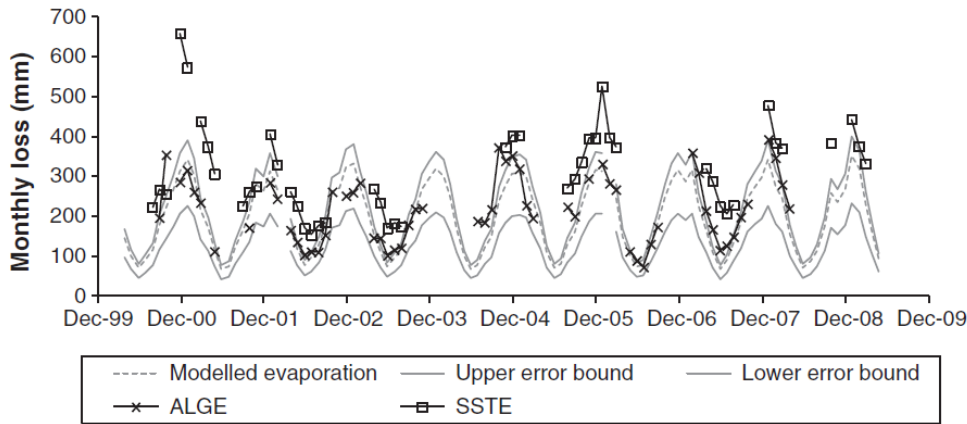
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493 **Figure 1.** Position of surveyed waterholes (crosses) on a grey-scale digital elevation model of the Neales
 494 River catchment. Areas of high elevation are white, and areas of low elevation are dark grey. The dashed
 495 ovals outline reaches with poor channelisation and no significant residual pools. Waterhole names are
 496 shown in Table I. Also shown are the positions of mound springs (x, unpublished data courtesy of Dr
 497 Wendy Welsh, Australian Bureau of Rural Sciences), named free-flowing bore drains (Big Blythe and One
 498 Mile, squares), small dams (triangles) and water-level monitoring sites (circles, non-waterhole sites
 499 named in text are AR – Arckaringa Creek and OC – Ockenden Creek).



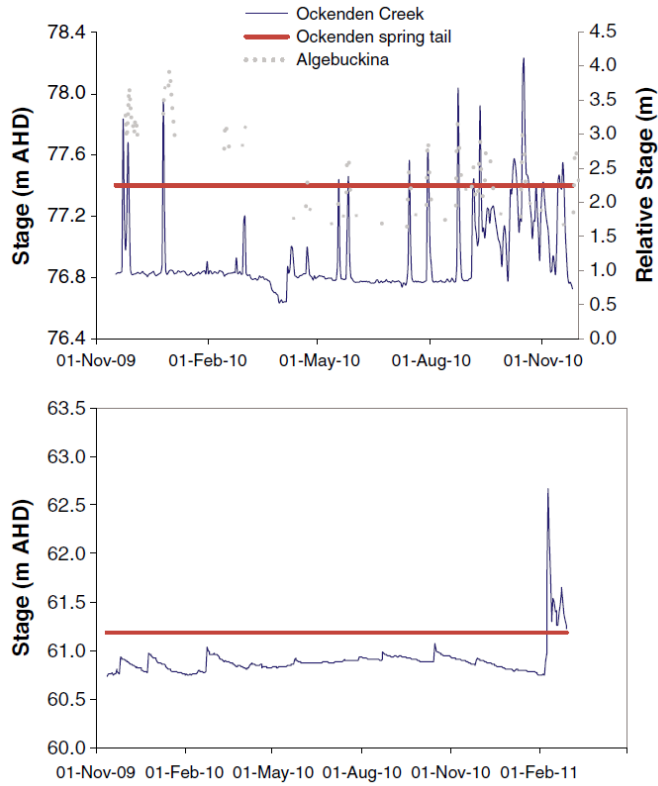
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501 **Figure 2.** Cease-to-flow depths for waterholes in the Neales River catchment. The range in values
 502 measured for Algebuckina is shown by the error bars. Codes for waterholes are shown in Table I.
 503 Horizontal bars show modelled open-water evaporation losses with error range for 1 year (black solid
 504 and dashed lines) and 2 years (grey solid and dashed lines) without inflow.



505

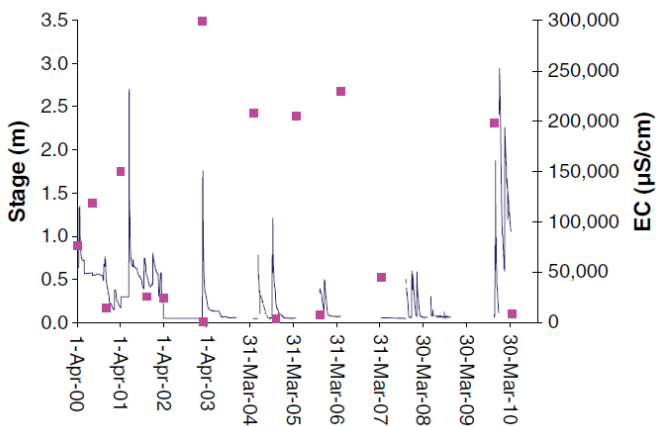
506 **Figure 3.** Observed loss rates from Algebuckina and South Stewart waterholes versus modelled open-
 507 water evaporation rates using the Penman combination equation. Shown are the modelled evaporation
 508 rates and upper and lower error bounds.



509

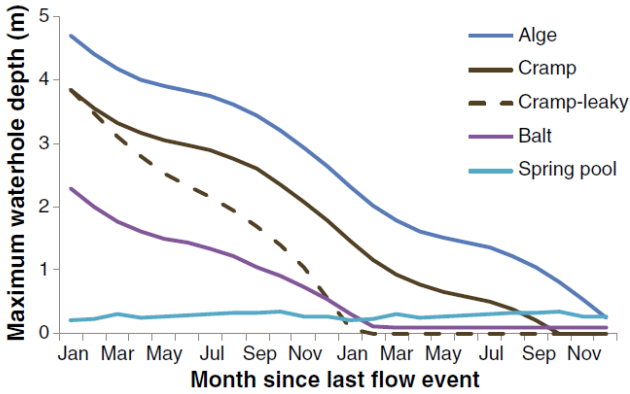
510 **Figure 4.** Top panel – hydrograph at Ockenden Creek (solid line) and Algebuckina Waterhole (dashed
 511 line). Horizontal bar shows level at which flow in Ockenden Creek connects with wetland formed by
 512 Ockenden Spring. Lower panel – hydrograph at North Freeling spring pool with horizontal bar showing
 513 approximate bankfull level. Stage given in Australian Height Datum.

514



515

516 **Figure 5.** Stage hydrograph and water salinity measurements at the Peake Creek crossing pool.



517

518 **Figure 6.** Plots show general relationship for evaporation loss with time. The upper plot shows the
 519 modelled evaporation loss rates. The lower plot shows the mean monthly observed loss rates for the
 520 leaky waterholes (SS, ST, CC), which include evaporation and leakage losses. Each curve shows the loss
 521 rate starting in a different month (i.e. 'Jan' curve shows loss rate if flow ceases in December and
 522 evaporative loss commences in January). The cease-to-flow depths of a selection of the deeper
 523 waterbodies are shown (see Table I for codes). Where these lines intersect, the loss rate curves show
 524 when that particular waterhole will dry out after flow ceases.

525 **Table I.** Fish species sampled in the Neales River catchment (Costelloe et al., 2004).

Family	Species	Size range (mm)	Tropic group
Atherinidae	<i>Craterocephalus eyresii</i>	<70	Microphagic carnivore
Clupeidae	<i>Nematalosa erebi</i>	<360	Detritivore (herbivore)
Gobiidae	<i>Chlamydogobius eremius</i>	<60	Microphagic omnivore
Melanotaeniidae	<i>Melanotaenia splendida tatei</i>	<125	Microphagic omnivore
Percichthyidae	<i>Macquaria ambigua sp.B</i>	<510	Macrophagic carnivore
Plotosidae	<i>Plotosidae Neosilurus hyrtlii</i>	<280	Macrophagic carnivore
	<i>Porochilus argenteus</i>	<260	Macrophagic carnivore
Terapontidae	<i>Amniataba percoides</i>	<160	Microphagic carnivore
	<i>Leiopotherapon unicolor</i>	<280	Macrophagic carnivore
Poeciliidae	<i>Scortum barcoo</i>	<410	Macrophagic omnivore
	<i>Gambusia holbrooki</i> ^a	<55	Microphagic carnivore

526 ^a Denotes exotic species.

527 **Table II.** Waterhole morphology characteristics (nm – not measured).

Waterhole	Type	Maximum cease to flow depth (m)	Bankfull width (m)	Bankfull depth (m)	Length (m)
Afghan (AF)	River channel	1.20	32	2.2	450
Angle Pole (AN)	River channel	2.16	24	2.7	670
Shepherds (SH)	River channel	1.70	22	2.3	730
Hookey (HO)	River channel	2.56	34	3.9	530
Mathieson (MA)	River channel	2.73	59	3.3	630
Stewart (ST)	River channel	3.23	52	3.7	940
South Stewart (SS)	River channel	2.53	23	3.9	470
Cramps Camp (CC)	River channel	3.85	44	4.4	1130
Fish Hole (FH)	Tributary	1.16	47	1.8	380
Hagans Hole (HH)	Tributary	1.20	nm	nm	740
Algebuckina (AL)	River channel	4.70	70	7.9	2700
South Cliff (SC)	River channel	2.50	85	3.3	490
Cliff (CL)	River channel	1.28	24	2.2	700
Tardetakarinna (TA)	River channel	2.20	40	3.6	1600
Warrarawoona (WA)	River channel	2.00	55	4.7	1260
North Freeling (NO)	Spring pool	0.30	32	nm	750
Baltucoodna (BA)	River channel	2.30	37	4.4	960
Peake Crossing (PE)	River channel	1.50	54	4.7	690
Cootanoorina (CO)	River channel	2.10	50	2.5	920
Birribiana (BI)	River channel	1.80	83	2.5	470
Eaglehawk (EA)	Dam	3.13	80	nm	80
Slate Hole (SH)	Dam	3.83	42	nm	70

528 *Locations shown in Figure 1.*

529

530 **Table III.** Waterhole electrical conductivity characteristics from data collected in the period 2000–2011.

Waterhole	<i>n</i>	Mean	Standard deviation	Median	Min	Max
Afghan (AF)	2	207	—	—	171	242
Angle Pole (AN)	2	173	—	—	143	203
Shepherds (SH)	1	227	—	—	—	—
Hookey (HO)	6	317	191	255	189	700
Mathieson (MA)	6	305	169	242	179	631
Stewart (ST)	9	3647	5047	1618	194	15,560
South Stewart (SS)	13	564	382	460	194	1317
Cramps Camp (CC)	4	674	451	548	284	1315
Fish Hole (FH)	6	846	607	574	468	2045
Hagans Hole (HH)	1	760	—	—	—	—
Algebuckina (AL)	15	8661	9377	6420	372	32,748
South Cliff (SC)	9	1112	922	617	494	3096
Cliff (CL)	2	1798	426	3170	—	—
Tardetakarinna (TA)	6	76,669	95,578	41,824	11,891	260,300
Warrarawoona (WA)	3	4852	3568	6477	761	7319
North Freeling (NO)	3	6337	1358	6709	4831	7470
Baltucodna (BA)	3	45,911	32,853	53,836	9821	74,080
Peake Crossing (PE)	15	123,424	141,152	76,666	4905	520,550
Cootanoorina (CO)	5	242	110	167	155	388
Birribiana (BI)	3	329	134	280	226	481
Eaglehawk (EA)	1	811	—	—	—	—
Slate Hole (SH)	2	166	—	—	160	172

531 *All measurements in $\mu\text{S cm}^{-1}$ corrected to 25 °C.*

532

533 **Table IV.** Number of observed flow events and corrected for data availability.

Reach	Data availability (%)		Flow events		Corrected flow events		No flows recorded	
	Feb00 to Oct09	Nov09 to Apr11	Feb00 to Oct09	Nov09 to Apr11	Feb00 to Oct09	Nov09 to Apr11	Feb00 to Oct09	Nov09 to Apr11
Catchment	—	—	24	22	—	—	—	—
Upper Neales	79.8	96.4	14	19	17.5	19.7	5	3
Lower Neales	80.3	95.7	14	20	17.4	20.9	6	1
Upper Peake	73.6	96.4	11	10	14.9	10.4	7	11
Lower Peake	66.2	34.2	15	8	22.7	23.4	2	5

534 *Final two columns show number of events that were not recorded in a reach when flows were occurring in other reaches.*