AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS
*Aquatic Conserv: Mar. Freshw. Ecosyst.* (2014)
Published online in Wiley Online Library
(wileyonlinelibrary.com). DOI: 10.1002/aqc.2478

# *Assessing the status of a disjunct population of the endangered crayfish* Euastacus bispinosus *in a karst rising-spring habitat in southern Australia*

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## ABSTRACT

1. Many species of freshwater crayfish of the endemic Australian genus *Euastacus* are threatened with extinction. Routine monitoring of most *Euastacus* species is minimal, and a subsequent lack of information has hampered prioritization of conservation and management efforts.

2. The present study investigated population parameters and temporal changes in abundance in a disjunct population of *Euastacus bispinosus*. This occurs at the western extent of the range of the species in the state of South Australia (SA) and inhabits a groundwater-dependent karst habitat distinct from the larger population in the Victorian Glenelg basin.

3. *Euastacus bispinosus* was recorded at sites within seven karst rising-springs as well as two isolated locations (sinkhole and cave), which expanded the extent of occurrence of the species in SA, but the area of occupancy remains limited. Most subpopulations contained low numbers, had little or no signs of recruitment, were dominated by large crayfish, and demonstrated a high incidence of gonopore aberrations. Declines in abundance were observed across these subpopulations between 2006 and 2011, although these declines were not statistically significant.

4. Of critical importance to the conservation of the species in SA is ensuring that groundwater discharge is maintained in karst rising-springs, hydrological connectivity is enhanced between subpopulations and degradation within habitats is reversed. Further monitoring and research is necessary to gain a clear understanding of the status of subpopulations of the species over time.


Received 13 January 2014; Revised 26 March 2014; Accepted 11 May 2014

KEY WORDS: groundwater-dependent ecosystems; freshwater crayfish; endangered species; conservation evaluation; population fragmentation

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## INTRODUCTION

Freshwater crayfish are a diverse group, with currently more than 640 species described worldwide, distributed across all continents except Antarctica and occupying wide-ranging habitats (Crandall and Buhay, 2008). In these habitats they are valuable indicators of environmental change and form important links in the transformation of energy through aquatic foodwebs (Reynolds and Souty-Grosset, 2011). However, at least one-third of all freshwater crayfish species are threatened with extinction with the two major common threats being over-exploitation by humans and degradation of habitat (Collen *et al*., 2014). Freshwater crayfish inhabiting groundwater-dependent karst systems appear particularly at risk because of localized impacts but also broader groundwater extraction (Walsh, 2000; Boulton *et al*., 2003; Taylor *et al*., 2007). For instance, almost all North American crayfish species associated with karst caves are considered threatened (Culver *et al*., 2000). In south-east South Australia, karst systems discharge groundwater under pressure to form surface water bodies resembling small lakes and streams (Allison and Harvey, 1983). The term 'karst rising-spring' (hereafter KRS) has recently been adopted by Keith *et al*. (2013) to describe this habitat.

The endemic Australian genus *Euastacus* provides a prime example of the threats posed to freshwater crayfish, with 40 of the 50 species considered threatened according to IUCN Red List criteria (Furse and Coughran, 2011b; Coughran and Furse, 2012). This is true of *Euastacus bispinosus* Clark, 1941, which is a slow-growing and long-lived (~26 years: Honan and Mitchell, 1995a) species endemic to the Glenelg River Basin in south-west Victoria and KRS in coastal drainages of south-east South Australia (Figure 1) (Zeidler, 1982; Morgan, 1986). Across its range, *E. bispinosus* has undergone reductions in distribution and abundance owing to over-fishing, hydrological alteration and habitat degradation, which has triggered the closure of the recreational fishery (TSSC, 2011). A recent conservation assessment has escalated the conservation status to endangered under Federal environmental legislation (the EPBC Act 1999: TSSC, 2011) and vulnerable globally (under the IUCN Red List: Coughran and Furse, 2010). The distribution of the species is now considered to be heavily fragmented and it persists as discrete populations

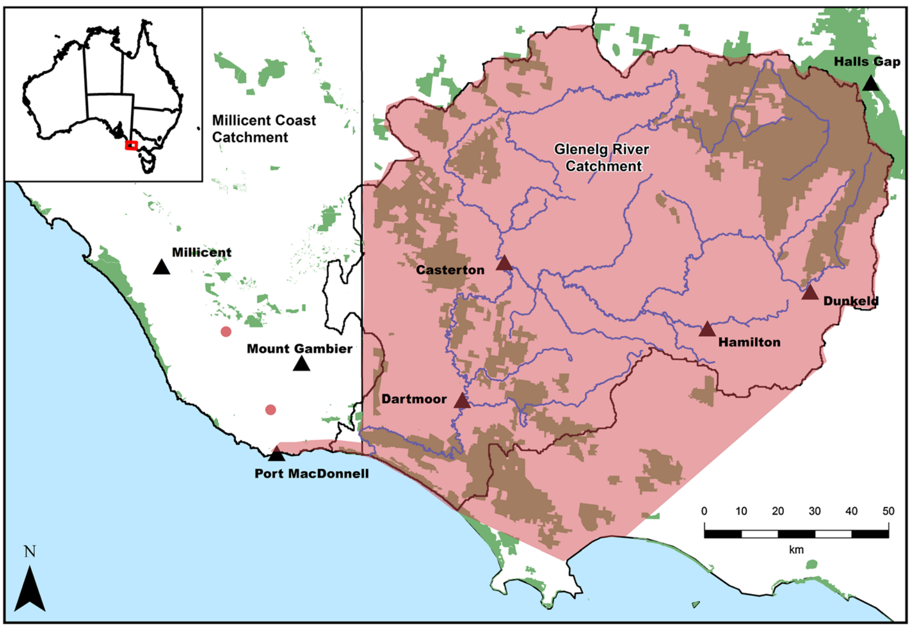


Figure 1. Approximate distribution of *Euastacus bispinosus* in south-west Victoria and south-east South Australia (red polygon). Surveys focused on the narrow section of coastal drainages between the SA border and Port MacDonnell as well as two isolated locations (red dots). National parks and state forests are shown by green polygons, and the larger waterways of the Glenelg Basin in Victoria are shown in dark blue.

 

across much of its range (Honan, 2004; Hammer and Roberts, 2008). The most westerly population of the species (hereafter referred to as the South Australian (SA) population), is of particular conservation concern as it has an extremely small extent of occurrence (24 km$^2$) that is disjunct from nearby populations in the Glenelg River Basin (minimum 9 km away) owing to changes in hydrology in the region (Boutakoff, 1963; Hammer and Roberts, 2008; Miller *et al.*, 2013, in press).

The SA population is largely restricted to KRS habitat that occurs along a narrow (3 km) section of coastal drainages (Zeidler, 1982; Hammer and Roberts, 2008). These KRS were once hydrologically linked through the seasonal inundation of the surrounding peat swamp (Eardley, 1943; Stephens, 1943). Extensive drainage and native vegetation clearance from the 1840s to the present day for agriculture (SEWCDB, 1993) has disrupted connectivity and, as a result, individual KRS and their associated flora and fauna are now largely isolated within an intensively irrigated agricultural landscape. This has contributed to the recent assessment of KRS as a critically endangered ecological community (Keith *et al.*, 2013). The SA population of *E. bispinosus* persists as discrete subpopulations (i.e. populations hydrologically isolated from other nearby populations) (Hammer and Roberts, 2008), with females maturing at considerably smaller sizes and aberrant crayfish (those possessing both male and female gonopores) much more common than in Victorian populations (Honan and Mitchell, 1995b). Genetic analyses have revealed very little differentiation between these subpopulations, although this is likely to be due to a founder effect and subsequent genetic drift rather than high gene flow (Miller *et al.*, in press).

Despite their conservation status, ecological importance and charismatic nature, knowledge gaps in our understanding of population parameters and threatening processes exist for most *Euastacus* species (Furse and Coughran, 2011a, c). Evaluation of population trends and information on life-history characteristics is required to manage threatened *E. bispinosus* and other threatened *Euastacus* species effectively. For example, the early identification of a decline in abundance permits intervention to reverse declines before populations become locally extinct, while knowledge of the reproductive characteristics of a species allows managers to identify potential hindrances to population growth. This study sought to:

1. resurvey known subpopulations of the species and detect new populations;
2. document information on population parameters (sex ratio, size distribution, frequency of gonopore aberrations), including those relating to reproduction (presence of berried females, size at onset of reproduction) and recruitment (presence of juveniles); and
3. investigate changes in the abundance of subpopulations between 2006 and 2011 so as to elucidate population trends.

## METHODS

### Study region

The study focused on KRS scattered throughout a narrow section of coastal drainages between the SA–Victoria border and Port MacDonnell (Figure 1, Table 1). In these habitats, clear (little or no suspended solids), fresh (typically <1000 μS cm$^{-1}$) and cool (approximately 13–17°C year-round) groundwater is discharged from the bottom of pools (pool sites) 1–10 m deep, from which water flows along creeks, modified to act as drainage channels (hereafter creek sites), to the ocean (Allison and Harvey, 1983). Hammer and Roberts (2008) recorded *E. bispinosus* from 13 of 17 surveyed sites within KRS from 5–16 December 2006 (austral summer). In the present study, sites were re-surveyed where *E. bispinosus* had previously been recorded as well as new locations thought suitable for the species in an attempt to identify new populations. The 13 known sites were re-surveyed during the austral winter (21–27 August 2011) and summer (5–10 December 2011) to explore seasonal differences, with the comparison between years (2006, 2011) achieved for summer only. There were 16 new sites surveyed, including pool and creek sites comprising seven discrete rising-spring habitats (Table 1). Two additional KRS (Blacks Pond in the Eight Mile Creek subpopulation and Donovans Drain in Piccaninnie Ponds

 

Table 1. Details of the survey locations within subpopulations of the SA population of *Euastacus bispinosus*. Locations in bold were surveyed in both 2006 (summer) and 2011 (winter, summer); locations not in bold were surveyed only in 2011 and underlined locations are those where crayfish were detected. Where crayfish were detected at a site in 2006 they were also always detected in 2011

| Subpopulation | Number of survey sites | Location of sites |
|---|---|---|
| Clarke Park | 3 | **-38.0528, 140.6797**; -38.0429, 140.6817; -38.0564, 140.6851 |
| Cress Creek | 3 | -38.0380, 140.7143; -38.0386, 140.7154; **-38.0502, 140.7152** |
| Eight Mile Creek | 9 | -38.0366, 140.7899; **-38.0398, 140.7981**; **-38.0307, 140.8071**; **-38.0265, 140.7904**; -38.0274, 140.7911; **-38.0281, 140.7928** **-38.0297, \|140.7945**; **-38.0353, 140.8006**; **-38.0447, 40.7992**; |
| Deep Creek | 4 | **-38.0385, 140.7676**; **-38.0383, 140.7831**; **-38.0433, 140.7775**; **-38.0433, 140.7776** |
| Hitchcox Drain | 2 | -38.0222, 140.8230; -38.0250, 140.8264 |
| Gouldens Waterhole | 1 | -37.9453, 140.6836 |
| The Pines | 1 | -37.7551, 140.5465 |
| Jerusalem Creek | 2 | **-38.0454, 140.7274**; -38.0454, 140.7298 |
| Tea-tree sinkhole | 1 | -38.0336, 140.9190 |
| Horse and Cart sinkhole | 1 | -38.0342, 140.9186 |
| Nene Valley | 1 | -38.0307, 140.5505 |
| Snuggery Spring | 1 | -37.6524, 140.4431 |
| Piccaninnie Ponds | 1 | -38.0368, 140.8941 |

Conservation Park) and two isolated locations (Gouldens Waterhole, a sinkhole; and The Pines, a cave) were also surveyed between June and December 2012.

## Crayfish surveys

In both 2006 and 2011, pool and creek sites were surveyed overnight using commercially available crab nets (Munyana, Wishart, Queensland; 60 mm stretch mesh, 0.76 m diameter steel hoops with two eye-shaped 0.18 × 0.12 m flexible entrances; opera nets, 5 mm stretch mesh, 635 × 445 mm base, 220 mm deep, 60 mm entrances) baited with ox liver, covered with shade cloth and containing one or two lengths of PVC pipe of 50 mm diameter as refuges for small crayfish. Nets were placed to sample both shallow and deep locations and, in creek sites, were placed close to riparian vegetation and instream substrate previously identified as optimal habitat (Honan, 2004). Nets were set in the afternoon (16.00–19.00) and retrieved the following morning (07.00–11.00) (average soak time: ~15 h). In very shallow pools and creek sites (where netting was unsuitable), backpack electrofishing (LR-24, Smith-Root Inc) was used (250 V; 70 Hz, 7% duty cycle and 800–1000 s). For sites surveyed in both 2006 and 2011, similar sample effort was employed between years for netting (in total, 1995 netting hours were achieved in summer 2006, 1830 netting hours in winter 2011 and 1725 netting hours in summer 2011) and electrofishing (in total, 1.24 electrofishing hours were achieved in summer 2006, 1.43 in winter 2011 and 1.1 in summer 2011). Surveys targeted all suitable habitat at each site, and survey effort varied based on the number of nets that could be deployed given the physical size of the waterway (based on 5 m$^2$ area per net: Hammer and Roberts, 2008) with a minimum of five nets (Cress Creek, a small shallow site), and a maximum of 11 (Ewens Pond 2, a large, deep pond). Sites surveyed only in 2011 were subject to a similar sampling regime.

Data collected on captured crayfish included occipital carapace length (OCL, mm, from eye socket to rear of the carapace), weight, sex, and stage of female maturity (Morgan, 1986). The presence of berried females (with eggs) was recorded along with the number of crayfish with gonopore aberrations. All aberrant crayfish were classified as ‘pseudo-female’ males as the male gonopore were always better developed (Honan and Mitchell, 1995b).

## Data analysis

Crayfish abundance was standardized to catch per unit effort (CPUE) both for netting (individuals per net per night) and electrofishing (individuals per second) sampling. Statistical analysis was achieved using univariate permutational analysis of variance (PERANOVA: Anderson, 2001), based on Bray–Curtis distance of fourth-root transformed CPUE abundance, to assess differences between years (2006, 2011, random) for 13 sites across the five subpopulations sampled over time (analysed separately for netting and electofishing; summer data only). For Eight Mile Creek and Deep Creek subpopulations, length–frequency distributions were plotted using 10-mm OCL size classes and compared between years (2006, 2011) using the two sample Kolmogorov–Smirnov test (KS-test). The diversion of sex ratios from unity was tested using chi-squared analysis. Across the SA population, the percentage of sexually mature females in 10-mm OCL size classes was used to estimate the size at the onset of sexual maturity (SOM) according to a logistic equation (Hobday and Ryan, 1997):

$$M = 100 / \left[1 + (OCL/SOM_{50})^{b}\right]$$

where $M$ is the percentage of females in a size class, OCL is the occipital carapace length (mm), $SOM_{50}$ is the length at which 50% of females are sexually mature (mm) and b is a constant.

## RESULTS

### Range of the species

*Euastacus bispinosus* was recorded at 23 of the 30 surveyed sites in 2011, with subpopulations in the five KRS surveyed in 2006 found to persist (Table 1). New sites were identified in two of these subpopulations (upper pool habitat in Cress Creek and Blacks Pond in the Eight Mile Creek subpopulation). The species was detected from four new subpopulations: Hitchcox Drain, Donovans Drain in Piccaninnie Ponds Conservation Park and isolated sinkhole (Gouldens Waterhole) and cave (The Pines) locations.

### Summary of population parameters

In total, 134 *E. bispinosus* (range 6–107 mm OCL) were sampled in 2006 (summer data) and 225 crayfish (n = 100 in winter and n = 125 summer; range 5–112 mm OCL) were sampled in 2011. In 2006 the sex ratio was significantly skewed towards females (0.62:1, $\chi^2 = 4.52$, $P < 0.05$) and 44% of males (or 16% of the total population) were aberrant whereas in 2011 the overall sex ratio was non-significantly skewed towards females (0.77:1, $\chi^2 = 1.34$, $P = 0.247$) and 38% of males (or 17% of the total population) were aberrant (summer data). Winter sampling in 2011 revealed 75% mature females, of which 45% (range 56–103 mm) were in berry whereas in summer 52% (2006) and 64% (2011) of females were mature and no berried females were observed. During winter 2011, berried females were recorded in five subpopulations (Eight Mile Creek, Deep Creek, Cress Creek, Hitchcox Drain and Donovans Drain). Two subpopulations contained no crayfish under 70 mm OCL (Hitchcox Drain and Jerusalem Creek). In contrast, one subpopulation (The Pines) occurred in the top (light) section of a cave environment, and only contained crayfish below 67 mm OCL and no mature females. Across the subpopulations, sex ratios ranged from being significantly skewed towards females (Hitchcox Drain, 0.2:1, $\chi^2 = 34.15$, $P < 0.0001$) to non-significantly skewed towards males (Cress Creek, 1.21:1, $\chi^2 = 0.77$, $P = 0.38$) whereas the mean percentage of aberrant males varied from zero (Hitchcox Drain, Clarke Park, Jerusalem Creek) to 53% (Cress Creek). Across the SA population, the size of onset of sexual maturity for 50% of the surveyed population ($SOM_{50}$) in 2011 was estimated at $63.5 \pm 0.7$ mm OCL ($r^2 = 0.99$) (Figure 2).

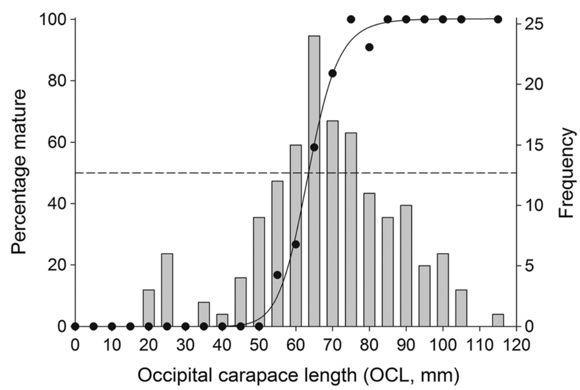


Figure 2. Frequency distribution of occipital carapace length (OCL) (grey bars), size of onset of sexual maturity for each size class (SOM, black dots) and the logistic equation (black line) and associated $SOM_{50}$ (dashed line) for female *Euastacus bispinosus* in the South Australian population (n = 157).

 

### Temporal trends

Although relative abundance declined at all but one pool site between summer 2006 and 2011, differences were not significant (netting CPUE, $F = 1.05$, $P = 0.258$; electrofishing CPUE, $F = 2.44$, $P = 0.174$) (Figure 3). The structure of the Eight Mile Creek subpopulation did vary significantly between years (KS-test, $D = 0.36$, $P < 0.01$), attributed to higher numbers of large crayfish >70 mm OCL being detected in 2006 (Figure 4). No significant differences were found in the structure of the Deep Creek subpopulation (KS-test, $D = 0.27$, $P = 0.33$).

## DISCUSSION

### Status of the South Australian population

The persistence of *E. bispinosus* in the five KRS in which they were detected in 2006 was confirmed in 2011, and four new subpopulations were located. A particularly noteworthy result was the detection of three outlying subpopulations of the species, one in a Ramsar listed wetland, Piccaninnie Ponds Conservation Park. Inclusion of these three new subpopulations considerably expands the core extent of occurrence (EOO: IUCN, 2013) of the SA population to approximately ~300 km$^2$ (from 24 km$^2$, representing a 1150% increase) but the area of occupancy (AOO: IUCN, 2013) remains much smaller (as little as ~2 km$^2$ of aquatic habitat). Genetic analyses have shown that the crayfish in the isolated locations are *E. bispinosus* and that there is little differentiation between these populations and those in the KRS (Miller *et al.*, in press). Deliberate translocations are known to have occurred in at least one of these sites (Gouldens Waterhole) and this is the most parsimonious explanation for the occurrence of the species in the isolated cave (The Pines). At this location, the species was observed in the top (light) section associated with a large submerged cave system. As yet it is unclear whether the species is resident throughout the cave, and it is atypical for crayfish species to occupy both karst cave and surface habitat (Finlay *et al.*, 2006; Taylor *et al.*, 2010). Further surveys are required to ascertain whether *E. bispinosus* is one such species. Regardless, the lack of distinctiveness of the isolated populations suggests that, if necessary, future population translocations can take place in SA from these isolated populations without risk of compromising unique genotypes.

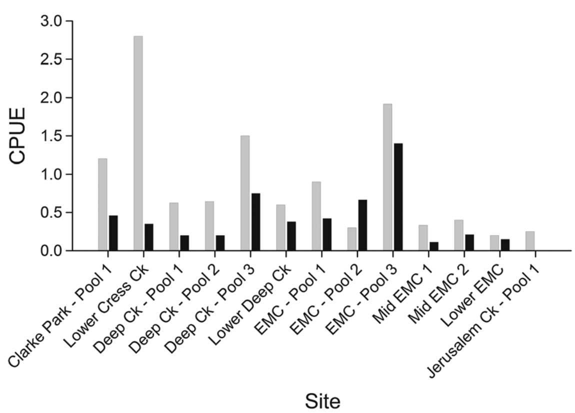


Figure 3. Differences in *Euastacus bispinosus* abundance (CPUE) between summer 2006 (grey bars) and summer 2011 (black bars) in pool and creek sections for each subpopulation in karst rising-spring habitat of south-east South Australia.

The SA population was largely confined to a series of spring pool and creek sites in the Eight Mile Creek and Deep Creek systems (~60% of all recorded crayfish). Despite the relative robustness of the age-class distributions (i.e. most age-classes represented, evidence of juveniles) of these subpopulations, the fact that such a large proportion of the SA population is confined to two relatively small waterways in a critically endangered habitat (Keith *et al.*, 2013) illustrates the vulnerability of the SA population to stochastic events and, perhaps, poaching. Although it is widely known that *E. bispinosus* is protected and signs have been installed in public areas, anecdotal evidence indicates that poaching still occurs, although the intensity is not known.

All other subpopulations, with the possible exception of those found in the sinkholes, may be considered even more vulnerable. The species was recorded from the KRS on Hitchcox Drain (although only large crayfish >70 mm OCL were detected) despite spring discharge having ceased in recent summers, and complete drying of this pond and the upper section of the Hitchcox Drain in January 2013 (authors, pers. observation). Although crayfish were detected in summer 2013 (authors, unpublished data), this site emphasizes

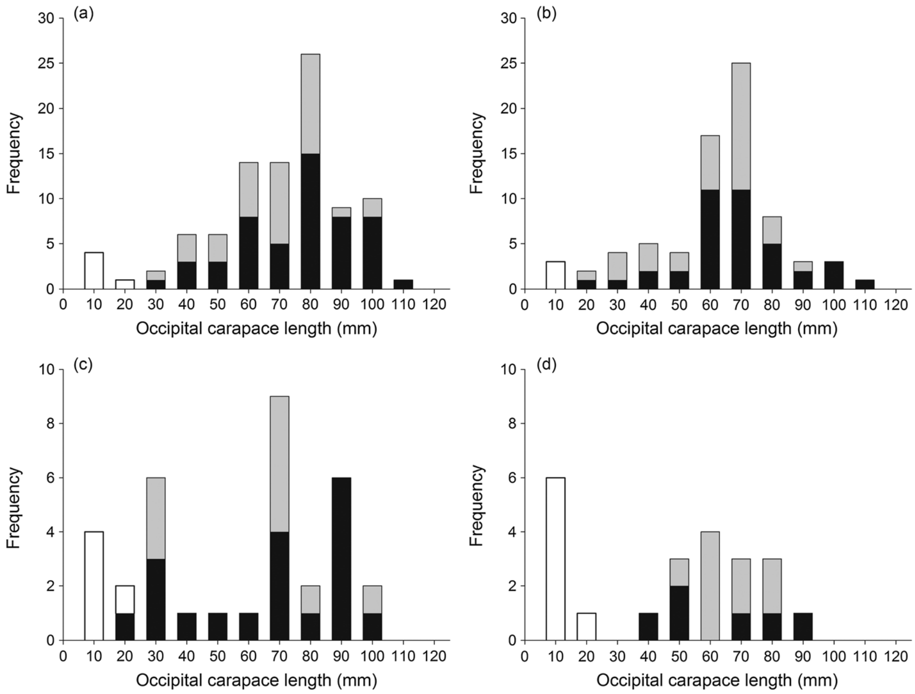


Figure 4. Length–frequency distributions of *Euastacus bispinosus* from Eight Mile Creek (a, 2006; b, 2011) and Deep Creek (c, 2006; d, 2011) subpopulations across years in rising-spring karst habitat of south-east South Australia. Females are represented by grey bars, males by black bars and juveniles by white bars.

the vulnerability of subpopulations in smaller KRS habitat that are threatened by reduced stream flow caused by groundwater extraction for irrigated agriculture, and drying associated with climate change (Hobday and Lough, 2011; Keith *et al.*, 2013). Burrowing is a characteristic feature of freshwater crayfish species (Horwitz and Richardson, 1986), and the presence of only large adults after a drying event suggests that adults may be capable of avoiding desiccation by burrowing into moist sediment whereas juveniles may be more vulnerable (Johnston *et al.*, 2008) but this hypothesis needs further investigation. It is not known whether other subpopulations with few juveniles have also experienced similar drying events, but the finding of only three large crayfish in winter 2011, and none in summer 2011 (or in summer 2013; authors, unpublished data), in the once relatively abundant subpopulation in Jerusalem Creek (Honan and Mitchell, 1995b) suggests that drying may have occurred. Since this study ended, the Cress Creek subpopulation has been threatened by flow cessation for only the second recorded time. Although the species remains present in this location (authors, unpublished data), long-term persistence is considered doubtful.

These findings, together with recent genetic analyses that found extremely low genetic diversity and evidence of significant inbreeding (Miller *et al.*, in press) suggest that the SA population is at very high risk of extinction in the near future (i.e. under 10 years) (IUCN, 2013), which supports assertions that long-lived and slow-growing freshwater crayfish are inherently vulnerable to extinction (Purvis *et al.*, 2000), and that aquatic karst fauna is particularly at risk (Walsh, 2000).

## Comparison with other populations of the species

This study confirmed several distinctions between SA and Victorian populations of the species: females appear to mature at a smaller size in SA compared with Victoria (SA, $SOM_{50}$, 63.5 mm OCL; Victoria, ~85 mm OCL: Honan and Mitchell, 1995b) and aberrant crayfish are more common in SA (38% of males in the present study compared with <1% in Victorian populations: Honan and Mitchell, 1995b). In addition, the proportion of gravid mature females during the breeding season in South Australia (44%) was less than half that of Victorian populations (93–95%, Honan and Mitchell, 1995b; authors, unpublished data). These differences may be explained by the energy conservation hypothesis, where species in karst caves may exhibit slower growth rates, smaller brood sizes and infrequent reproduction as a result of lower energy availability (Poulson and White, 1969; Streever, 1996). Although surface KRS habitats will receive energy inputs via photosynthesis that cave systems will not, the possibility that the different reproductive life-history characteristics evident in KRS are due to existence in a karst environment is intriguing and suggests that the SA population may be locally adapted to what is essentially a different habitat from lowland stream and river habitat in the Glenelg River catchment. This raises the possibility that the SA population, in possessing distinct traits, may be valuable to the evolutionary potential of the species in the face of continued environmental change. The isolated locations, which may be buffered from the threats posed to KRS, may also provide a refuge (sensu Pârvulescu *et al.*, 2013) for the species.

## CONCLUSIONS

This study found that subpopulations exhibited low crayfish abundance, few or no signs of recruitment, were dominated by large crayfish, had a high incidence of gonopore aberration and a low proportion of berried females. Coupled with recent findings of extremely low genetic diversity in all subpopulations and a high level of threat to the KRS habitat of the species, the findings suggest that the disjunct SA population is at high risk of extinction and is likely to be considerably more threatened than the larger Victorian population. Continuing monitoring and research is necessary to gain a clearer understanding of the status of subpopulations within the SA range of the species, as well as to investigate further the influence of the karst environment on the life-history characteristics of the species. More pressing is the requirement to reverse the degradation of KRS habitat, ensure groundwater discharge rates are at least maintained at current rates, and to increase connectivity for the species. This would enable subpopulations to act as a metapopulation and buffer individual populations from local extinction.

## ACKNOWLEDGEMENTS

All sampling was conducted in accordance with relevant permits (DEWNR Wildlife Research permits: U25318 and E25963-1, PIRSA Fisheries permits: 9901926 and 9902414). Financial support for this study was provided by Friends of Mt Gambier Area Parks (Friends of Parks Inc.) via the Australian Government's Caring for our Country programme. We (and the species) are indebted to the pioneering work and continuing interest of Jodie Honan and specific comments she provided on the manuscript. Thanks to Kerrylyn Johnston for valuable general input, Peter Horne and Liz Rogers for information on the cave location, Chris Bice for assistance with statistical analysis, Michael Roberts for important contributions to the baseline study design and assessment, and Dave Mossop and numerous volunteers (Maureen Christie and Ian Mitchener, Helen Bawden, Jean Haywood, Noel Stratman, Lars Kellar (and family) Toby Read, Becky McCann, Cath Dickson, Steve Clarke, Tania Rajic and Angus Anderson) for assistance with field sampling. We gratefully acknowledge access to sampling sites by the South East Water Conservation and Drainage Board and local landowners. We thank two anonymous reviewers and the editor for comments that greatly improved the manuscript.

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