Austral Ecology A Journal of ecology in the Southern Hemisphere

Austral Ecology (2014) ••, ••–••

Hypoxic blackwater event severely impacts Murray crayfish (*Euastacus armatus*) populations in the Murray River, Australia

BERNARD MCCARTHY,[1,2]* SYLVIA ZUKOWSKI,[1,2†] NICK WHITEROD,[1,2†] LORENZO VILIZZI,[1,2‡] LEAH BEESLEY[3§] AND ALISON KING[3¶]

[1]*Murray-Darling Freshwater Research Centre,* [2]*La Trobe University, Mildura, and* [3]*Department of Sustainability and Environment, Arthur Rylah Institute for Environmental Research, Heidelberg, Victoria, Australia*

Abstract Prolonged flooding in 2010/11 ended a decade of drought and produced a large-scale hypoxic blackwater event across the southern Murray-Darling Basin, Australia. The hypoxic conditions caused fish kills and Murray crayfish *Euastacus armatus* to emerge from the water onto the river banks to avoid the poor water quality. This study examined the medium-term impact of this blackwater event on Murray crayfish populations in the Murray River, where approximately 1800 km of the main channel were affected by hypoxia. Murray crayfish populations were surveyed in July 2012, along a 1100-km section of the Murray River at 10 sites affected by hypoxic blackwater and six sites that were not affected, and data were compared with surveys of the same sites undertaken in July 2010, four months before the hypoxic blackwater event (before-after-control-impact experimental design). Murray crayfish abundance in 2012 (post-blackwater) was significantly lower at blackwater affected sites (81% reduction from 2010), but not at non-affected sites. The hypoxic blackwater impacted Murray crayfish of both sexes and all size-classes in a similar manner. The results demonstrate that prolonged periods of hypoxia can markedly impact populations of the long-lived and slow-growing Murray crayfish despite the species ability to emerge from hypoxic water. The findings highlight important challenges for the management of both the recreational fishery for this species and riverine flows in relation to hypoxic blackwater events.

Key words: BACI experimental design, dissolved oxygen, environmental flow, hypoxia.

INTRODUCTION

River–floodplain connectivity is important for the functioning of many rivers worldwide (Junk *et al.* 1989), and the mobilization of dissolved organic carbon (DOC) from the floodplain to the river channel is a key driver of riverine food webs (Thorp *et al.* 2008). However, the leaching of high loads of DOC can produce tea-coloured water termed 'blackwater' (Baldwin 1999; O'Connell *et al.* 2000). Blackwater conditions occur in many rivers worldwide, due to sandy soils not retaining the DOC leached from terrestrial vegetation (Janzen 1974; Meyer 1990). Discrete blackwater events may also occur in lowland rivers with forested floodplains that do not normally contain high DOC loads (Whitworth *et al.* 2012). In these circumstances, the leaching of DOC from inundated plant litter, particularly leaves (O'Connell *et al.* 2000), can stimulate high rates of microbial activity that may outstrip oxygen-generating processes within the water column ultimately leading to low dissolved oxygen (DO) concentrations or hypoxia (Howitt *et al.* 2007; Hladyz *et al.* 2011). The return of blackwater to the river from the floodplain can severely degrade riverine water quality and create a downstream plume of hypoxic water (Whitworth *et al.* 2012).

Riverine hypoxic blackwater events have been reported previously in the Paraguay River, Brazil (Hamilton *et al.* 1997), the Atchafalaya River and Rio Grande catchments, USA (Fontenot *et al.* 2001; Valett *et al.* 2005), as well as in Australian tropical floodplain rivers (Bishop 1980; Townsend & Edwards 2003) and in the Murray-Darling Basin (McKinnon 1995; McKinnon & Shepheard 1995; Baldwin *et al.* 2001; Howitt *et al.* 2007; Hladyz *et al.* 2011). Hypoxic blackwater events are an irregular but recurring feature in

*Corresponding author. Present address: Jujube Australia, 23 Loop Road, Nichols Point, Victoria 3501, Australia (Email: bernard@jujubeaustralia.com)

†Present address: Aquasave – Nature Glenelg Trust, 7 Kemp St, Goolwa Beach, South Australia 5214, Australia.

‡Ichth-Oz Environmental Science Research, 972 Irymple Ave, Irymple, Victoria 3498, Australia.

§Present address: Centre of Excellence in Natural Resource Management, University of Western Australia, Albany, Western Australia 6330, Australia.

¶Present address: Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, Northern Territory 0909, Australia.

Accepted for publication November 2013.

doi:10.1111/aec.12109

the Murray River and its tributaries, with five events recorded downstream of Barmah-Millewa Forest since 1990 (i.e. 1992, 2001, 2005, 2010/11 and 2012). River regulation is believed to have exacerbated the frequency and magnitude of hypoxic blackwater events through a greater accumulation of organic material on floodplains resulting from decreased flooding frequency, and altering flow seasonality that has increased the likelihood of inundation during warm periods (Whitworth *et al.* 2012).

Hypoxic blackwater events can adversely impact aquatic fauna, with fish and freshwater crayfish particularly susceptible to mortality or stress (Hamilton *et al.* 1997; Townsend & Edwards 2003; King *et al.* 2012). The tolerance and behavioural responses of fish to low DO and high DOC differs among species (McNeil & Closs 2007; McMaster & Bond 2008) and with size, due to larger fish possessing greater capacity for anaerobic adenosine triphosphate (ATP) production necessary for survival during hypoxia (Nilsson & Ostlund-Nilsson 2008). Freshwater crayfish are particularly at risk to prevailing DO concentrations due to their limited mobility (Ryan 2005), and show a range of tolerances and behaviours to hypoxia. For example, rusty crayfish *Orconectes rusticus* may partially emerge from shallow water to aerate their branchial chambers before re-submerging (McMahon & Wilkes 1983), whereas red swamp crayfish *Procambarus clarkii* (Wheatly *et al.* 1996), common yabby *Cherax destructor* (Morris & Callaghan 1998) and freshwater white-clawed crayfish *Austropotamobius pallipes* (Taylor & Wheatly 1981) may emerge fully to breathe air, with the latter species showing a tolerance to prolonged hypoxia of 3 mg L^{-1} (Demers *et al.* 2006). For Murray crayfish *Euastacus armatus* (von Martens, 1866) (Decapoda, Parastacidae), emergence occurs at approximately 2 mg L^{-1} based on field observations (McKinnon 1995; King *et al.* 2012), with juveniles (Stage 3) having a LC_{50} (lethal concentration to kill 50% of tested population) of 2.2 mg L^{-1} (Geddes *et al.* 1993).

High and unseasonal rainfall from August 2010 to March 2011 ended a decadal drought in the Murray-Darling Basin (van Dijk *et al.* 2013) and generated a series of flood events that inundated Murray River floodplains (Whitworth *et al.* 2012). The unseasonal inundation of large quantities of accumulated organic material on the floodplains, particularly at the 70 000 ha river red gum *Eucalyptus camaldulensis* dominated Barmah-Millewa Forest, during periods of warm water temperature (>20°C) led to a significant input of blackwater from the floodplain to the main channel (King *et al.* 2012; Whitworth *et al.* 2012). This in turn created a prolonged hypoxic blackwater event that persisted for nearly 6 months and extended for approximately 1800 river kilometres downstream (Figs 1,2) (Whitworth *et al.* 2012). To our knowledge the magnitude and duration of this event was considerably greater than any event previously recorded in the Murray River, and perhaps in the world.

Short-term effects (at the scale of weeks–months) of the 2010/11 hypoxic blackwater event on biota were evident by the mortality of Murray cod *Maccullochella peelii* and localized decline in abundance of some fish species in the vicinity of Barmah-Millewa Forest (King

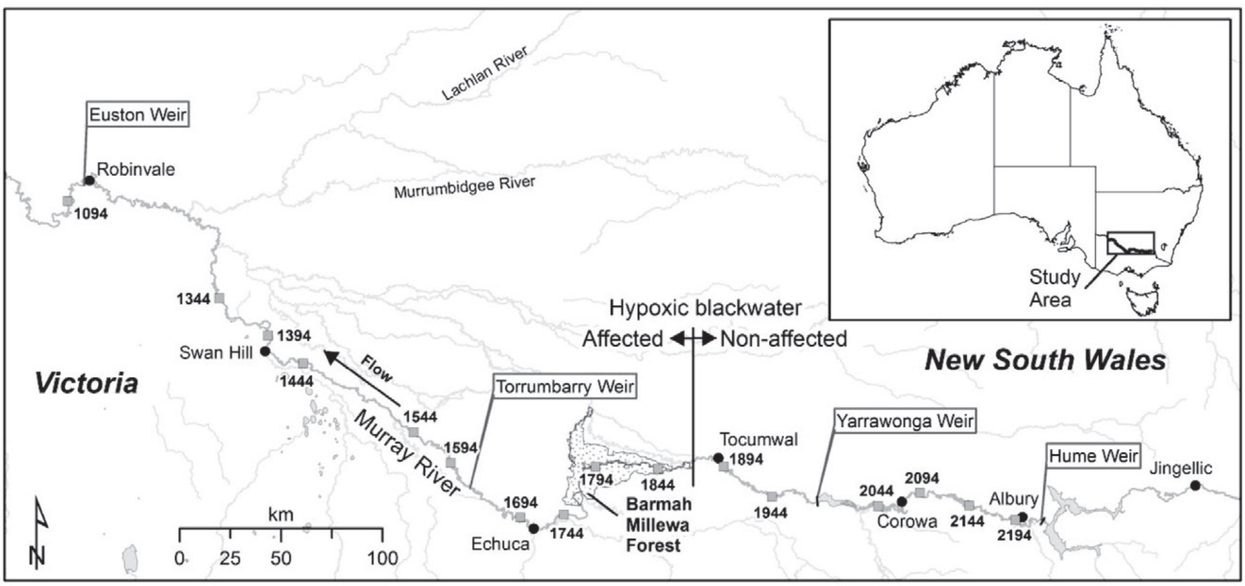

Fig. 1. Location of the 16 study sites along the Murray River, Australia, that were surveyed before (2010; Zukowski 2012) and after (2012; present study) the prolonged hypoxic blackwater event that was generated by the flooding of Barmah-Millewa Forest in 2010/11. Sites upstream and downstream of Barmah-Millewa Forest were considered to be 'non-affected' and 'affected' by the hypoxic blackwater respectively (river kilometre represents the distance upstream of the mouth of the Murray River).

doi:10.1111/aec.12109

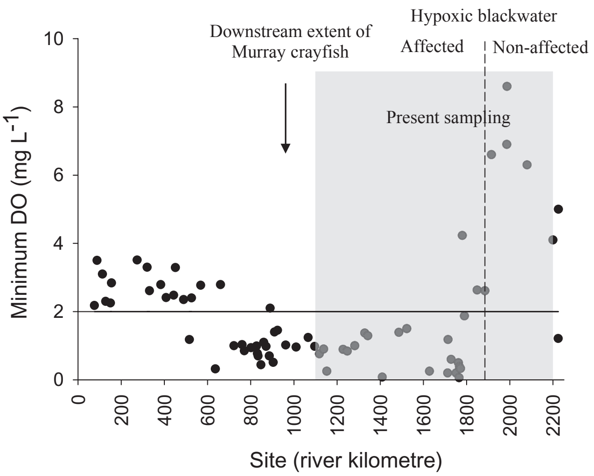

Fig. 2. Minimum dissolved oxygen (DO) concentrations recorded at sites along the Murray River during the period September 2010 to April 2011 inclusive (data sourced from multiple agencies as acknowledged in Whitworth *et al.* 2012). The 2 mg L^{-1} threshold (solid horizontal line) represents the approximate DO concentration for emergence of Murray crayfish from the water. The vertical (dashed) line represents the upstream extent of Barmah-Millewa Forest and designation of sites as 'affected' or 'non-affected' by the 2010/11 hypoxic blackwater event. The 16 study sites were located within river kilometres 1094 to 2194 (denoted by grey shading). Murray crayfish occur as far downstream as approximately 950 river kilometres (McCarthy 2005). Note that the <2 mg L^{-1} reading for the most upstream site is attributed to hypolimnetic water release from Lake Hume (Whitworth *et al.* 2012) (river kilometre represents the distance upstream of the mouth of the Murray River).

et al. 2012) and the lower Murray River (Leigh & Zampatti 2013). In the Barmah-Millewa Forest, mortality of freshwater crustaceans, including freshwater shrimp *Macrobrachium* spp. and yabbies *Cherax* spp., was also observed, as was the emergence of large numbers of Murray crayfish from the water onto the river bank at the beginning of the blackwater event (King *et al.* 2012) – a behaviour recorded previously in this area (McKinnon 1995). Emerged Murray crayfish were absent 2 months later, although the blackwater event was still occurring (King *et al.* 2012), and the medium-term (at the scale of 1–2 years) fate of the species remains unclear.

The Murray crayfish is the second largest freshwater crayfish in the world and an iconic species of many rivers of south-eastern Australia (McCormack 2012). It supports a popular recreational fishery in New South Wales and Victoria. However, the species has decreased in abundance and range over the past 50 years (Horwitz 1990, 1995), and is listed as threatened in Victoria (*Flora and Fauna Guarantee Act 1988*), protected in South Australia (*Fisheries Management Act 2007*), vulnerable in the Australian Capital Territory (*Nature Conservation Act 1980*) and recently as vulnerable in New South Wales (Fisheries Scientific Committee 2013). Yet, Murray crayfish are nationally listed as indeterminate (*Environment Protection and Biodiversity Conservation Act 1999*) and globally as data deficient (*International Union for Conservation of Nature*), highlighting that insufficient resources have been directed towards establishing its population status (Alves *et al.* 2010a).

The objective of this study was to assess the impact of the 2010/11 hypoxic blackwater event on Murray crayfish populations in the Murray River. A before-after-control-impact (BACI) experimental design was used to compare key population parameters (abundance, size, sex) at affected and non-affected sites before (July 2010; cf. Zukowski 2012) and after (July 2012) the event. We hypothesized that the blackwater event caused a significant reduction in Murray crayfish abundance in areas subjected to the hypoxic blackwater, with the effects uniform irrespective of size-class or sex.

METHODS

Study location

The Murray River flows 2530 km from the south-eastern highlands, through the southern Murray-Darling Basin, to the sea at Goolwa (Eastburn 1990). The Murray River is highly regulated by upland impoundments, low-level weirs and irrigation diversions along much of its length (Walker 2006). Barmah-Millewa Forest is a large and significant river red gum floodplain wetland system, located between Barmah and Ulupna Island (between 1762 and 1864 river kilometres upstream of the Murray mouth) on the Murray River (Fig. 1). Along the 1100-km study section (i.e. 1094 to 2194 river kilometres upstream of the Murray mouth), 16 sites were sampled (Fig. 1). The 16 sites were a subset of the 26 sites studied by Zukowski (2012) in July 2010 (the 'before' dataset in the present study) and represented those sites (i) where the catch per unit effort (CPUE) of Murray crayfish equalled or exceeded 0.1 individuals per net per hour (Zukowski 2012) and (ii) that were located downstream of Hume weir. All 16 sites surveyed in this study were located in free-flowing reaches of river outside of the direct impoundments created by weirs (Zukowski 2012).

Sites were designated as 'affected' or 'non-affected' by the 2010/11 hypoxic blackwater event based on minimum DO concentrations (Whitworth *et al.* 2012) (Fig. 2). Therefore, sites upstream of Barmah-Millewa Forest were designated as 'non-affected' by hypoxic blackwater and those within and downstream of Barmah-Millewa Forest were designated as 'affected' (Fig. 1). The 16 sites were paired to allow surveys of two sites on a single day and the order of sampling each pair of sites was randomly determined. Sampling was undertaken during the austral winter (2–13 July 2012), and coincided with the 'before' dataset (Zukowski 2012). Seasonal studies of Murray crayfish show that catches are highest during winter months (Zukowski *et al.* 2012).

doi:10.1111/aec.12109

Sampling protocol

A standardized sampling protocol and design was employed during the 'before' sampling (Zukowski 2012) and repeated for the 'after' sampling. Standard hoop nets were first deployed by boat at a site either in the morning (08.00–09.30 hours) or in early afternoon (12.30–15.00 hours). Nets were retrieved 1 h after deployment with the exception of site 2094, where the higher abundance of Murray crayfish and longer processing time increased the mean soak time to 1.2 h per net. Each hoop net consisted of 20 mm stretched mesh threaded over a single 0.8-m-diameter steel hoop to create a drop of approximately 0.3 m. An ox liver bait of 300 ± 50 g was tied to the central part of the net to attract Murray crayfish and was replaced at each site. Twenty-one nets were set at each site and separated by at least 40 m over a distance of about 2 km (set on both sides of the river). Nets were typically set 3–10 m from the water's edge along steep-sided banks (i.e. near outside bends and straight reaches of the river) where water was generally at its deepest. Each net was re-deployed approximately 10 m from its initial set position after the first lift. Two sets were conducted at each site, for a maximum of 42 net lifts. Nets that lost their bait or were snagged upon hauling were excluded from the abundance analysis.

Captured Murray crayfish were sexed, weighed (g) and their occipital carapace length (OCL, in mm: from eye-socket to rear of carapace) measured with callipers. Murray crayfish were temporarily marked (with a marker pen) to identify potential recaptures (none were obtained: author's unpubl. data 2012) before being returned to the water at the point of capture. Water temperature (°C), DO (mg L^{-1}), pH, electrical conductivity (μS cm^{-1}) and turbidity (nephelometric turbidity units; NTU) were measured with a Horiba U-50 water quality multi-probe (Australian Scientific Ltd, Kotara, New South Wales (NSW), Australia) at 0.2 m depth at the central part of the river at each site following the first deployment of nets.

Statistical analyses

Differences in Murray crayfish CPUE abundance (computed as number of individuals per net per hour; specifically, the total number of crayfish caught per site divided by the total number of net hours per site) between affected and non-affected sites before and after the blackwater event were tested by permutational univariate analysis of variance (PERANOVA: Anderson 2001). According to a BACI design (Green 1979), there were two factors: Year (2010: Before; 2012: After) and Location (Control: non-affected; Impact: affected), both fixed and crossed. Sites were the experimental units, consisting of 6 control and 10 impact located upstream and downstream of Barmah-Millewa Forest respectively. Following square-root transformation of CPUE data, a Euclidean distance was used to produce a distance matrix, with statistically significant effects for the main (i.e. Year and Location) and interaction (i.e. Year × Location) effects followed by *a posteriori* pairwise comparisons ($\alpha = 0.05$). Statistical analyses were carried out in PERMANOVA+ (Anderson *et al.* 2008) for PRIMER v6 (Clarke & Gorley 2006), with 9999 permutations of the raw data. Briefly, the advantage of PERANOVA compared with traditional parametric analysis of variance is that the stringent assumptions of normality and homoscedasticity in the data, which prove very often unrealistic when dealing with ecological datasets, are significantly relaxed (Anderson 2001; Anderson & Robinson 2001).

Log-linear analysis (Quinn & Keough 2002) was used to test for differential effects of hypoxic blackwater upon Murray crayfish size categories and sex. Prior to analysis, Murray crayfish were divided into two size categories based on an OCL threshold of 90 mm (i.e. 'small': <90 mm; 'large': ≥90 mm), which represents the NSW recreational fishing minimum legal length at the time of survey for harvesting Murray crayfish and the approximate size at maturity for females (Gilligan *et al.* 2007; Zukowski *et al.* 2012). Log-linear analysis was used to determine the effects of Year (2010: Before; 2012: After), Location (Control: non-affected; Impact: affected), Sex (Male, Female) and Size (Small, Large: defined as above) on the number of Murray crayfish sampled. A null model (i.e. with all frequencies being equal) was initially fitted and terms added sequentially starting from all possible combinations of individual factors, two-way interactions and three-way interactions, up to a saturated model (i.e. one including the highest four-way interaction term Year × Location × Sex × Size). The significance of terms included sequentially ($\alpha = 0.05$) was then tested by an analysis of deviance based on a chi-square test. Fitting of log-linear models was conducted with the R language and software environment for statistical computing and graphics v2.13.0 64-bit (R Development Core Team 2010), using library MASS v7.3-14 under a Poisson distribution.

RESULTS

A total of 182 Murray crayfish (ranging between 52 and 137 mm OCL and 75 and 1212 g) were sampled from non-affected sites whereas only 18 individuals (53 and 136 mm, 82 and 1151 g) were collected from those sites affected by the blackwater event. Abundance ranged between 0.18 and 2.13 individuals per net per hour at non-affected sites and 0 and 0.21 individuals per net per hour at affected sites (Fig. 3). There was a significant decrease in the abundance of Murray crayfish collected at the affected sites following the blackwater event, but not at the non-affected sites (Fig. 4; significant Year × Location interaction in Table 1). Of the 10 sites affected by the hypoxic blackwater event, mean Murray crayfish abundance decreased by 81% compared with 2010. From an individual site perspective, Murray crayfish abundance in 2012 was lower than 2010 at 9 of the 10 affected sites; at five of these sites no crayfish were detected in the post-blackwater survey (Fig. 4). Meanwhile, there was a 31% increase in mean abundance of Murray crayfish in 2012 relative to 2010 at the non-affected sites (increase at five of the six sites) (Figs 3,4), but this was not statistically significant (Table 1).

Of the 182 individual Murray crayfish sampled from the six non-affected sites in 2012, 82 were males ($n = 67$, <90 mm OCL; $n = 15$, ≥90 mm OCL) and

doi:10.1111/aec.12109

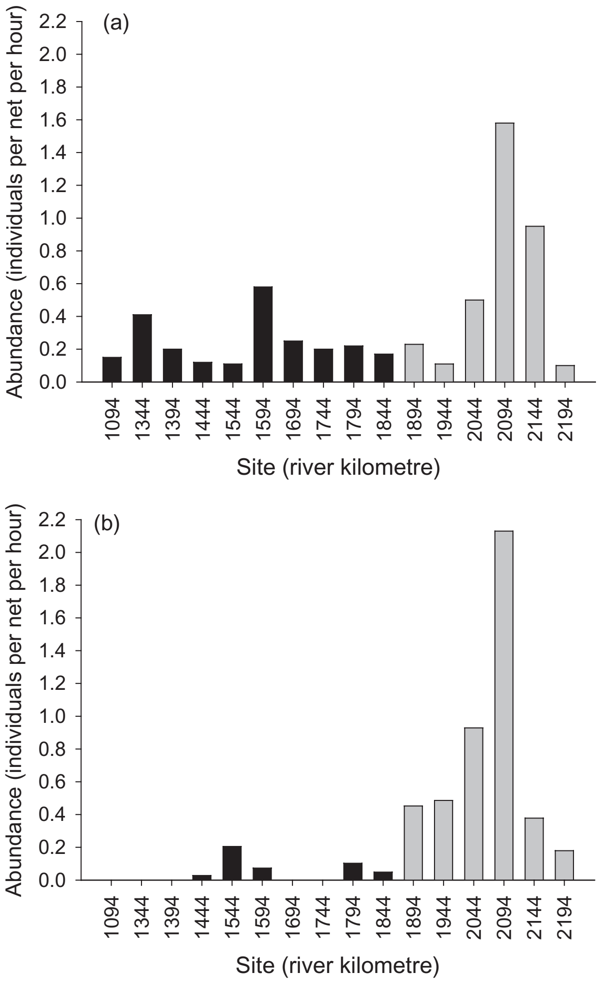

Fig. 3. Murray crayfish abundance (individuals per net per hour) at individual sites (a) before (2010; data from Zukowski 2012) and (b) after (2012; data from this study) the 2010/11 hypoxic blackwater event. Grey bars: non-affected sites; black bars: affected sites.

100 were females ($n = 66$, <90 mm OCL; $n = 34$, ≥90 mm OCL, with 27 gravid) (Fig. 5). Of the 18 individual Murray crayfish sampled at the 10 affected sites in 2012, 10 were males ($n = 8$, <90 mm OCL; $n = 2$, ≥90 mm OCL) and 8 were females ($n = 6$, <90 mm OCL; $n = 2$, ≥90 mm OCL, with three gravid) (Fig. 5). There was no detectable effect of the blackwater event on the number of Murray crayfish (either male or female) for small- or large-sized individuals (i.e. <90 mm, ≥90 mm) (non-significant Year × Location × Sex × Size term). There was also no detectable effect of the blackwater on Murray crayfish size, irrespective of sex (non-significant Year × Location × Size term) (Table 2; Fig. 5). However, larger crayfish (≥90 mm OCL) tended to be females (significant Sex × Size interaction term), and mean (± SE) crayfish size was greater in 2012 (83.1 ± 1.2 mm) across all sites compared with 2010 (75.5 ± 0.8 mm) (significant Year × Size interaction term) (Table 2).

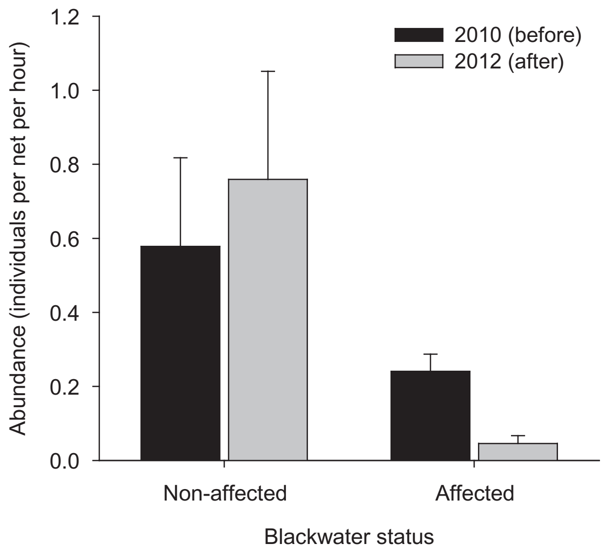

Fig. 4. Mean (+SE) Murray crayfish abundance (individuals per net per hour) before (2010: black shading; data from Zukowski 2012) and after (2012: grey shading; this study) the 2010/11 hypoxic blackwater event. Sites were non-affected ($n = 6$; upstream of Barmah-Millewa Forest) and affected ($n = 10$; downstream of Barmah-Millewa Forest) by hypoxic blackwater.

Water quality measurements were taken at each site, with all variables (temperature: 8.92–11.07°C; DO: 12.29–14.60 mg L^{-1}; pH: 7.51–7.92; electrical conductivity: 37–94 μS cm^{-1}; turbidity: 1–127 NTU) within acceptable limits for winter in lowland rivers (ANZECC 2000).

DISCUSSION

Hypoxic blackwater and Murray crayfish abundance

The present study provides strong evidence that the hypoxic blackwater event of 2010/11 had a significant medium-term impact on the Murray crayfish population in the Murray River. Reduced abundances were recorded across all size-classes at affected sites, whereas abundance remained similar at sites not affected by the hypoxic blackwater event. King *et al.* (2012) questioned whether the large numbers of Murray crayfish that emerged and then disappeared during the 2010/11 blackwater event had died or acclimatized to the hypoxic conditions and the findings of the present study suggest that the majority had perished.

The considerable population loss (estimated at 81%) found by this study, coupled with the large-scale geographical coverage of the blackwater event, which covered two-thirds of the current distribution of

doi:10.1111/aec.12109

Table 1. PERANOVA results for Murray crayfish √CPUE abundance (individuals per net per hour) before (2010) and after (2012) a hypoxic blackwater event at 6 non-affected and 10 affected sites upstream and downstream of Barmah-Millewa Forest respectively

Source of variation	d.f.	MS	$F^{\#}$	$t^{\#}$	$P^{\#}$
Year	1	0.07	1.21		0.285
Location	1	1.40	21.96		<0.001
Year × Location	1	0.39	6.17		**0.019**
2010 *vs.* 2012 (non-affected)				0.59	0.557
2010 *vs.* 2012 (affected)				4.89	**<0.001**
Residual	28	0.06			

A posteriori pairwise comparisons are given for the statistically significant effects of interest ($\alpha = 0.05$, in bold type). $F^{\#}$: permutational F value; $t^{\#}$: permutational t-test value; $P^{\#}$: permutational probability value. See also Figure 3. CPUE, catch per unit effort; MS, mean square; PERANOVA, permutational univariate analysis of variance.

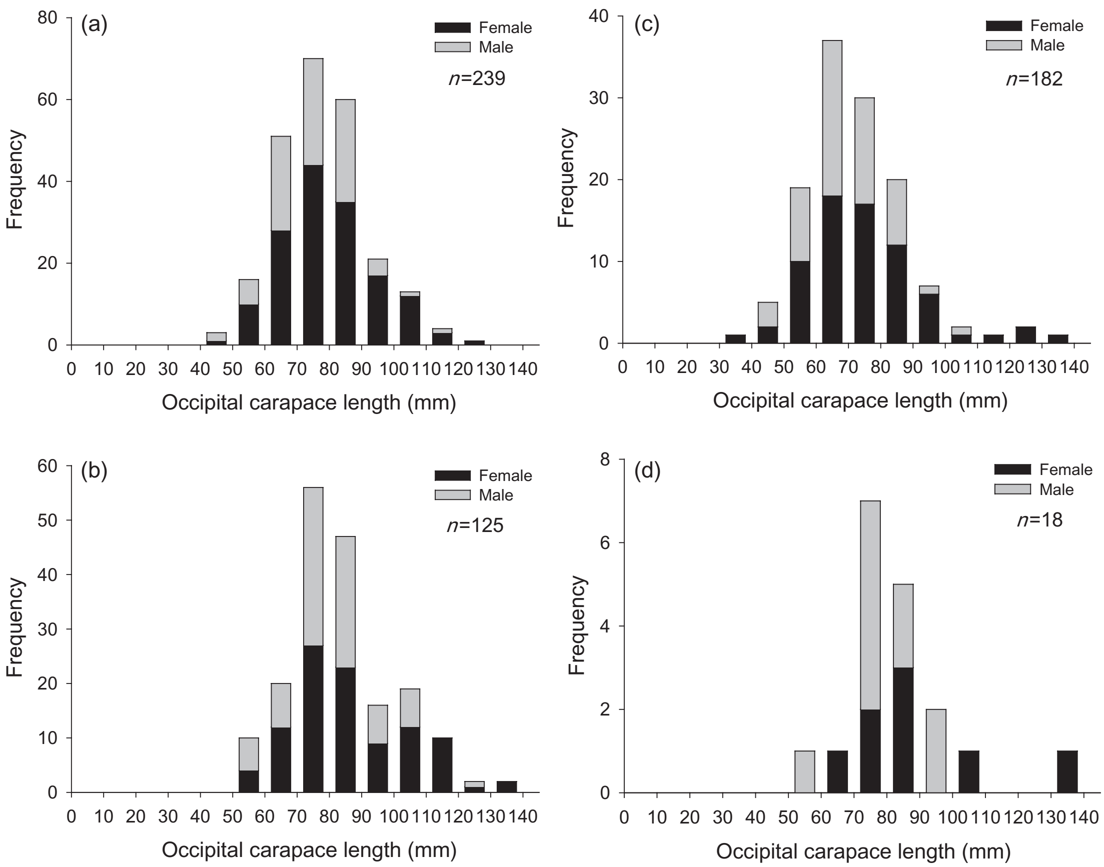

Fig. 5. Length-frequency distributions for Murray crayfish populations at non-affected sites (a) before (2010; Zukowski 2012) and (b) after (2012; this study) the hypoxic blackwater event and at affected sites (c) before and (d) after the event. Grey bars: males; black bars: females (river kilometre represents the distance upstream of the mouth of the Murray River). Note the different *y*-axis scales used to allow clearer interpretation of sex ratios with size.

Murray crayfish along the Murray River (Walker 1982; McCarthy 2005; Zukowski 2012), highlights the significance of this one event and has important ramifications for this species. Murray crayfish have also been undergoing serious population declines in distribution and abundance since the 1950s (Horwitz 1990, 1995), making the importance of this event even more critical. Murray crayfish are considered locally extinct from the lower 950 km of the Murray River, most likely due to the highly regulated nature of this stretch of river (Walker 1982; McCarthy 2005), and were not detected from upstream impoundments in 2010 (i.e. Euston weir pool and Lake Hume: Zukowski 2012). Therefore, only approximately 50% of the original

doi:10.1111/aec.12109

Table 2. Log-linear analysis results for the number of Murray crayfish sampled before and after the 2010/11 hypoxic blackwater event at 6 non-affected and 10 affected sites upstream and downstream of Barmah-Millewa Forest respectively

Source of variation	d.f.	Deviance	Resid. d.f.	Resid. Dev.	P (>|Chi|)
[Null]	1		15	537.98	
Year	1	48.384	14	489.60	<0.001
Location	1	143.199	13	346.40	<0.001
Sex	1	16.420	12	329.98	<0.001
Size	1	239.729	11	90.25	<0.001
Year × Location	**1**	**49.356**	**10**	**40.90**	**<0.001**
Year × Sex	1	2.588	9	38.31	0.108
Location × Sex	1	2.040	8	36.27	0.153
Year × Size	**1**	**12.288**	**7**	**23.98**	**<0.001**
Location × Size	1	2.499	6	21.48	0.114
Sex × Size	**1**	**19.547**	**5**	**1.94**	**<0.001**
Year × Location × Sex	1	0.054	4	1.88	0.816
Year × Location × Size	1	0.201	3	1.68	0.654
Year × Sex × Size	1	1.413	2	0.27	0.235
Location × Sex × Size	1	0.006	1	0.26	0.936
Year × Location × Sex × Size	1	0.262	0	0.00	0.609

Statistically significant effects ($\alpha = 0.05$) in bold type (notably, significant interaction terms override significant individual factors in the interaction). All factors with two levels: Year = 2010 (before), 2012 (after); Location = non-affected by hypoxic blackwater (control), Affected by hypoxic blackwater (impact); Sex = male, female; Size = small (<90 mm occipital carapace length (OCL)), large (≥ 90 mm OCL).

habitat within the Murray River was considered suitable for sustaining Murray crayfish populations prior to the blackwater event, and much of this area was heavily affected by hypoxic blackwater.

Field observations (McKinnon 1995; King *et al.* 2012) and laboratory experiments (Geddes *et al.* 1993) demonstrate that Murray crayfish are intolerant to low DO (<2 mg L^{-1}). The 2010/11 blackwater event clearly exceeded the tolerance threshold for the species, with DO concentrations <2 mg L^{-1} recorded along much of the study region for several months (Whitworth *et al.* 2012). One obvious explanation for the reduction in abundance is that a high proportion of Murray crayfish could not sustain normal physiological functioning in the face of chronic low DO levels, and perished. It is also possible that leachates within the blackwater may have exacerbated oxygen stress by reducing oxygen exchange across the surface of the gills (Janzen 1974; Gehrke *et al.* 1993; McMaster & Bond 2008), although this remains unstudied in Murray crayfish.

Another explanation for the reduction in crayfish abundance is emergence and subsequent predation. King *et al.* (2012) observed significant numbers of Murray crayfish emerging from the Murray River at affected sites during the 2010/11 blackwater event, as did McKinnon (1995) during an event in 1992. While it remains unknown what proportion of the population emerged, the severity and longevity of the blackwater event makes it likely that a large fraction would have used this behavioural mechanism to improve their oxygen uptake. Emergence may be a good strategy to avoid hypoxia in the short term, but its efficacy likely declines with time spent out of water as crayfish become increasingly exposed to predation from terrestrial animals (Furse & Coughran 2011) and from illegal harvesting by humans. Indeed, Murray crayfish were reportedly harvested in high numbers during the emersion event in 1992 (McKinnon 1995) and while there were no direct reports of this in 2010/11 by King *et al.* (2012), anecdotal accounts during the field study indicated some degree of harvesting (anonymous recreational fisher, pers. comm. 2012). Emerged Murray crayfish also become vulnerable to desiccation, although they were reported to remain close to water and showed regular re-immersion behaviour at least at the start of the blackwater event (McKinnon 1995; King *et al.* 2012), presumably to maintain hydration. There are also likely to be physiological costs associated with emergence behaviour. For example, the common yabby displays a fivefold increase in cardiac output during emersion (Morris & Callaghan 1998). These, in combination with the timing of emersion in November–December 2010 when air temperatures were relatively high, suggest that prolonged emersion would have placed Murray crayfish at a high risk of mortality.

Consideration also needs to be given to a second flood event that occurred along the Murray River in early 2012 and in the period between the 2010 (Zukowski 2012) and 2012 sampling of the present study. This flood event also inundated Barmah-Millewa Forest and generated a second hypoxic blackwater event in the Murray River from late March to

doi:10.1111/aec.12109

early April 2012 that was generally less hypoxic and more localized than for 2010/11 (Whitworth & Baldwin 2012). However, DO concentrations near zero were recorded in the Murray River immediately downstream of Barmah-Millewa Forest (Whitworth & Baldwin 2012) (although no emergence behaviour of Murray crayfish was reported), and this second blackwater event may therefore have contributed to population decline of Murray crayfish at some sites.

This study found no evidence that hypoxic blackwater differentially impacted Murray crayfish because of their size or sex. However, the limited number of crayfish caught from affected sites (total of 18) means that caution should be exercised when making inferences at the population level. In addition, small Murray crayfish are difficult to sample using conventional netting methods in rivers (Alves *et al.* 2010b), so it may take several years to assess whether there were differential impacts of the hypoxic blackwater upon the small size-classes. That said, the present results are supported by both King *et al.* (2012) and McKinnon (1995) who reported a broad size range (including small juveniles to large adults) of emerged Murray crayfish, indicating that individuals of different sizes were affected in a similar manner by hypoxia.

Broader implications

The focus of this study was the Murray River due to the occurrence of an invaluable 'before' dataset (Zukowski 2012) that sampled Murray crayfish approximately four months before the occurrence of the hypoxic blackwater event in 2010/11. However, this event (and also that of early 2012; Whitworth & Baldwin 2012) also affected other river systems across the southern Murray-Darling Basin where Murray crayfish occur, in particular the Edward and Murrumbidgee Rivers (Whitworth *et al.* 2012). This raises an important question about the ability to extrapolate the results of this study to Murray crayfish populations in other river systems also affected by blackwater. There were anecdotal reports of Murray crayfish emergence in these other river systems (Alison King, pers. comm. 2012) and consequently there is a reasonable probability that the hypoxic blackwater had a negative impact on the Murray crayfish population more broadly. Unfortunately, rigorous evaluation of the impacts of hypoxic blackwater in these other systems will be hampered by the absence of suitable 'before' data.

This study has highlighted how a single stressor event can have a severe negative impact on aquatic animals, and significantly compound the decline in already suppressed populations. The long-term prospects of recovery for the impacted Murray crayfish populations downstream of Barmah-Millewa Forest remain unclear, but recovery should be aided by the recent closure of the recreational fishery in blackwater affected areas of the Murray and Murrumbidgee Rivers (NSW DPI 2013; Victorian DEPI 2013). Population recovery is anticipated to be slow for this poor dispersing, slow-growing and late maturing species (i.e. k-selected life history (Pianka 1970)), and targeted monitoring and population modelling will be important to manage Murray crayfish recovery. However, predictions of more frequent hypoxic blackwater events in south-eastern Australia under climate change projections (Morrongiello *et al.* 2011) may undermine population recovery. Management strategies, such as the use of environmental water, may be critical to prevent or mitigate the impacts of these future stressor events (King *et al.* 2012; Kerr *et al.* 2013).

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Sustainability and Environment for funding this study, in particular Julia Reed and Jane Doolan for their encouragement and instigation of the project. The authors thank Charles Sturt University for the loan of the hoop nets, Kerry Whitworth, Darren Baldwin and Janice Kerr (Murray-Darling Freshwater Research Centre (MDFRC)) for the provision of DO data and Simon McDonald (Spatial Data Analysis Network, Charles Sturt University) for technical assistance with Figure 1. The manuscript was improved by valuable comment from Michelle Kavanagh (MDFRC), two reviewers and the editor. All sampling was conducted in accordance with NSW Fisheries Scientific Collection Permit P12/0009-1.0 and P12/0006-1.0.

REFERENCES

Alves N., Coughran J., Furse J. & Lawler S. (2010a) *Euastacus armatus*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1 [Cited 13 March 2013.] Available from URL: http://www.iucnredlist.org

Alves N., Merrick J. R., Kohen J. & Gilligan D. (2010b) Juvenile recruitment of the Murray crayfish, *Euastacus armatus* (Decapoda: Parastacidae), from south-eastern Australia. *Freshwater Crayfish* **17,** 167–75.

Anderson A. J., Gorley R. N. & Clarke K. R. (2008) *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E Ltd, Plymouth.

Anderson M. J. (2001) Permutation tests for univariate or multivariate analysis of variance and regression. *Can. J. Fish. Aquat. Sci.* **58,** 626–39.

Anderson M. J. & Robinson J. (2001) Permutation tests for linear models. *Aust. NZ J. Stat.* **43,** 75–88.

ANZECC (2000) *National Water Quality Management Strategy. Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. Australia and New Zealand Environment and

Conservation Council, and Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

Baldwin D. S. (1999) DOM and dissolved P leached from fresh and 'terrestrially'-aged River Red Gum leaves – implications for assessing river–floodplain interactions. *Freshwater Biol.* **41,** 675–85.

Baldwin D. S., Howitt J. & Edwards M. (2001) Blackwater event. *Austasia Aquac.* **15,** 21.

Bishop K. A. (1980) Fish kills in relation to physical and chemical changes in Magela Creek (East Alligator River System, Northern Territory) at the beginning of the tropical wet season. *Aust. Zool.* **20,** 485–500.

Clarke K. R. & Gorley R. N. (2006) *PRIMER V6: User Manual/Tutorial*. PRIMER-E Ltd, Plymouth, UK.

Demers A., Souty-Grosset C., Trouilhé M., Füreder L., Renai B. & Gherardi F. (2006) Tolerance of three European native species of crayfish to hypoxia. *Hydrobiologia* **560,** 425–32.

Eastburn D. (1990) The river. In: *The Murray* (eds N. Mackay & D. Eastburn) pp. 2–15. Murray–Darling Basin Commission, Canberra.

Fisheries Scientific Committee (2013) *Final Determination, the Murray Crayfish – Euastacus Armatus as a Vulnerable Species*. Fisheries Scientific Committee, NSW Department of Primary Industries, Sydney.

Fontenot Q. C., Rutherford D. A. & Kelso W. E. (2001) Effects of environmental hypoxia associated with the annual flood pulse on the distribution of larval sunfish and shad in the Atchafalaya River Basin, Louisiana. *Trans. Amer. Fish. Soc.* **130,** 107–16.

Furse J. M. & Coughran J. (2011) An assessment of the distribution, biology, threatening processes and conservation status of the freshwater crayfish, genus *Euastacus* (Decapoda, Parastacidae), in continental Australia. II. Threats, conservation assessments and key findings. In: A. Asakura (ed.), New frontiers in crustacean biology: Proceedings of the TCS Summer Meeting, Tokyo, 20–24 September 2009. *Crustaceana Monographs* **15,** 253–63.

Geddes M. C., Musgrove R. J. & Campbell N. J. H. (1993) The feasibility of re-establishing the River Murray crayfish, *Euastacus armatus*, in the lower River Murray. *Freshwater Crayfish* **9,** 368–79.

Gehrke P. C., Revell M. B. & Philbey A.W. (1993) Effects of river red gum, *Eucalyptus camaldulensis*, litter on golden perch, *Macquaria ambigua*. *J. Fish Biol.* **43,** 265–79.

Gilligan D., Rolls R., Merrick J., Lintermans M., Duncan P. & Kohen J. (2007) Scoping the knowledge requirements for Murray crayfish (*Euastacus armatus*). Report to the Murray-Darling Basin Commission. NSW Department of Primary Industries, Cronulla.

Green R. H. (1979) *Sampling Design and Statistical Methods for Environmental Biologists*. Wiley, Chichester.

Hamilton S. K., Sippel S. S. J., Calheiros D. F. & Melack J. M. (1997) An anoxic event and other biogeochemical effects of the Pantanal Wetland on the Paraguay River. *Limnol. Oceanogr.* **42,** 257–72.

Hladyz S., Watkins S. C., Whitworth K. L. & Baldwin D. S. (2011) Flows and hypoxic blackwater events in managed ephemeral river channels. *J. Hydrol.* **401,** 117–25.

Horwitz P. (1990) The conservation status of Australian freshwater crustacea. Report Series 14, Australian National Parks and Wildlife Service.

Horwitz P. (1995) The conservation status of Australian freshwater crayfish: review and update. *Freshwater Crayfish* **10,** 70–80.

Howitt J., Baldwin D., Rees G. & Williams J. (2007) Modelling blackwater: predicting water quality during flooding of lowland river forests. *Ecol. Modell.* **203,** 229–42.

Janzen D. H. (1974) Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica* **6,** 69–103.

Junk W. J., Bayley P. B. & Sparks R. E. (1989) The flood pulse concept in river–floodplain systems. *Can. Spec. Publ. Fish. Aquat. Sci.* **106,** 110–27.

Kerr J. L., Baldwin D. S. & Whitworth K. L. (2013) Options for managing hypoxic blackwater events in river systems: a review. *J. Environ. Manage.* **114,** 139–47.

King A. J., Tonkin Z. & Lieshcke J. (2012) Short-term effects of a prolonged blackwater event on aquatic fauna in the Murray River, Australia: considerations for future events. *Mar. Freshwater Res.* **63,** 576–86.

Leigh S. & Zampatti B. (2013) Movement and mortality of Murray cod (*Maccullochella peelii*) during overbank flows in the lower River Murray, Australia. *Aust. J. Zool.* **61,** 160–69.

McCarthy B. (2005) Distribution of Murray crayfish (*Euastacus armatus*) in the Mallee region 2004. Technical Report 2/2005. Murray-Darling Freshwater Research Centre, Mildura.

McCormack R. B. (2012) *A Guide to Australia's Spiny Freshwater Crayfish*. CSIRO Publishing, Canberra.

McKinnon L. & Shepheard N. (1995) Factors contributing to a fish kill in Broken Creek. *Vic. Natur.* **112,** 93–9.

McKinnon L. J. (1995) Emersion of Murray crayfish, *Euastacus armatus* (Decapoda: Parastacidae), from the Murray River due to post flooding hypoxia. *Proc. R. Soc. Vic.* **107,** 31–8.

McMahon B. R. & Wilkes P. R. H. (1983) Emergence responses and aerial ventilation in normoxic and hypoxic crayfish *Orconectes rusticus*. *Physiol. Zool.* **56,** 133–41.

McMaster D. & Bond N. (2008) A field and experimental study on the tolerances of fish to *Eucalyptus camaldulensis* leachate and low dissolved oxygen concentrations. *Mar. Freshwater Res.* **59,** 177–85.

McNeil D. G. & Closs G. P. (2007) Behavioural responses of a south-east Australian floodplain fish community to gradual hypoxia. *Freshwater Biol.* **52,** 412–20.

Meyer J. L. (1990) A blackwater perspective on riverine ecosystems. *Bioscience* **40,** 643–51.

Morris S. & Callaghan J. (1998) The emersion response of the Australian Yabby *Cherax destructor* to environmental hypoxia and the respiratory and metabolic responses to consequent air-breathing. *J. Comp. Physiol. B* **168,** 389–98.

Morrongiello J. R., Beatty S. J., Bennett J. C. *et al*. (2011) Climate change and its implications for Australian freshwater fish. *Mar. Freshwater Res.* **62,** 1082–98.

Nilsson G. E. & Ostlund-Nilsson S. (2008) Does size matter for hypoxia tolerance in fish? *Biol. Rev. Camb. Philos. Soc.* **83,** 173–89.

NSW DPI (2013) New Murray crayfish rules for 2013 [Cited 5 May 2013.] Available from URL: http://www.dpi.nsw.gov.au/aboutus/news/all/2013/murray-crayfish-rules

O'Connell M., Baldwin D. S., Robertson A. I. & Rees G. (2000) Release and bioavailability of dissolved organic matter from floodplain litter: influence of origin and oxygen levels. *Freshwater Biol.* **45,** 333–42.

Pianka E. R. (1970) On *r*- and *K*- selection. *Am. Nat.* **104,** 592–97.

Quinn G. P. & Keough M. J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

doi:10.1111/aec.12109

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna [Cited 8 October 2012.] Available from URL: http://www.R-project.org

Ryan K. (2005) *The home range behaviour of the Murray crayfish,* Euastacus armatus *(Decapoda: Parastacidae), in the Murrumbidgee River, Australian Capital Territory* (Unpublished Honours Thesis). University of Canberra, Canberra.

Taylor E. W. & Wheatly M. G. (1981) The effect of long-term aerial exposure on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotambius pallipes*. *J. Exp. Biol.* **92,** 109–24.

Thorp J. H., Thoms M. C. & Delong M. D. (2008) *The Riverine Ecosystem Synthesis: Toward Conceptual Cohesiveness in River Science*. Elsevier, Amsterdam.

Townsend S. A. & Edwards C. A. (2003) A fish kill event, hypoxia and other limnological impacts associated with early wet season flow into a lake on the Mary River floodplain, tropical northern Australia. *Lakes Reserv. Res. Manage.* **8,** 169–76.

Valett H. M., Baker M. A., Morrice J. A. *et al.* (2005) Biogeochemical and metabolic responses to the flood pulse in a semiarid floodplain. *Ecology* **86,** 220–34.

van Dijk A. I. J. M., Beck H. E., Crosbie R. S. *et al.* (2013) The Millennium Drought in southeast Australia (2001–2009): natural and human causes and implications for water resources, ecosystems, economy, and society. *Water Resour. Res.* **49,** 1040–59.

Victorian DEPI (2013) *Victorian Recreational Fishing Guide 2013*. Victorian Department of Environment and Primary Industries (DEPI), Melbourne.

von Martens E. (1866) On a new species of Astacus. *Ann. Mag. Nat. Hist.* **17,** 359–60.

Walker K. F. (1982) The plight of Murray crayfish in South Australia. Red gum: conservation & environment in S.A. 6, 2–6. Conservation Council of South Australia, Adelaide. *Redgum (Conservation Council of South Australia)* **6,** 2–6.

Walker K. F. (2006) Serial weirs, cumulative effects: the Lower River Murray, Australia. In: *Ecology of Desert Rivers* (ed. R. T. Kingsford) pp. 248–79. Cambridge University Press, Cambridge.

Wheatly M. G., de Souza S. C. R. & Hart M. K. (1996) Related changes in hemolymph, acid-base status, electrolytes and ecdysone in intermoult crayfish (*Procambarus clarkii*) at 23°C during extracellular acidosis induced by exposure to air, hyperoxia or acid. *J. Crust. Biol.* **16,** 267–77.

Whitworth K. L. & Baldwin D. S. (2012) Blackwater in the southern Murray-Darling Basin during 2012 flood events. Report prepared for the Murray-Darling Basin Authority by The Murray-Darling Freshwater Research Centre, MDFRC Publication 16/2012.

Whitworth K. L., Baldwin D. S. & Kerr J. L. (2012) Drought, floods and water quality: Drivers of a severe hypoxic blackwater event in a major river system (the southern Murray–Darling Basin, Australia). *J. Hydrol.* **450–451,** 190–98.

Zukowski S. (2012) *Impacts of fishing regulations on the sustainability of Murray crayfish (*Euastacus armatus*), Australia: Social and biological perspectives* (PhD Thesis). Charles Sturt University, Albury.

Zukowski S., Watts R. & Curtis A. (2012) Linking biology to fishing regulations: Australia's Murray crayfish (*Euastacus armatus*). *Ecol. Manage. Restoration* **13,** 183–90.

doi:10.1111/aec.12109