Zooplankton response to flooding of a drought refuge and implications for the endangered fish species *Craterocephalus fluviatilis* cohabiting with alien *Gambusia holbrooki*

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Abstract Disruption to a river's natural flow regime changes its ecological character, which becomes unfavourable for previously adapted biota. The zooplankton particularly are affected, and survival of larval and juvenile fish is largely determined by their availability. Alien fishes can also impact on recruitment in native fishes, sometimes through competition. In this regard, the invasive eastern Gambusia *Gambusia holbrooki* is linked to the decline of several fish species. It can have a substantial influence in shaping plankton communities, which implies that it competes with native fish that rely on the microfauna. The effects of river regulation and over abstraction of water in the Murray–Darling Basin, south-eastern Australia, were exacerbated by drought from 1997 to 2010. Consequently, the endangered Murray hardy-head *Craterocephalus fluviatilis* underwent substantial population decline and extirpations. The purpose of this study is to determine if a link exists between zooplankton response to flooding of a drought refuge and the recruitment success of *C. fluviatilis* in the presence of *G. holbrooki*. Flooding triggered sharp and substantial increases in the zooplankton and their eggs, which was the sole food of *C. fluviatilis*. This apparently benefitted the recruitment of *C. fluviatilis*, and sometimes alleviated diet overlap with *G. holbrooki*. Conversely, the zooplankton in a nearby non-flooded refuge was low in abundance and diversity, and all fish species were extirpated. The findings indicate that the flooding of drought refugia with relatively small volumes of water can be timed with ecological cues that would otherwise be desynchronized in highly regulated rivers, particularly during drought.

Keywords Diet · Recruitment · Small-bodied fish · Salinization · Murray–Darling Basin

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Introduction

Flow regimes of unmodified rivers correspond with climatic variation and are defined by substantial variability in the volume, timing and duration of flow, which are major ecological drivers (Ward et al. 1999). Disruptions to natural flow regimes change a river's ecological character. For example, reductions in the natural frequency of flooding are unfavourable for some zooplankton species (Boulton and Lloyd 1992), particularly those without a dormant stage (Hairston 1996). Similarly, flow modification can influence recruitment of aquatic macrophytes on floodplains (Nielsen et al. 2007), and impact on spawning cues for fish (e.g. Franssen et al. 2007). Fish are sensitive to the

effects of altered flow regimes, particularly during drought (Matthews and Marsh-Matthews 2003), so are ideal biological indicators.

Zooplankton are important prey for early life stages of fish and play a key role in their recruitment (Miller et al. 1990; Bremigan and Stein 1997). Shifts in zooplankton assemblages can be attributed to predation by fish and invertebrates (e.g. Boulton and Lloyd 1992), but abiotic factors often are influential (e.g. salinization: Nielsen et al. 2007). Many zooplankton species are adapted to variable climate and intermittent flooding, which often includes a stage of propagule dormancy in dry sediments (Shiel et al. 1998). The zooplankton on floodplains also display rapid succession after flooding, typically moving from a rotifer-dominated assemblage to one dominated by the larger cladocerans (Jenkins and Boulton 2003). Notably, early life development in local fish populations often coincides with zooplankton succession, whereby young-of-the-year (YOY) fish can increasingly forage on larger prey to maximize energy intake (Maazouzi et al. 2011). The nature of zooplankton, such as their sensitivity to shifts in water level and salinity, makes them ideal for ecological studies (Lampert 1997).

To sustain populations, short-lived (<2 years) fish species rely heavily on annual replacement of adults through the successful recruitment of YOY cohorts. The survival rate of larval and juvenile stages is intrinsically linked to recruitment levels and is largely determined by food availability (starvation) and predation (Houde 1987; Bremigan and Stein 1997). Certainly, breeding by some fish species is triggered by flooding that increases zooplankton abundance (Cushing 1990). Hence, it is important to examine the main prey of YOY fish to determine the underlying factors influencing survivorship and recruitment (Bremigan and Stein 1994). Intuitively, this also requires an understanding of how zooplankton assemblages respond to environmental change.

Alien fishes can also have an impact on recruitment of native fishes, mostly through predation or competition for food (Ross 1991). The invasive eastern Gambusia *Gambusia holbrooki* is linked to the decline of several fish species through competition for prey and its aggressive nature (Pyke 2008). For example, it can have a substantial influence in shaping plankton communities (Margaritora et al. 2001), which implies that it competes with native fishes that rely on the microfauna. The species can endure extremes in dissolved oxygen

(1–11 mg L^{-1}), salinity (0–41 g L^{-1}), pH (4.5–9) and temperature (0–45 °C) and has a high reproductive potential (Pyke 2008). Notably, the invasiveness of *G. holbrooki* is limited at high salinity (>18 g L^{-1}: Alcaraz and García-Berthou 2007), which can reduce its impact on euryhaline fishes (e.g. Alcaraz et al. 2008).

Rivers in the Murray–Darling Basin (MDB), southeastern Australia, had naturally sporadic seasonal flows prior to major channel modifications from 1920 to 1940 (Walker 2006). The 2,560-km-long River Murray is now intensively regulated by several large capacity reservoirs in the headwaters, and eleven weirs from just above the Darling River confluence to the mouth. The effects of river regulation and over abstraction of water on MDB ecosystems are substantial (Walker 2006) and were exacerbated by drought from 1997 to 2010 (Murphy and Timbal 2008). For example, rapid drawdown of water levels in lakes at the terminus of the MDB resulted in habitat disconnection, salinization and drying (Wedderburn et al. 2012). Consequently, ecological specialists declined in small-bodied (<10 cm total length) fish assemblages, especially diadromous and threatened freshwater species (Wedderburn et al. 2012).

The benefits of watering floodplains for the recruitment of some threatened fishes are notable (e.g. Tonkin et al. 2008). However, the specific influential factors often are unidentified, but likely relate to improvements in aquatic plant populations that provide shelter and spawning substrate (e.g. Nielsen et al. 2007), and increased prey abundance (e.g. zooplankton emergence from sediments: Boulton and Lloyd 1992). In the MDB, most threatened fish species are unstudied in this regard, including the endemic, small-bodied Murray hardyhead *Craterocephalus fluviatilis*. The species is *Endangered* under the International Union for Conservation of Nature's Red List of Threatened Species due to substantial population decline and localized extirpations over recent decades.

The reasons for the decline of *C. fluviatilis* are uncertain, but might relate to extreme salinization and interactions with alien fishes, including redfin perch *Perca fluviatilis* and *G. holbrooki* (Lintermans 2007). It has a scattered distribution in the River Murray, often cohabiting in saline (>7 g L^{-1}) off-channel sites only with *G. holbrooki* and flathead gudgeon *Philypnodon grandiceps* (Wedderburn et al. 2007). The reasons for its absence in freshwater habitats (<0.4 g L^{-1}), where fish species diversity is greatest, remain unclear, but

predation and competition warrant investigation (Wedderburn et al. 2008). Although the species can have an extended breeding-recruitment period (September–April) under favourable conditions, *C. fluviatilis* mostly reproduces in late spring–early summer (September–November) and lives for 1–2 years (Lintermans 2007).

The purpose of this study is to determine if a link exists between zooplankton response to flooding and the recruitment success of *C. fluviatilis* whilst cohabiting with *G. holbrooki* in a drought refuge. Given that newly inundated habitat apparently promotes the abundance of *C. fluviatilis* (S. Wedderburn, unpublished data), we tested the proposition that flooding increases the abundance and diversity of zooplankton prey that benefits its recruitment. Based on the premise that *C. fluviatilis* is impacted by *G. holbrooki* because of competition for food, we also tested the proposition that they consume the same zooplankton prey. During drought, sampling was conducted at an isolated site in Boggy Creek near the terminus of the River Murray, which received environmental water allocations on two occasions to conserve one of the few remaining populations of *C. fluviatilis*. Comparisons were made with a nearby unmanaged isolated waterbody on Mundoo Island, which was also inhabited by *C. fluviatilis* and *G. holbrooki*. This study also interprets the importance of research to enable proper planning for water use in regulated catchments where the frequency and intensity of drought are forecast to increase over coming decades (Lake and Bond 2007).

Materials and methods

Study sites

The MDB covers 1,063,000 km^2 and discharges into Lake Alexandrina and Lake Albert (750 km^2) before flowing through five tidal barrages into the estuary (Eastburn 1990). Prior to a severe drought from 1997 to 2010, barrages were operated to hold lake water levels at approximately 0.75 m Australian Height Datum (AHD). During the drought, lake water levels lowered to −0.6 m AHD and littoral, off-channel waterbodies fringing the lakes dried or salinized following disconnection (Wedderburn et al. 2012).

'Boggy' is a small channel (ca. 5 m × 200 m) that branches off Boggy Creek on Hindmarsh Island (Fig. 1). The site received environmental water allocations (EWAs), via pumping overland from Lake Alexandrina, on two occasions during the study: 0.68 ML from 1 to 6 December 2009 and 5.30 ML over 3 weeks commencing on 27 January 2010. Situated approximately 4 km from Boggy, 'Mundoo' is a small unmanaged waterbody (ca. 5 m × 50 m) in a channel system on Mundoo Island. The study sites became isolated in the summer of 2006–2007 due to water level recession in Lake Alexandrina, but remained wetted by local rainfall and ground water. Boggy and Mundoo had comparable environmental characteristics (e.g. water chemistry, depth) at the beginning of the study, but conditions later differed as a result of their distinct hydrological regimes.

Water quality and nutrients

Water quality parameters were measured in the middle of the day using WP-81 and WP-84 metres (TPS Pty Ltd., Brisbane, Australia): salinity (g L^{-1}), pH and temperature (°C). Water levels were monitored using permanent gauge sticks. Chlorophyll-*a* (Chl-*a*) was measured at each site by taking 3 × 600 mL samples of water from 30 cm below the surface. Water samples were filtered in the laboratory, and Chl-*a* concentrations were determined by measuring absorption at 665 and 750 nm with a U2000 spectrophotometer (Hitachi, Tokyo, Japan). Nutrient concentrations were determined for samples collected on 8 December 2009, 19 January 2010, 19 February 2010 and 19 March 2010: Ammonia as N, Nitrate + Nitrite as N, Total Nitrogen (TKN), Filterable Reactive Phosphorus as P (filtered through 0.22 μm) and Total Phosphorus.
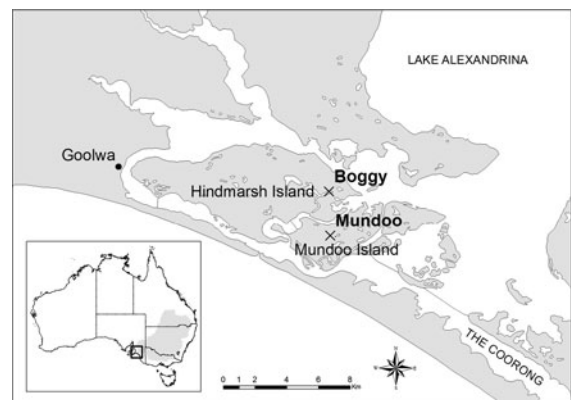

Fig. 1 Location of Boggy and Mundoo study sites

Zooplankton

Quantitative and qualitative zooplankton samples were collected on 4 November 2009, 8 December 2009 (after the first EWA) and monthly thereafter: 18 December 2009, 19 January 2010, 19 February 2010 (after the second EWA) and 19 March 2010. Zooplankton were collected using a 4 L Haney trap (three collections to attain a 12 L sample) for a quantitative count and with 3×5 m tows (36 μm tow net) and 5 min sweeping among vegetation with a Frey net (36 μm) for qualitative examination.

Qualitative zooplankton samples were allowed to settle, and the entire settled volume was pipetted using a wide bore 10-mL glass bulbed pipette into a 125-mm-square gridded Greiner tray. Gentle agitation distributed the settled volume across the tray. The tray was scanned row-by-row on a dark-field dissecting microscope. All identifiable zooplankters were enumerated and identified until 200–300 individuals were encountered, which is considered an adequate number to provide a reliable percentage composition from randomly distributed plankton and to feasibly identify the majority of taxa collected in the sample (Elliot 1977). The remainder of the tray was scanned for any missed species, which were recorded as *present*.

Quantitative samples (12 L volumes concentrated in the field to 200 mL in 70 % ethanol solution, returned to the lab in 200-mL PET bottles) were decanted into a 200-mL measuring cylinder, and the volume was recorded. The cylinder was then capped with Parafilm, inverted three times to distribute the contents and a 1-mL Gilson pipette sample taken from the centre of the agitated solution. The sample was run into a Pyrex gridded Sedgewick-Rafter cell, the contents counted in their entirety on a compound microscope and zooplankters identified using keys in Shiel (1995). The number of zooplankton carrying eggs was also recorded. The density of zooplankton in the 1 mL aliquot was multiplied by the sample volume to provide an estimate of the density in the volume (zooplankton L^{-1}).

Fish

To assess relative abundance of all fishes and the recruitment of *C. fluviatilis*, fish were sampled at Boggy and Mundoo in November 2009 and March 2010 using three 6-m single-leader fyke nets (5-mm half mesh) set overnight, 10 m apart and perpendicular to the bank. Given the environmental similarities of the two study sites in November, fyke nets were set in comparable physical habitats (e.g. depth, density of submerged aquatic vegetation), then at the same locations in March. All fish were identified to species (Lintermans 2007) and counted. The total lengths (TL) of *G. holbrooki* and *C. fluviatilis* were measured. Given the endangered status of *C. fluviatilis*, only the TL of up to the first 50 fish from each fyke net were measured so that timely release prevented mortality.

For dietary analyses, ten *C. fluviatilis* were collected from Boggy on 19 January and 19 March using a seine net (2×1 m, 1 mm mesh), killed and preserved in 70 % ethanol. Seine shots were used only to collect fish for dietary analyses (i.e. not combined with the fyke net data) and were repeated in the littoral zone until the required number of fish was collected. In the first 1 cm of the foregut (items unrecognizable beyond this point), the zooplankton prey was identified to species and the total numbers recorded. The same process was conducted for *G. holbrooki*, but the entire gut was analysed. The low abundance of *G. holbrooki* early in the study (seasonal pattern) meant that only seven fish were collected in January, but 10 fish were collected in March.

Data analyses

The estimated zooplankton densities from quantitative trap samples, for each site on each sampling occasion, were plotted to compare the percentage composition by number of each major zooplankton group present. Species richness for each site on each sampling occasion was examined from the Haney trap, tow net and Frey net samples. Fish dietary data (numbers of each zooplankton item) were analysed by Non-metric Multi-dimensional Scaling (NMS) ordination using the Relative Sørensen distance metric, in PC–ORD (ver. 4.36, McCune and Mefford 2002). The data were also plotted to compare the percentage composition by number of each prey item for *C. fluviatilis* and *G. holbrooki*. Levels of dietary overlap between *C. fluviatilis* and *G. holbrooki* were tested using Schoener's index (based on counts of prey items), which provides a relative measure of extent to which the same prey items are used (Bowen 1996). Overlap values range from 0 (no overlap) to 1 (complete overlap), and >0.6 is biologically significant (Wallace and Ramsey 1983). The levels of prey

Fig. 2 Salinity (**a**), total nitrogen concentration (**b**) and Chl-*a* ▶ concentration with standard error bars (**c**) at Boggy and Mundoo (*dashed lines* represent timing of EWAs at Boggy)

selectivity for *C. fluviatilis* and *G. holbrooki* were determined using the Strauss index, where values range from −1 (perfect selection against a prey type) to 1 (perfect selection for a prey type) (Bowen 1996). Recruitment in *C. fluviatilis* was examined using length–frequency charts.

Results

Water quality

Salinity in Boggy was 17.40 g L^{-1} (water temperature = 23.7 °C) in November 2009, but was reduced to 5.98 g L^{-1} (16.1 °C) by the first EWA in December 2009 (Fig. 2a). Salinity then increased to 17.90 g L^{-1} (23.7 °C) prior to the second EWA in January 2010, after which it remained at 8.67–10.48 g L^{-1} (21.4–23.1 °C) for the remainder of the study. Salinity in Mundoo continually increased from 8.84 to 55.30 g L^{-1} (16.7–23.7 °C, respectively) during the study. Water levels at the gauge fluctuated between 0 and 50 cm at Boggy, and the EWAs prevented the site from drying. Water levels at the gauge in Mundoo continuously declined from 49 cm in November to 6 cm in March.

Concentrations of ammonia (≤0.03 mg L^{-1}), nitrate + nitrite (≤0.01 mg L^{-1}), filterable reactive phosphorus (≤0.07 mg L^{-1}) and total phosphorus (≤0.45 mg L^{-1}) remained low in both sites throughout the study. Total nitrogen concentrations remained relatively constant in Boggy during the study, with a peak in January (6.43 mg L^{-1}) prior to the second EWA (Fig. 2b). Conversely, there was a sharp increase to an extremely high total nitrogen concentration (16.90 mg L^{-1}) in Mundoo between December and February.

Chl-*a* concentrations generally remained below 30 µg L^{-1} in both sites (Fig. 2c), with three exceptions. First, it was relatively high in Boggy on 19 January 2010 (mean = 31.47 µg L^{-1}) but had dropped considerably a month later following the second EWA (mean = 6.26 µg L^{-1}). Second, although the 5.3 ML EWA prior to 19 February apparently reduced Chl-*a* concentration (mean <10 µg L^{-1}) in Boggy, it peaked in March 2010
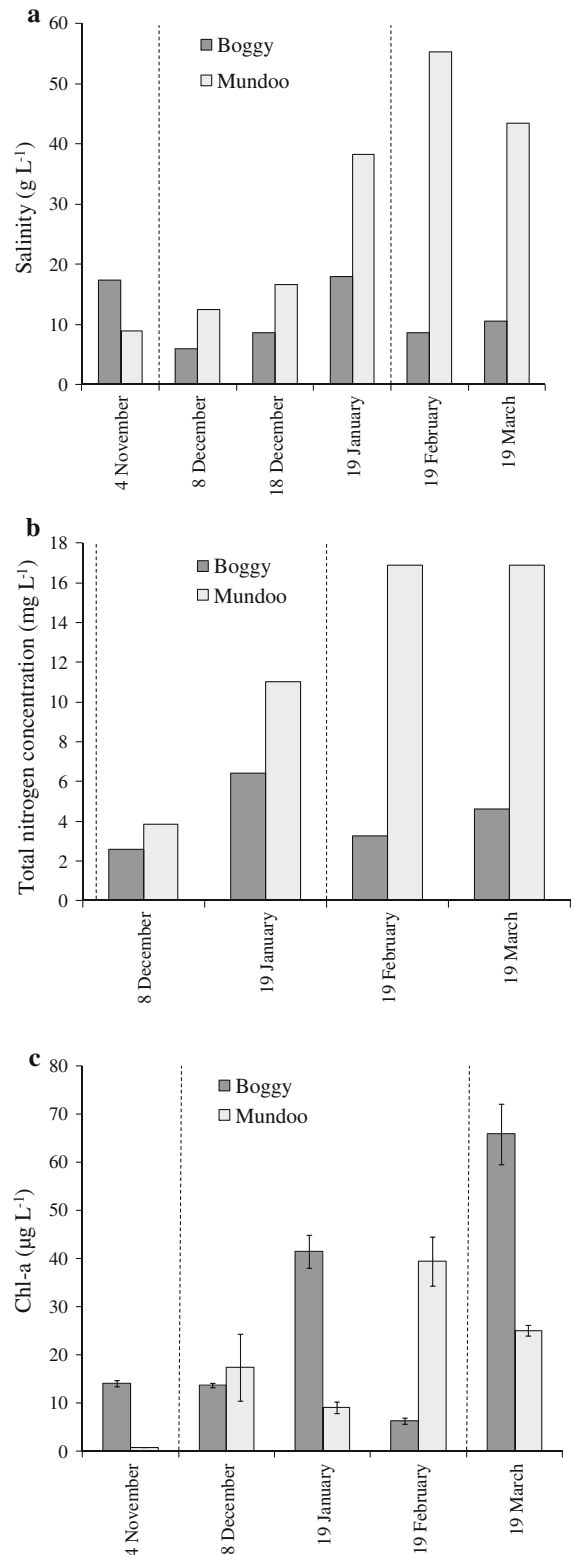
Table 1 Zooplankton taxon recorded (•) in the Boggy Creek (flooded) and Mundoo Island (unmanaged) study sites using quantitative (Q) and (QU) qualitative sampling techniques

Taxon	Boggy		Mundoo	
	Q	QU	Q	QU
Protista				
Ciliophora				
cf. *Cothurnia* sp.	–	–	•	–
Indeterminate ciliate	•	•	–	•
Arcellidae				
Arcella sp.1	•	•	•	•
Arcella cf. *hemisphaerica*	–	•	•	•
Cyphoderiidae				
Cyphoderia sp.	•	–	–	–
Foraminifera				
sp.1	–	•	–	–
Rotifera				
Bdelloidea				
Indeterminate bdelloid	•	•	–	•
Brachionidae				
Brachionus ibericus	•	•	–	–
B. plicatilis	•	•	–	•
Brachionus quadridentatus cluniorbicularis	–	–	–	•
Brachionus rotundiformis	•	•	–	•
Epiphanidae				
Proalides sp.	•	•	–	•
Hexarthridae				
Hexarthra brandorffi	•	•	–	•
Hexarthra fennica	•	•	–	•
Lecanidae				
Lecane sp.	–	•	–	–
Lecane cf. *obtusa*	•	–	–	–
Lecane thalera	•	–	•	•
Lecane luna	–	–	–	•
Lepadellidae				
Colurella adriatica	•	•	•	•
Lepadella sp.	•	–	–	–
Proalidae				
Proales sp.	•	–	–	–
Trichocercidae				
Trichocerca sp.	•	•	–	–
Trichosphaeriidae				
Filinia sp.	–	•	–	–
Indeterminate rotifer	•	–	•	•
Copepoda				
Calanoida				
Gladioferens pectinatus	–	•	–	–
Sulcanus conflictus	•	•	–	–
Cyclopoida				
Halicyclops cf. *ambiguus*	•	•	–	•
Mesocyclops sp.	•	•	•	•

Table 1 continued

Taxon	Boggy		Mundoo	
	Q	QU	Q	QU
Harpacticoida				
Mesochra sp.1	•	•	•	•
Mesochra sp.2	–	•	–	•
Cladocera				
Chydoridae				
Alona sp.	–	–	–	•
Daphniidae				
Daphnia carinata	–	•	–	–
Macrotrichidae				
Macrothrix sp.	–	–	•	•
Ostracoda				
Australocypris sp.	–	•	–	–
cf. *Diacypris* sp.	•	•	–	•
cf. *Heterocypris* sp.	–	•	–	–
Limnocythere sp.	–	•	–	•
Indeterminate juvenile	•	•	•	•

(mean = 65.79 µg L^{-1}). Third, Chl-*a* increased sharply in Mundoo between January and February (mean = 9.04 and 39.38 µg L^{-1}, respectively) before decreasing by March 2010 (mean = 25.02 µg L^{-1}).

Zooplankton

Approximately 44 zooplankton taxa were recorded (Table 1). Rotifers dominated (>60 %) the zooplankton assemblage in Boggy for the majority of the study, except ostracods were the most abundant group in March 2010 (Fig. 3a). Many rotifers (42 %) carried eggs immediately after the first EWA, and many free-floating eggs were present, thereby demonstrating that parthenogenetic egg production and sexual reproduction had occurred. Although rotifers dominated the zooplankton assemblage at Mundoo in November 2009 (>60 %, none carrying eggs), protists dominated (>60 %) thereafter (Fig. 3b). There was a ciliate (Protista) bloom in January 2010, which accounts for the highest density of zooplankton recorded at Mundoo (2,605 zooplankton L^{-1}).

Zooplankton density increased substantially in Boggy following the first EWA and remained relatively high throughout the study until a reduction between February and March (Fig. 4a). Conversely, zooplankton densities at Mundoo remained
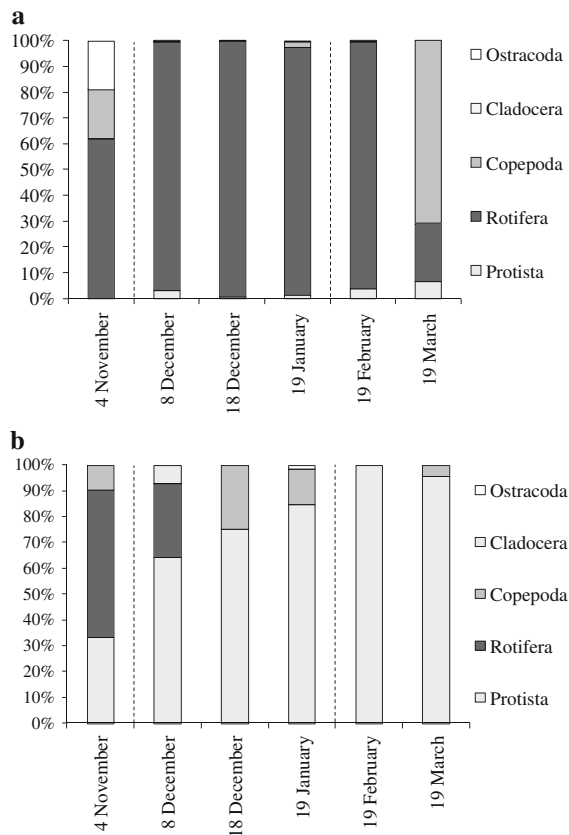
Fig. 3 Proportions of the five zooplankton groups in samples from Boggy (**a**) and Mundoo (**b**) (*dashed lines* represent timing of EWAs at Boggy)

Fig. 4 Estimated zooplankton densities (**a**) and number of zooplankton species in samples (**b**) at Boggy and Mundoo (*dashed lines* represent timing of EWAs at Boggy)

consistently low, but a moderate increase was apparent in January before declining for the remainder of the study.

The number of zooplankton species was lower in samples from Boggy in November 2009 compared to samples from Mundoo (Fig. 4b). Following the first EWA in early December, the number of zooplankton species remained higher in samples from Boggy compared to Mundoo for the remainder of the study. The number of zooplankton taxa peaked at 18 species in samples from Boggy in February 2010 following the second EWA. Conversely, the number of zooplankton taxa in samples from Mundoo steadily declined from 14 to four species during the study.

Significantly, a possible new ostracod genus near *Heterocypris* (Ostracoda: Cyprididae) was captured at Boggy, but this requires confirmation through a taxonomic investigation. This study also identified the first record for the halophile rotifer *Hexarthra*
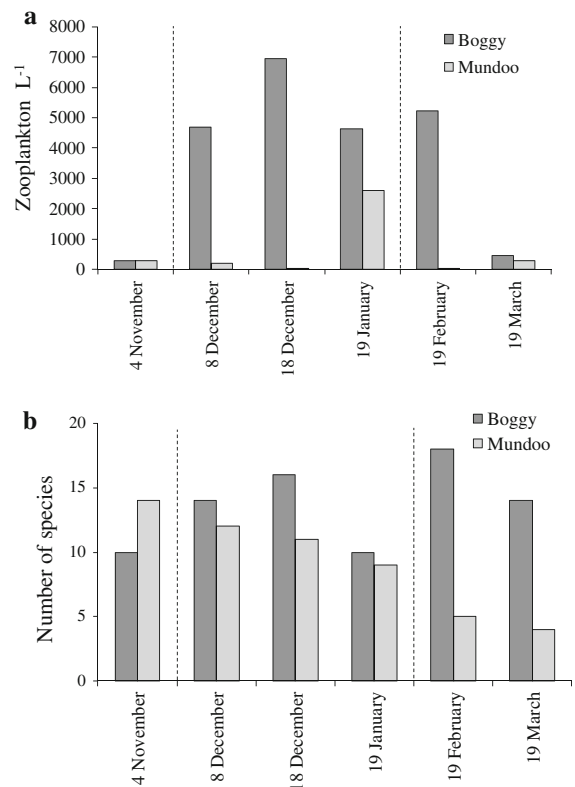
brandorffi Koste, 1977 (Rotifera: Hexarthridae) in Australia. Further, several zooplankton species could not be referred to known taxa and now require formal taxonomic description (R. Shiel, unpublished data).

Fish assemblages

Relatively low numbers of *C. fluviatilis*, *G. holbrooki* and western blue-spot goby *Pseudogobius olorum* were captured at Boggy in November 2009 (Table 2). By March 2010, *C. fluviatilis* was moderately abundant, and *G. holbrooki* was prolific and in breeding condition, but no other fish species were recorded. A large proportion of YOY *C. fluviatilis* was apparent in November 2009, and an adult cohort was evident in March 2010, indicating successful recruitment (Fig. 5). An abundant adult population of *C. fluviatilis* inhabited Mundoo in November 2009, but YOY were absent (Fig. 6). Other fish species captured at Mundoo in November 2009 were extremely high numbers of

Table 2 Number of each fish species recorded at the study sites in November (spring) and March (autumn)

Fish species	Boggy		Mundoo	
	November	March	November	March
C. fluviatilis	16	98	162	0
A. microstoma	0	0	785	0
P. olorum	39	1	20	0
G. holbrooki	7	334	29	0

smallmouth hardyhead *Atherinosoma microstoma*, which included YOY, and low numbers of *P. olorum* and *G. holbrooki*. Despite considerable sampling effort with the seine net (>10 × 5 m hauls), a single *G. holbrooki* was the only fish captured at Mundoo in January. Thereafter, no fish were captured at Mundoo during the study.

Fish diets

There was no dietary overlap between *C. fluviatilis* and *G. holbrooki* in January, but overlap was significant in March (Schoener's index value = 0.30 and 0.79, respectively). In January, 31 % of food items in *C. fluviatilis* (mean TL = 28.5 mm [range = 22.0–31.5 mm]; mean gape = 2.14 mm) were harpacticoids (*Mesochra* spp.), which constituted only 10 % of its diet in March (Fig. 7). Copepod eggs comprised 37 % of its food items in January, but were absent from March samples. In March, the diet of *C. fluviatilis* (mean TL = 50.2 mm [range = 42.0–58.0 mm]; mean gape = 2.87 mm) was predominantly rotifers (83 %), mostly the halophile *Brachionus plicatilis* and its eggs. In January, *C. fluviatilis* actively selected for adult harpacticoids (Strauss index value = 0.27), and the eggs of rotifers (0.24) and copepods (0.37), whilst strongly selecting

against adult rotifers (−0.89) (Table 3). In March, *C. fluviatilis* actively selected rotifers (0.25) and their eggs (0.36), whilst selecting against copepod nauplii (−0.53).

In January, *G. holbrooki* (mean TL = 32.0 mm [range = 22.5–50.1 mm]; mean gape = 3.16 mm) consumed moderate numbers of predominantly harpacticoid adults (33 % of diet) and eggs (59 %). In March, *G. holbrooki* (mean TL = 40.0 mm [range = 33.0–56.5 mm]; mean gape = 3.42 mm) consumed higher numbers of rotifers (28 %) and their eggs (50 %). In January, the species actively targeted adult harpacticoids (Strauss index value = 0.33) and their eggs (0.59), whilst strongly selecting against adult rotifers (−0.96). In March, *G. holbrooki* actively selected rotifer eggs (0.50), whilst selecting against copepod nauplii (−0.53).

The dietary data yielded a two-dimensional ordination (stress 16 %), indicating two distinct groups for *C. fluviatilis* and two scattered groups for *G. holbrooki* (Fig. 8). The plot displays partial overlap of *G. holbrooki* and *C. fluviatilis* in March, which corresponds to the significant Schoener's index value. Multi-response permutation procedure confirmed that the four groups are significantly different ($P < 0.001$). Corixidae is the prey most strongly associated with fish diet on Axis 2 (correlation between salinity and axis score: $r = 0.69$), towards two outlying *G. holbrooki* sampled in January 2010. There is a contrary trend for harpacticoid nauplii ($r = −0.41$) and copepod eggs ($r = −0.41$), towards all *C. fluviatilis* collected in January 2010. Rotifer adults ($r = 0.52$) and eggs ($r = 0.59$) and ostracods ($r = 0.32$) display a correlation along Axis 1, towards all *C. fluviatilis* and three *G. holbrooki* sampled in March 2010. Conversely, terrestrial insects ($r = −0.40$) are correlated with *G. holbrooki* sampled on both occasions.

Fig. 5 Length–frequency distributions of *C. fluviatilis* captured at Boggy in November 2009 (*left*; $n = 15$) and March 2010 ($n = 74$)
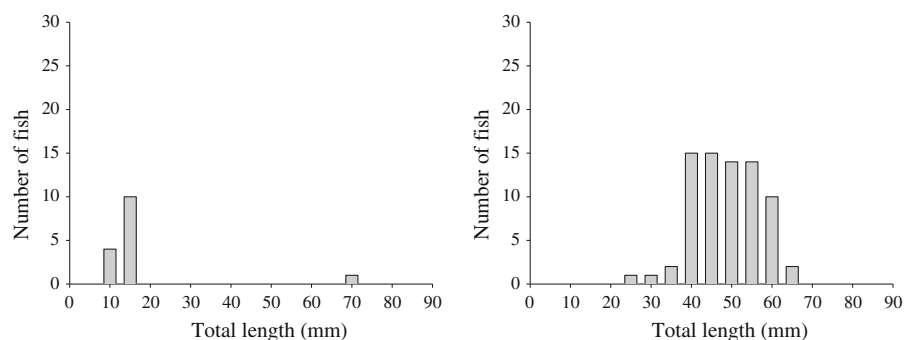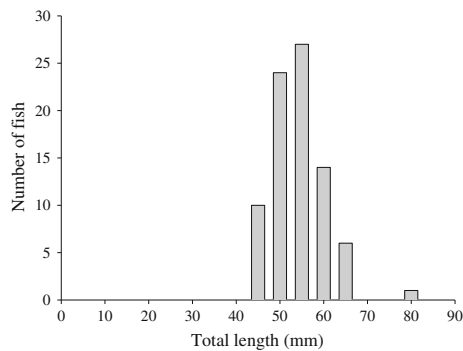
Fig. 6 Length–frequency distribution of *C. fluviatilis* captured at Mundoo in November 2009 (*n* = 82)

Fig. 7 Proportions of major food items in *C. fluviatilis* and *G. holbrooki* at Boggy

Discussion

Flooding of the receding drought refuge using environmental water allocations apparently triggered sharp and substantial increases in the zooplankton, which was the sole food source of *C. fluviatilis*. Concurrently, *C. fluviatilis* underwent successful recruitment. In contrast, zooplankton densities in the unwatered refuge were always lower and diversity decreased steadily throughout the study, whilst all fish species were extirpated. Therefore, our findings support the proposition that flooding of drought refugia promotes diversity and abundance of the zooplankton, which benefits recruitment in *C. fluviatilis*. Although our study was limited to a comparison of only two environmentally similar sites, the contrast between recruitment outcomes for *C. fluviatilis* is clear.

Decline in the zooplankton at Mundoo coincided with deterioration of water quality during evaporation, including extreme salinization and high total organic nitrogen levels. The sub-lethal effects of salinization are difficult to assess, but given that *C. fluviatilis* has a tolerance to high salinity (>85 g L^{-1}: Wedderburn et al. 2008) it was unlikely to be the direct cause of mortality. Instead, starvation was possibly a key factor contributing to its extirpation. In Mundoo, its preferred zooplankton prey, confirmed in gut samples from Boggy, consistently occurred at low densities or were absent. The shortage of prey was possibly exacerbated by the high abundance of fish in Mundoo during the early stages of the study, especially *A. microstoma*, which has a similar diet and upper salinity tolerance to *C. fluviatilis* (Lintermans 2007; Wedderburn et al. 2008). Dissolved oxygen was not measured in this study, but low levels can eliminate fish species that are
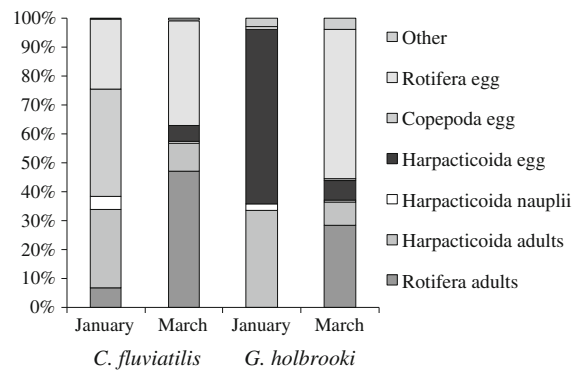
unable to respire at the water's surface and those that can (e.g. *G. holbrooki*) if their energetic requirements are not met (McNeil and Closs 2007). Therefore, it is possible that anoxia was a factor in addition to starvation or was solely responsible for the extirpation of all fish species in Mundoo.

Flooding in Boggy apparently triggered the hatching of zooplankton from the sediment egg bank, which led to their reproductive boom. The desiccation-resistant (resting) eggs of zooplankton can persist in sediments until rewetted, sometimes for decades. Resting egg densities on the River Murray floodplain can exceed 1,200 eggs per cubic centimetre of sediment (Shiel et al. 2001), and similar findings have been made in other parts of the world (e.g. Europe, Japan: Hairston 1996). Importantly, a variety of
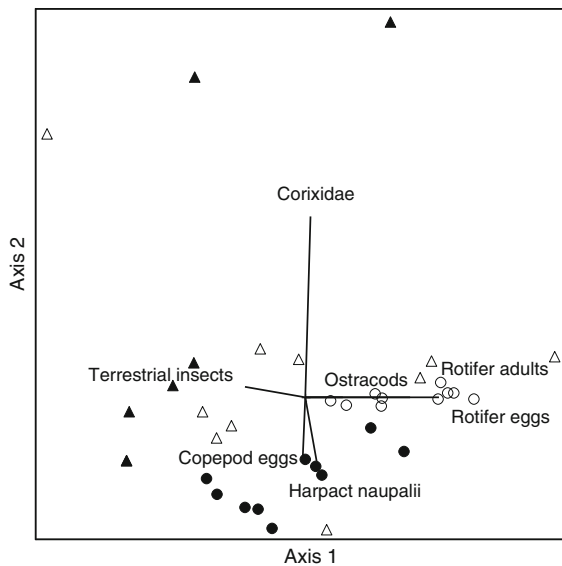
Table 3 Selectivity index values for the main prey of *C. fluviatilis* and *G. holbrooki* in Boggy

Prey item	*C. fluviatilis*		*G. holbrooki*	
	January	March	January	March
Rotifera adults	−0.89	0.25	−0.96	0.06
Rotifera eggs	0.24	0.36	0.01	0.50
Harpacticoida adults	0.27	0.09	0.33	0.08
Harpacticoida nauplii	0.05	0.01	0.02	0.01
Harpacticoida eggs	0	0.05	0.59	0.07
Copepoda adults	0	0.02	0	0.03
Copepoda eggs	0.37	0	0	0.01
Copepoda nauplii	−0.02	−0.53	−0.02	−0.53
Ostracoda adults	0	0.01	0	0.01
Corixidae	0	0	0.01	0
Terrestrial insects	0	0	0.02	0

Fig. 8 Ordination with correlations for dietary items overlaid (stress 0.16), and fish species indicated for *C. fluviatilis* in January (*filled circle*) and March (*open circle*), and *G. holbrooki* in January (*filled triangle*) and March (*open triangle*)

hydrological patterns (wet and dry phases) are required in off-channel sites to maintain zooplankton diversity and the egg bank (Brock et al. 2003). Given that the highest rates of mortality in fishes occur at the larval stage (Shan et al. 2009), and this has a direct link to recruitment success (Bremigan and Stein 1994), our findings suggest that zooplankton egg banks can be utilized for the benefit of early life stages of endangered fishes during prolonged drought by infrequently flooding at the appropriate seasonal timing. This appears to be relevant especially for *C. fluviatilis* in the presence of halophilic rotifers.

Salinities recorded in the current study were high compared with early drought conditions (<2 g L^{-1}: Wedderburn et al. 2007; Wedderburn et al. 2012). There is an apparent link between increasing salinity and reduced recruitment levels for *C. fluviatilis* in an off-channel site in Victoria, caused by a reduction in zooplankton prey (I. Ellis, unpublished data). The current study confirms that many MDB zooplankton species (e.g. the rotifer *B. plicatilis*: Walker 1981) are adapted to elevated salinity (max. 17.4 g L^{-1} at Boggy). However, the decrease in diversity and abundance in the zooplankton at Mundoo corresponded to extreme salinization (max. 55.3 g L^{-1}). Our findings indicate that the flooding of drought

refugia has further benefits for zooplankton and the fish that rely on them for food, especially because it abates extreme salinization and nitrification. However, this proposition requires testing on a larger spatial and temporal scale, because the current study was limited to a comparison of only two sites in one season.

The preference of *C. fluviatilis* for zooplankton eggs and harpacticoids over the abundant adult rotifers in January might relate to nutritional value or ease of foraging, or a combination of both. For example, Busch et al. (2011) showed that the growth of Atlantic cod (*Gadus morhua*) larvae was significantly less for fish fed with adult rotifers (*Brachionus* spp.) compared to those fed on copepods, because the latter have higher levels of protein and fatty acid. Whilst growth and nutrition has been well studied for aquaculture species (see Huntingford et al. 2012), less attention has been given to endangered small-bodied fishes. Research regarding the nutritional value of the zooplankton gained by flow regime manipulation would greatly assist management of endangered fishes in refugia. We propose that zooplankton blooms caused by intermittent flooding and drying provide early life stages of fish with a selection of food, including the more nutritional eggs (e.g. of rotifers) and larger adult microfauna (e.g. harpacticoids) that enhance survivorship and recruitment.

Selective feeding by fishes may be passive (accessible or conspicuous prey), active (limit prey choice) or the result of morphological constraints such as gape size (Raubenheimer et al. 2012). The second EWA led to the predominance of rotifers in Boggy during February. In March, the apparent shift in food selectivity by *C. fluviatilis* to adult rotifers and their eggs might represent a switch from active to passive feeding that relates to prey density. However, the avoidance of copepods in March is curious, given their higher nutritional value over rotifers (Busch et al. 2011). Possibly, *C. fluviatilis* was targeting rotifer eggs that were attached to adult rotifers or the highly mobile copepods were less accessible, but these propositions require further testing by analysing larger sample sizes in dietary analyses.

Gambusia holbrooki often consumed the same food as *C. fluviatilis*, but its more varied diet in January included macroinvertebrates and terrestrial insects. This corresponds to a study at Broken River in north-eastern Victoria, Australia, where *G. holbrooki* consumed large and small prey items (approximately

0.1–1.9 mm wide: King 2005). Indeed, *G. holbrooki* will selectively target larger prey if they are present (Wells 1969). The apparent dietary shift by *G. holbrooki* from zooplankton and terrestrial insects in January to mostly zooplankton in March, thereby overlapping the diet of *C. fluviatilis*, might relate to changes in prey density following the second EWA. In January, *G. holbrooki* differed from *C. fluviatilis* by actively selecting harpacticoids and their eggs. Notably, *G. holbrooki* also avoided consuming the abundant adult rotifers. In March, *G. holbrooki* mostly targeted rotifer eggs. The diminishing zooplankton community in March corresponds to predation by the abundant adult *C. fluviatilis* and *G. holbrooki* populations, but seasonal factors might have been influential. The high levels of Chl-*a* in March suggest that food was not a limiting factor for the zooplankton.

Considering the findings of our dietary analyses, we reject the proposition that competition from *G. holbrooki* for zooplankton prey prevents recruitment in *C. fluviatilis* in drought refugia when the watering regime is suitably managed. However, the strength of our findings is limited by the relatively small numbers of fish used for the dietary analysis. Competition also might be significant where intermittent flooding is absent, but this was untested because fish were extirpated in Mundoo before samples could be collected for dietary study. Incidentally, fish were absent in the diet of *G. holbrooki*, thereby suggesting that predation on *C. fluviatilis* did not occur or was minimal in Boggy. Similarly, Ivantsoff and Aarn (1999) detected fish in the diet of only 18 of 631 *G. holbrooki* when it cohabited with other small-bodied fishes in the Orara River, eastern Australia.

The substantially higher abundance of *G. holbrooki* in March compared to November was probably seasonal rather than flood-induced, because the same pattern was observed in other nearby off-channel sites during the drought (Wedderburn et al. 2012). The species began breeding in January when conditions were favourable (e.g. water temperature >19 °C: King and Warburton 2007). The findings correspond to a study in the Ovens River, Australia, where the abundance of *G. holbrooki* larvae peaked in December (King et al. 2003). It also preferred low-flow conditions for reproduction, and there was no significant change in larval abundance due to flooding (King et al. 2003). Likewise, during serial floods in the unregulated Lake Eyre Basin, central Australia, native fishes appear to have a recruitment advantage over *G. holbrooki* (Puckridge et al. 2000). Relatively low numbers of *G. holbrooki* were present in Boggy in November, and its larvae were not detected until January. Conversely, post-larval *C. fluviatilis* were present in November, so the zooplankton bloom created by flooding in December before *G. holbrooki* established provided abundant food that may have increased survivorship in the cohort. It would be important to test this proposition, and overcome the limitations of the current study, by sampling *C. fluviatilis* more regularly throughout the early life stages for dietary analyses.

Conclusions

The findings of the present study suggest that the timing of flooding in drought refugia is important to maximize conservation outcomes. Specifically for short-lived, small-bodied fishes, it should coincide with spawning and recruitment for the increased survivorship of early life stages by reducing or eliminating starvation. The high abundance and diversity of zooplankton, and their egg production, created by flooding also apparently alleviates competition for food with *G. holbrooki*. This is pertinent given the worldwide invasiveness of *G. holbrooki* (Pyke 2008). Flooding also maintains suitable water quality and obviously increases the volume of available habitat. Importantly, by sustaining isolates of endangered fish species in drought refugia, wider population recovery may be possible when river flows return and connectivity is regained. This approach is more desirable than re-stocking programmes, because of disease, genetic and domestication issues (Lynch and O'Hely 2001). Using relatively small volumes of water, EWAs provide the opportunity to correspond flooding with ecological cues for threatened fishes that would otherwise be desynchronized in highly regulated rivers, particularly during drought.

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