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Ecological aspects related to reintroductions to avert the extirpation of a freshwater fish from a large floodplain river

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Abstract The factors leading to reintroduction success are rarely determined in species translocation programmes attempting to prevent extirpations. Examining interactions between translocated fish and ecological aspects will provide information to increase success of future reintroduction efforts. The zooplankton are a key food source for small-bodied fishes; therefore, the examination of prey availability may contribute useful insight. Fish that are ecological specialists may have preference for invertebrates associated with specific habitat conditions. Food availability for ecological specialists also may be influenced by the presence of alien fishes, through competition for prey and habitat exclusion, including invasive Eastern Gambusia (*Gambusia holbrooki*) which is linked to the decline of numerous small-bodied fishes. The Murray–Darling Basin in south-eastern Australia is a highly regulated floodplain system where native fishes have declined over recent decades, especially ecological specialists adapted to ephemeral wetlands with natural flow regimes. Yarra Pygmy Perch (*Nannoperca obscura*) is an ecological specialist that was extirpated in 2008 during a prolonged drought. The objective of this study is to examine dietary aspects of reintroduced Yarra Pygmy Perch as related to zooplankton prey availability and the potential for competition with Eastern Gambusia. The study demonstrated that prey was being consumed by Yarra Pygmy Perch within 24 h of release and provides evidence of overlap in diet with cohabiting Eastern Gambusia. The findings provide direction for further study and a new understanding regarding reintroduction ecology of threatened small-bodied wetland fishes to aid species recovery and persistence.

Keywords Extinction · Recovery · Diet · Microcrustaceans · Percichthyidae · *Gambusia holbrooki*

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Introduction

Species translocations may involve augmentations of existing populations, reintroductions into former habitats or assisted colonisation to help prevent extirpations (Armstrong et al. 2015; Corlett 2016; IUCN/SSC 2013). Prevention is deemed successful when self-sustaining populations are established, but success is variable depending on a range of considerations (Pérez et al. 2012). Most obviously, reintroduction success rarely occurs when threats persist, inadequate numbers are released or habitat conditions are poorly managed (Cochran-Biederman et al. 2015; Fischer and Lindenmayer 2000). More commonly, the reasons for reintroduction failure are undetermined and similar mistakes may compromise future recovery efforts (Pérez et al. 2012). In this regard, examining interactions between the released fish and ecological aspects, such as habitat condition, prey availability and diet or the presence of alien fish, will provide information to increase success of future reintroduction efforts (Bearlin et al. 2002; Cochran-Biederman et al. 2015; Tatár et al. 2016).

The zooplankton are a key food source for early life stages of fishes and for all life stages of short-lived (< 5 years), small-bodied (adults < 100 mm long) freshwater fishes (Bremigan and Stein 1994; Miller et al. 1990). Several key factors shape zooplankton assemblages. In floodplain wetlands, for example, fresh inflows can trigger emergence from propagules (e.g. Furst et al. 2014; Tan and Shiel 1993), or increases in salinity in drying wetlands can trigger the emergence of halophilic rotifers from sediments (e.g. Gilbert 2017; Skinner et al. 2001). Turbidity also influences zooplankton by regulating the risk of predation (Estlander et al. 2009; Geddes 1984; Jones et al. 2016). Zooplankton, therefore, are ideal for ecological studies due to their sensitivities to the influences of water level fluctuations, water quality and biotic interactions (Lampert 1997). Shifts in zooplankton assemblages also are attributed to predation by fish, which can be intensified by alien species (Jack and Thorp 2002; Lieschke and Closs 1999). Therefore, the examination of zooplankton prey availability for a small-bodied fish may contribute towards understanding the reasons for success or failure of reintroductions.

Fish that are ecological specialists may have preference for invertebrates associated with specific habitat conditions, so habitat alterations may alter prey availability or catchability. For example, the decline of Northern Pike (*Esox lucius*) in the Baltic Sea is related to increased turbidity which reduces the ability of larvae to capture their preferred zooplankton prey (Salonen and Engström-Öst 2013). Food availability for ecological specialists also may be influenced by the presence of alien fishes through competition for prey and habitat exclusion (Ross 1991). The invasive Eastern Gambusia (*Gambusia holbrooki*) is linked to the decline of numerous small-bodied fishes through competition and its aggressive nature (Nicol et al. 2015; Pyke 2008). Eastern Gambusia alters zooplankton assemblages because it targets larger species (cladocerans and copepods) which are predators of the smaller rotifers (e.g. Haiahem et al. 2017; Margaritora et al. 2001). Further evidence suggests ecological specialists are intolerant (reduced occurrence, abundance and health) to the influences of Eastern Gambusia (Macdonald et al. 2012). This implies that Eastern Gambusia has an impact on the recruitment of ecological specialists, which make up the majority of threatened fishes worldwide (Cambray and Bianco 1998; Galat and Zweimüller 2001), so it is particularly relevant for reintroduction programmes.

The influences of river regulation, reduced river flows and alien fishes in the Murray–Darling Basin (MDB) have impacted on native fishes, especially ecological specialists adapted to ephemeral wetlands with natural flow regimes (Gehrke et al. 1995; Wedderburn et al. 2017). Most floodplain wetlands are now permanently dry or inundated, and the latter provide ideal conditions for alien Eastern Gambusia (e.g. Conallin et al. 2012; Macdonald et al. 2012). The impacts of Eastern Gambusia are particularly pronounced on small-bodied species (e.g. Saddlier et al. 2013), which constitute the majority of native fishes inhabiting the MDB (Wedderburn et al. 2017). In Lake Alexandrina, at the terminus of the MDB, Yarra Pygmy Perch (*Nannoperca obscura*) is an ecological specialist that was extirpated in 2008 during a prolonged drought (Wedderburn et al. 2012). Despite reintroductions of almost 6000 fish immediately following drought (Bice et al. 2014), the lack of sustained population recovery demonstrates the survival of Yarra Pygmy Perch is being inhibited (Wedderburn et al. 2014). Consequently, it was shifted from Vulnerable to Endangered under the International Union for Conservation of Nature's Red List of

Threatened Species (Whiterod et al. 2019). Over the same period, following a reduction during the severe drought conditions, Eastern Gambusia has increased in abundance and range in Lake Alexandrina and nearby tributary streams (Whiterod et al. 2015). Eastern Gambusia is considered a key threat to Yarra Pygmy Perch (Saddlier and Hammer 2010); therefore, the apparent failure to establish self-sustaining populations in wetlands might, in part, relate to the presence of Eastern Gambusia in its obligate habitat (cf. Houde 1987).

This study follows the fate of Yarra Pygmy Perch reintroduced to three wetland sites fringing Lake Alexandrina at the start of the breeding season in October 2015. The objective of this study is to examine dietary aspects of reintroduced Yarra Pygmy Perch as related to zooplankton prey availability and the potential for competition with Eastern Gambusia. Based on the premise that Eastern Gambusia can shape zooplankton assemblages (Boulton and Lloyd 1992; Margaritora et al. 2001; Pyke 2008), changes in its abundance and diet were examined to determine its potential impact on the microfauna. It is proposed that Eastern Gambusia consumes the same prey as Yarra Pygmy Perch and that its high abundance changes the structure of zooplankton assemblages to the detriment of the threatened fish species.

Materials and methods

Study sites

The heavily regulated Murray–Darling Basin (MDB) covers over one million square kilometres of south-eastern Australia. It discharges into Lake Alexandrina then flows past several small islands and through five barrages into the Coorong and out to the Southern Ocean through the Murray Mouth. Widespread drought from 1997–2010 placed this lower river reach under severe environmental stress which culminated in significant water level recession from 2007 to 2010. There were severe impacts on the ecological character of the region, including the extirpation of several small-bodied fishes (Wedderburn et al. 2012). Three reintroduction sites were selected in the south-western region of Lake Alexandrina which is approximately 650 km^2 and up to 6 m deep. The sites were selected using a criteria accounting for where the species was relatively abundant prior to the impacts of drought in 2008 and the prevailing habitat and fish community (Wedderburn and Hammer 2003). Site 5 is a drainage channel ($\sim$ 3 m wide) that has been deepened by excavation to access water for agriculture. The channel runs between Steamers Drain and Holmes Creek—the latter is a major channel leading to the Murray estuary through Mundoo Barrage. Aquatic habitats are heavily dominated by Hornwort (*Ceratophyllum demersum*), and there are moderate abundances of Water Milfoil (*Myriophyllum salsugineum*) and Cumbungi (*Typha domingensis*). Sites 34 and 68 are approximately 1 km apart in Shadows Lagoon. Site 34 is in the north-eastern section of the lagoon where aquatic vegetation includes abundant Ribbon Weed (*Vallisneria australis*) and low abundance of Cumbungi. Site 68 is in the south-western section of Shadows Lagoon adjacent to a channel that was excavated in the austral summer of 2014–15 to allow flow to Hunters Creek which winds down to the Murray estuary. Habitats include low to moderate abundances of Ribbon Weed, Water Milfoil, Water Primrose (*Ludwigia peploides*) and Cumbungi.

Habitat quality measures

Changes in wetland water levels were recorded against a fixed point predetermined for each site at commencement of the study. Managed water levels in Lake Alexandrina over the study period receded from 0.85 m Australian Height Datum (AHD; sea level) in October 2015 to 0.55 m AHD in March 2016. The level of Lake Alexandrina, given its shallow nature, has substantial influence on water levels at the lake-fringing wetlands which are hydrologically disconnected when lake levels fall below approximately 0.25 m AHD. Electrical conductivity (EC) units (µS cm^{-1}), pH and water temperature (°C) were recorded at each site on each sampling occasion using a TPS WP–81 hand-held meter (TPS Pty Ltd, Australia). Secchi depth was measured as a surrogate of turbidity, and where the depth was greater than measurable value, the highest value was used for data analyses. The proportion of area covered by aquatic plants was estimated at each site.

Zooplankton

Quantitative zooplankton sampling occurred on five occasions at each site in 2015: surveys *A* (3 September), *B* (1 October), *C* (27–29 October), *D* (3–5 November) and *E* (24–26 November). During each survey, zooplankton were collected at each site with 3 × 4 L Haney traps, and the total 12 L was filtered through a 60 μm mesh tow net. The mesh is fine enough to collect small zooplankton (e.g. rotifers), which often make up the majority of individuals and overall biomass in samples (Riccardi 2010). The concentrate was captured in a 250-ml PET bottle at the end of the tow net and immediately preserved in 100% ethanol. The zooplankton sampling equipment was cleaned in a bucket with dilute detergent and rinsed with chlorinated tap water prior to sampling at each site to prevent cross-contamination. At each site on each survey, samples were collected from (1) open water and (2) aquatic vegetation. The zooplankton samples were decanted into a 250-ml measuring cylinder and the volume recorded. The cylinder was then capped with Parafilm and inverted three times to distribute the contents, and 1-ml Gilson pipette sample was taken from approximately the centre of the agitated sample. The 1 ml was run into a Pyrex gridded Sedgewick-Rafter cell, the contents counted in their entirety and zooplankters identified on an Olympus BH-2 compound microscope (Nomarski optics). 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, and the number of zooplankters L^{-1} was calculated. Taxonomic guides were Shiel (1995) and the series *Guides to Identification of the Microinvertebrates of the Continental Waters of the World* (SPB Academic Publishing).

Fish

Three hundred adult Yarra Pygmy Perch (> 30 mm total length) were released at each site on 26, 27 and 28 October 2015 at sites 5, 34 and 68, respectively. Fish were surveyed 1, 7 and 28 days after the release dates (surveys *C*, *D* and *E*) using five fyke nets (6 m single leader, 5 mm mesh) set overnight and placed approximately 10 m apart perpendicular to the bank. Fish assemblages were again surveyed at each site in March 2016 (survey *F*). Grids (50 mm) at the entrances of nets excluded turtles and larger fish that might harm threatened fish. All fish were identified to species and counted.

During fish surveys *C*, *D* and *E*, 10 Eastern Gambusia and up to 10 Yarra Pygmy Perch were collected for dietary examinations. Yarra Pygmy Perch were collected from the fyke nets because other capture methods were ineffective. Eastern Gambusia were captured using a dip net during the day and excluded from fyke net survey counts. Fish were immediately killed using an overdose of AQUI-S® anaesthetic (AQUI-S New Zealand Ltd, Wellington) and placed into 70% ethanol. In the laboratory, total length was measured to the nearest millimetre for all fish before removal of their gastrointestinal tract. Food items were identified to the highest practical taxonomic resolution.

Data analyses and interpretation

The densities of each zooplankton genus or microfauna group (zooplankter^{-1}) were analysed by non-metric multidimensional scaling (NMS) ordination using the relative Sørensen distance metric, in PC–ORD (version 6: McCune and Mefford 2011) to examine associations between zooplankton and habitat characteristics. Diets also were described using 'frequency of occurrence' (proportion of fish with a particular food item in the gut) to determine the consistency of food selection (Bowen 1996).

Results

Habitat conditions

The increase in EC from November to March at each site was the most notable variation observed in the six measured habitat variables, which was pronounced at the two sites in Shadows Lagoon (Table 1). Conversely, pH declined over time at each site, but was always within the normal range for wetlands in Lake Alexandrina (Wedderburn et al. 2014). Secchi depth was always higher at site 5, and the higher turbidity at the sites in Shadows Lagoon was comparable between sites 34 and 68 during the study. Water temperature was comparable between sites, with a tendency to increase as summer progressed. Water level fluctuated more widely in site 5 than at the two sites in Shadows

Table 1 Habitat measures at each site during surveys *A*, *B*, *C*, *D* and *E* in October–November 2015 and survey *F* in March 2016

Habitat measure	Site 5 Steamers						Site 34 Shadows						Site 68 Shadows					
	A	*B*	*C*	*D*	*E*	*F*	*A*	*B*	*C*	*D*	*E*	*F*	*A*	*B*	*C*	*D*	*E*	*F*
EC (μS cm^{-1})	940	1018	1025	1037	1046	1306	1315	1447	1432	1409	1446	2890	1286	1270	1284	1304	1358	2434
pH	8.79	8.34	8.17	8.48	7.75	6.81	8.15	8.08	8.56	8.63	7.80	8.12	9.02	9.00	8.88	9.02	7.58	7.08
Secchi depth (cm)	> 100	> 100	> 84	> 85	> 61	88	16	30	27	43	29	40	19	31	32	32	33	26
Temperature (°C)	12.8	13.4	17.5	18.6	15.9	20.9	13.8	19.4	18.9	20.5	20.5	26.8	13.5	18.4	18.6	17.9	21.9	20.9
Depth (cm)	48	48	70	78	33	73	46	46	47	51	49	50	45	45	48	48	45	47
Aquatic plants (%)	70	70	70	80	80	90	50	60	70	80	80	60	30	30	30	40	40	44

Lagoon where it remained relatively constant despite a managed 30 cm water level reduction in Lake Alexandrina. The percentage cover of aquatic plants remained relatively constant at the sites, with minor fluctuation at site 34 in Shadows Lagoon where Ribbon Weed (50–85% cover) was the sole species. Aquatic plants at site 5 consisted of abundant Water Milfoil and Cumbungi (70–80% cover combined), whereas site 68 consisted of low abundances of Water Milfoil, Cumbungi and Water Primrose (30–40% cover combined).

Zooplankton

Over 200 zooplankton taxa from five major orders were recorded in water samples: Protista ($\sim$ 67 taxa); Rotifera ($\sim$ 95 taxa); Cladocera ($\sim$ 24 taxa); Copepoda ($\sim$ 12 taxa); and Ostracoda (6 taxa) (Fig. 1). Macroinvertebrates predominantly included Insecta (mostly Chironomidae), with smaller numbers of Cnidaria, Nematoda, Turbellaria, Mollusca, Gastrotricha, Oligochaeta, Amphipoda, Decapoda and Arachnida. Eggs were abundant in many samples, but the source was undetermined; however, unusually high numbers reflect detached calanoid and cyclopoid egg sacs. There were extreme high densities of protists recorded at site 5, more so in the vegetation samples, making the overall zooplankton densities approximately 10 times higher than the two sites in Shadows Lagoon. Consequently, Protista was removed from the site comparison for clarity (but remained in the ordination), which was deemed acceptable because they were absent in the diets of fish (see below). Zooplankton densities in Shadows Lagoon were somewhat higher at site 34 than at site 68, whereas the general composition of major groups is comparable between the two sites. Rotifers, cladocerans and copepods were the most abundant. Cladocerans were at similar densities in open water and vegetation samples in the sites of Shadows Lagoon, and a similar pattern is evident for rotifers. The cladocerans, however, were more abundant in the vegetation samples from site 34 and 68 in Shadows Lagoon. Macroinvertebrates were present in low numbers in most samples despite being untargeted in the surveys.

The ordination shows a distinct separation of site 5 from the sites in Shadows Lagoon and displays higher salinities correlated with sites 34 and 68 (Fig. 2). Rotifers of the genera *Brachionus*, *Filinia* and

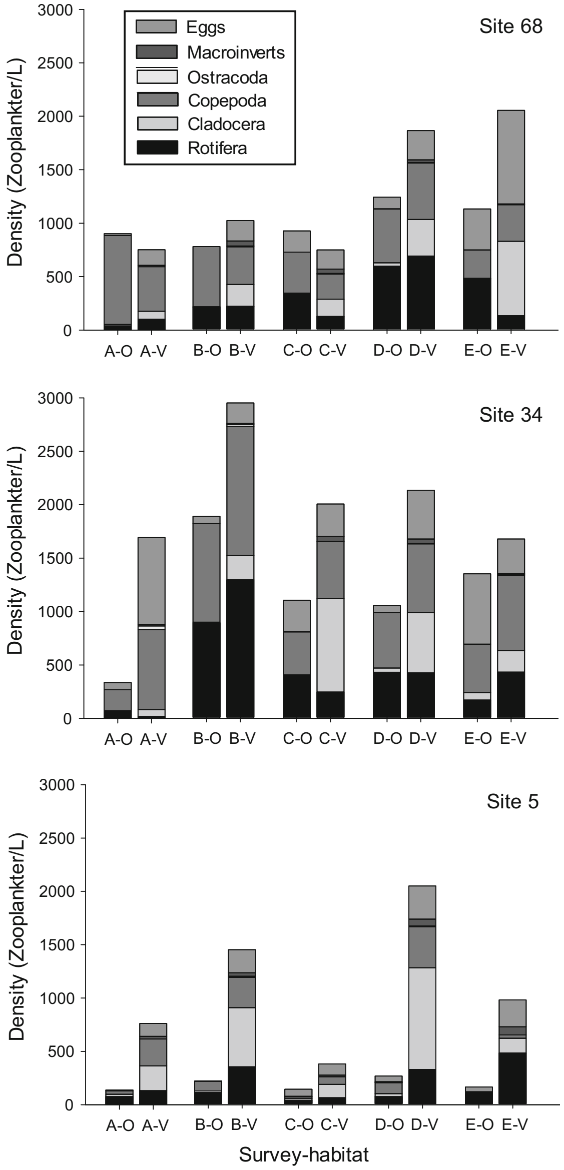

Fig. 1 Densities and composition of major zooplankton groups (excluding Protista), macroinvertebrates and unidentified eggs in surveys *A* to *E* collected from open water (O) and vegetation (V) in October–November 2015 at Yarra Pygmy Perch reintroduction sites

Keratella predominate with the early life stages of copepods in open water samples from Shadows Lagoon, while copepods of the genus *Gladioferens* and unidentified eggs were associated with water samples collected in vegetation. Conversely, site 5 is characterised by the dominance of protists and several rotifer genera (*Trichocerca*, *Lepadella*, *Mytilina*) correlated with higher Secchi depth.

Fishes

A total of 1562, 1267 and 2171 fish were captured at sites 5, 34 and 68, respectively, consisting of 11 native and four alien species (Table 2). The diadromous Common Galaxias (*Galaxias maculatus*) dominated the catch at site 5 in surveys C, *D* and *E* in October–November 2015, and Eastern Gambusia dominated the catch for survey F in March 2016. Three gudgeons dominated the catch at site 34 in surveys C, *D* and *E*, but Eastern Gambusia was the most abundant fish in March. Common Galaxias and Flathead Gudgeon (*Philypnodon grandiceps*) dominated the catch at site 68, except in March when Eastern Gambusia was highly abundant. Low numbers of Yarra Pygmy Perch were recaptured in surveys C, *D* and *E* at all sites (26, 10 and 17 fish overall each survey, respectively), but the species was undetected during survey *F* in March 2016.

The diets of 38 recaptured Yarra Pygmy Perch were examined, but six of them had empty guts (Table 3). Rotifers were absent in the diet of Yarra Pygmy Perch collected from all sites. At site 5, in order of highest frequency of occurrence, the diets of Yarra Pygmy Perch consisted of cladocerans, macroinvertebrates, copepods, eggs and ostracods. Yarra Pygmy Perch collected from the two sites in Shadows Lagoon had a high occurrence of macroinvertebrates in the diet, and cladocerans and copepods occurred at moderate frequencies. Very few individual Yarra Pygmy Perch consumed ostracods or unidentified eggs.

The diets of 90 Eastern Gambusia were examined, and only four had empty guts (Table 4). Cladocerans and macroinvertebrates made up the greatest proportion of the diets of Eastern Gambusia throughout the study at all sites, with lower frequencies of occurrence for copepods, ostracods and terrestrial invertebrates. Approximately a third of Eastern Gambusia collected from sites 34 and 68 in Shadows Lagoon had consumed leaves of a free-floating aquatic plant, Tiny Duckweed (*Wolffia australiana*). Rotifers were absent in the diet of all Eastern Gambusia.

Sixty-seven different food items were consumed by the fishes (Table 5). Yarra Pygmy Perch and Eastern Gambusia each had consumed 46 different food items of which 25 (predominantly cladocerans) were

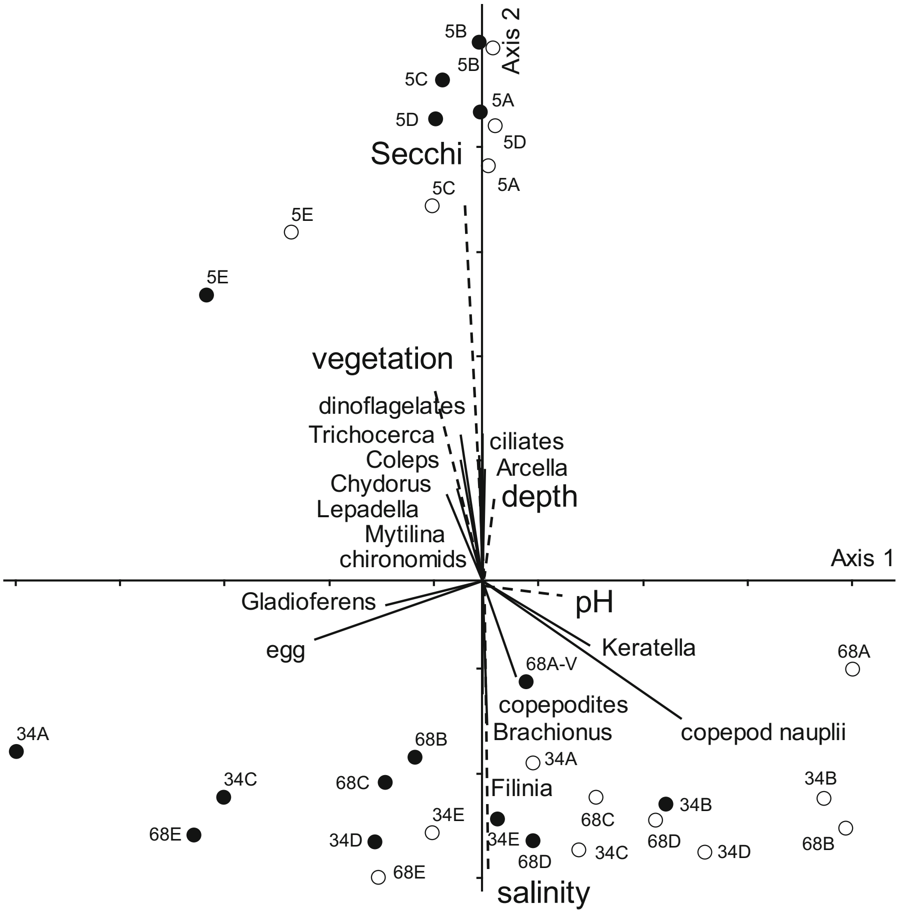

Fig. 2 Two-dimensional ordination (stress = 7.0) of all zooplankton samples with overlayed vectors proportional to and directed towards main zooplankton genera or food type (solid lines) and habitat characteristics (dashed lines), and open water (open circles) and vegetation (filled circles) surveys *A*, *B*, *C*, *D* and *E* indicated for sites 5, 34 and 68. Zooplankton groups and genera are dinoflagelates, ciliates, *Coleps* and *Arcella* (Protista), *Trichocerca*, *Lepadella*, *Mytilina*, *Brachionus*, *Keratella* and *Filinia* (Rotifera), *Chydorus* (Cladocera), copepodites, copepod nauplii and *Gladioferens* (Copepoda) and chironomids (Insecta)

recorded for both species. Ten of the same food items were recorded in the guts of both fish species at the same site during the same survey. Yarra Pygmy Perch had consumed 31, 19 and 10 food items at sites 5, 34 and 68, respectively. Eastern Gambusia had consumed 30, 28 and 22 food items, respectively.

Discussion

There is strong evidence that Yarra Pygmy Perch is the first freshwater fish extirpated from the Murray–Darling Basin in south-eastern Australia (Wedderburn et al. 2019), and reintroductions will be essential to recover the species. The present study followed an attempt to reintroduce the species to the wild and demonstrated that adequate food was available which was being consumed within 24 h of release. The findings also suggest overlap in diet with cohabiting Eastern Gambusia, but this requires confirmation due to small sample sizes for Yarra Pygmy Perch. There were also differences between the fishes with regard to prey items consumed, but it was undetermined whether this was due to differences in food preferences or competitive exclusion by Eastern Gambusia. Overall, the current study provides direction for further study and a new understanding regarding reintroduction ecology of threatened small-bodied wetland fishes to aid species recovery and persistence.

Table 2 Total abundance of each fish species captured at three sites in surveys *C*, *D* and *E* in October–November 2015 and survey *F* in March 2016

Common name	Scientific name	Site 5 Steamers				Site 34 Shadows				Site 68 Shadows			
		C	*D*	*E*	*F*	*C*	*D*	*E*	*F*	*C*	*D*	*E*	*F*
Yarra Pygmy Perch	*Nannoperca obscura*	22	2	2	0	4	3	3	0	13	3	1	0
Southern Pygmy Perch	*Nannoperca australis*	0	0	1	0	0	0	0	7	0	0	0	0
Flathead Gudgeon	*Philypnodon grandiceps*	0	0	0	0	50	24	33	97	36	26	19	11
Dwarf Flathead Gudgeon	*Philypnodon macrostomus*	0	0	0	0	9	10	3	34	3	4	2	0
Carp Gudgeon	*Hypseleotris* spp.	2	2	1	0	3	2	1	33	1	2	1	0
Australian Smelt	*Retropinna semoni*	0	0	0	0	24	1	0	0	4	0	0	6
Bony Herring	*Nematalosa erebi*	0	0	0	0	0	0	0	0	0	0	0	52
Congolli	*Pseudaphritis urvillii*	2	0	0	0	0	3	2	0	6	3	22	0
Common Galaxias	*Galaxias maculatus*	48	29	587	75	4	1	4	0	197	44	63	14
Smallmouth Hardyhead	*Atherinosoma microstoma*	0	0	0	0	0	0	0	0	1	1	0	7
Western Blue-spot Goby	*Pseudogobius olorum*	1	0	0	0	18	3	1	5	68	75	22	2
Common Carp	*Cyprinus carpio*	0	0	13	0	16	19	5	10	6	7	28	13
Goldfish	*Carassius auratus*	0	0	0	4	2	0	1	4	1	6	2	3
Redfin Perch	*Perca fluviatilis*	0	0	0	0	0	2	5	2	7	4	4	22
Eastern Gambusia	*Gambusia holbrooki*	31	8	96	636	17	0	1	801	16	20	72	1251

Table 3 Frequency of occurrence (%) of each food group in the diets of Yarra Pygmy Perch at each site in surveys *C*, *D* and *E* (*n* in parentheses for each survey)

Food item	Site 5			Site 34			Site 68		
	C (10)	*D* (2)	*E* (2)	*C* (4)	*D* (3)	*E* (3)	*C* (10)	*D* (3)	*E* (1)
Cladocera	100	0	100	100	33	0	30	0	0
Copepoda	100	0	50	75	33	0	10	0	0
Ostracoda	60	0	0	50	0	0	30	0	0
Macroinvertebrates	100	0	100	100	33	100	80	0	0
Terrestrial invertebrates	10	0	0	0	0	0	0	0	0
Eggs	60	0	50	25	0	0	10	0	0
Empty gut	0	100	0	0	0	0	20	100	100

The diet of Yarra Pygmy Perch is broadly described including microcrustaceans and other invertebrates (Saddlier and Hammer 2010). The current study is the first to identify specific food items which included a diverse array of taxa in the Order Cladocera (Chydoridae, Daphniidae), Copepoda (Calanoida, Cyclopoida) and Ostracoda, and several orders of macroinvertebrates. Most of the prey types consumed by Yarra Pygmy Perch were large zooplankton species associated with clearer waters and abundant aquatic plants, particularly cladocerans in the *Chydorus* genus. These habitat conditions also tended to have higher species richness of zooplankton including several rotifers that were absent in the diet of Yarra Pygmy Perch, possibly due to their small size. The complex aquatic plants apparently provided habitat qualities for a diverse array of zooplankton in various sizes and abundances which apparently afforded Yarra Pygmy Perch sufficient food (cf. Lucena-Moya and Duggan 2011; Nunn et al. 2012; Shiel 1976). The diets of adult Yarra Pygmy Perch were assessed in the current study, so it is conceivable that young-of-the-year fish

Table 4 Frequency of occurrence (%) of each food group in the diets of Eastern Gambusia at each site in surveys *C*, *D* and *E* ($n = 10$ for all surveys)

Food item	Site 5			Site 34			Site 68		
	C	*D*	*E*	*C*	*D*	*E*	*C*	*D*	*E*
Cladocera	80	100	70	50	90	90	50	80	60
Copepoda	20	90	0	0	10	40	0	0	0
Ostracoda	20	30	0	10	30	0	10	0	10
Macroinvertebrates	40	80	100	90	90	90	80	80	40
Terrestrial invertebrates	80	30	0	10	50	30	0	20	40
Eggs	30	0	0	0	0	10	10	10	10
Wolffia australiana	0	0	0	70	50	30	40	10	20
Fish	0	0	0	0	0	0	0	20	0
Empty gut	10	0	0	0	0	0	10	20	0

(i.e. < 25 mm total length in September–October) may consume smaller prey including the rotifers. This is an important aspect for further study to determine the life history requirements with regard to food availability necessary for reintroduction success.

Worldwide, the alien Eastern Gambusia is an aggressive and prolific live-bearing fish that can negatively impact on native fish populations (Pyke 2008). In the current study, it consumed many of the same prey items as Yarra Pygmy Perch, at the same sites, which is consistent with its invasion in other parts of the world (e.g. Mediterranean coastal marshes: Cardona 2006). Eastern Gambusia increasingly dominated catches over summer and was prolific by the end of the study in March. There are numerous examples of Eastern Gambusia and Western Mosquitofish (*Gambusia affinis*) impacting the small-bodied fishes through multiple negative interactions, including direct predation, competition for food and changed reproductive behaviour (e.g. Macdonald et al. 2012; Magellan and García-Berthou 2016; Mills et al. 2004). For example, Yarra Pygmy Perch are likely to utilise areas of high aquatic plant abundance to avoid Eastern Gambusia (Magellan and García-Berthou 2016) and predatory Redfin Perch (*Perca fluviatilis*) (Wedderburn and Barnes 2016). Findings of the current study suggest that when Eastern Gambusia is prolific over summer there would be substantial impact on the growth and survival of Yarra Pygmy Perch, especially young-of-the-year fish. For example, during October to December, when Eastern Gambusia was consuming *Daphnia* at all sites, it is conceivable that Yarra Pygmy Perch was excluded from this suitable food source. Eastern Gambusia has the potential to impair reintroduction success of Yarra Pygmy Perch and other small-bodied species through competition for food at times of high abundance.

Yarra Pygmy Perch is an ecological specialist with obligate habitat conditions required throughout its life history (Woodward and Malone 2002). Specialist fishes are sensitive to changes associated with hydrological alterations due to their requirements for reproduction, movement, obligate habitat conditions, specific prey and their vulnerability to predation and competition (Aarts et al. 2004; Magaña 2012; White et al. 2008). The current findings show that water clarity (Secchi depth), EC (surrogate for 'salinity') and the composition and abundance of aquatic plants were key habitat variables influencing zooplankton assemblages. The weight of influence of these factors likely increased as water levels receded by approximately 30 cm from October to March, but this aspect was not examined. There were also differences in zooplankton assemblages between the open water and vegetation samples. Cladocerans, a key food for Yarra Pygmy Perch, had the greatest contrast in density between the microhabitats, being more abundant in vegetation as expected (Estlander et al. 2009).

The current study has increased ecological knowledge concerning conservation of Yarra Pygmy Perch to avert its extirpation from the MDB. The findings provide valuable insight despite failure of the reintroductions (cf. Fischer and Lindenmayer 2000). In an examination of the literature, Cochran-Biederman

Table 5 Food items for Yarra Pygmy Perch and Eastern Gambusia distinguished to the lowest practical taxonomic resolution for each site (5, 34 and 68) and each survey (*C*, *D* and *E*)

Food item	Yarra Pygmy Perch	Eastern Gambusia
Zooplankton		
Cladocera		
Bosminidae		
Bosmina sp	–	34*E*
Chydoridae	34*C*	34*E*
Alonine	5*E*	5*D*, 34*D*, 34*E*, 68*C*
Camptocercus sp	5*C*	
Camptocercus australis	5*C*	5*D*
Chydorus spp.	5*C*, 5*E*, 34*D*	5*C*, 5*D*, 5*E*, 34*C*, 34*D*, 34*E*, 68*C*
Dunhevedia crassa	5*C*, 34*C*	–
Graptoleberis sp	5*C*, 68*C*	34*E*
Graptoleberis testudinaria	34*C*, 68*C*	–
Kurzia sp	5*C*	5*D*
Kurzia latissima	–	5*D*
Picripleuroxus quasidenticulatus	–	5*D*, 34*D*, 68*E*
Pleuroxus sp	5*C*	5*D*
Pleuroxus inermis	5*C*	34*D*
Pseudochydorus globosus	–	34*D*
Daphniidae		
Ceriodaphnia sp	5*C*, 34*C*	5*C*, 5*D*, 34*C*, 34*D*, 34*E*, 68*C*, 68*E*
Ceriodaphnia cornuta	68*C*	34*E*, 68*C*, 68*E*
Ceriodaphnia ephippium	34*C*	5*C*
Daphnia sp	5*C*	–
Daphnia carinata	–	5*D*
Daphnia lumholtzi	–	5*D*
Diaphanosoma sp	5*C*, 34*C*	–
Simocephalus sp	5*C*, 34*C*	5*C*, 5*D*, 5*E*, 34*C*, 34*D*, 34*E*, 68*C*, 68*E*
Simocephalus exspinosus	5*C*	–
Macrotrichidae		
Macrothrix sp	–	68*C*
Moinidae		
Moina sp.	34*C*	5*E*
Copepoda		
Calanoida	5*C*	–
Calanoida copepodite	34*C*	–
Centropagidae		
Gladioferens sp1	5*E*	–
Gladioferens sp2	5*C*	5*D*, 34*E*
Gladioferens spinosus	34*C*, 34*D*	–
Gladioferens copepodites	68*C*	–
Cyclopoida	5*C*, 5*E*, 34*C*	5*C*, 34*D*, 34*E*
Acanthocyclops sp	–	5*D*
Acanthocyclops vernalis	5*C*	–
Cyclopoida copepodite	–	5*D*, 34*D*

Table 5 continued

Food item	Yarra Pygmy Perch	Eastern Gambusia
Eucyclops sp	5*E*	–
Eucyclops australiensis	5*C*	–
Harpacticoida	5*C*	–
Ostracoda		
Unidentified ostracod	34*C*, 68*C*	5*C*, 5*D*, 68*C*
Candonopsis sp	5*C*	–
Stenocypris sp	5*C*	–
Unidentified cypridopsid	5*C*, 68*C*	–
Limnocythere sp	5*C*	–
Newnhamia fenestrata	–	34*C*, 34*D*, 68*E*
Macroinvertebrates		
Amphipoda	5*C*, 34*C*, 34*D*, 68*C*	5*D*, 34*C*, 34*D*, 34*E*, 68*C*, 68*E*
Coleopteran larvae	–	34*E*, 68*E*
Decapoda		
Atyidae		
Paratya australiensis	5*C*, 34*C*	5*D*
Diptera		
Chironomidae	5*C*, 5*E*, 34*C*, 34*E*, 68*C*	5*C*, 5*D*, 5*E*, 34*C*, 34*D*, 34*E*, 68*E*
Pupae (unidentified)	5*C*	5*C*, 5*D*, 68*C*
Gastrapoda	–	5*C*, 34*C*, 34*E*, 68*C*
Hemiptera		
Corixidae	34*C*	5*C*, 5*D*, 34*C*, 34*D*, 34*E*, 68*C*
Dytiscidae	5*C*	–
Mollusca		
Ferrissia sp (freshwater limpet)	–	34*D*
Nemertea (ribbon worm)	–	34*C*, 68*E*
Odonata		
Ephemeroptera	5*C*	5*D*, 34*D*, 34*E*
Zygoptera nymphs	34*E*	5*D*
Zygoptera?	68*C*	–
Oligochaeta		
Naididae (oligochaete worm)	–	68*C*
Terrestrial invertebrates		
Ant	–	5*C*, 34*D*
Coleopteran	–	5*C*
Oribatid mites	5*C*	34*C*
Spider	–	5*C*, 5*D*, 34*D*, 34*E*, 68*E*
Indeterminate insect	–	68*D*
Miscellaneous items		
Eggs (unidentified source)	5*C*, 5*E*, 34*C*, 68*C*	5*C*, 34*E*, 68*C*, 68*D*, 68*E*
Fish (unidentified)	–	68*D*
Wolffia australiana (plant leaves)	–	34*C*, 34*D*, 34*E*, 68*C*, 68*D*, 68*E*

et al. (2015) found that inadequately addressing the initial cause of decline was the best predictor of failed freshwater fish reintroductions. The extirpation of Yarra Pygmy Perch in 2008, however, was the result of drought and over-abstraction of water which are unmanageable in its obligate wetlands (Wedderburn et al. 2012). In the case of Yarra Pygmy Perch, therefore, their proposition can be applied to addressing the likely causes of reintroduction failure. In light of this, in concert with finding of the current study, the key factors for successful reintroduction of a small-bodied wetland fish are (1) stocking numbers, frequency and duration (cf. Southern Pygmy Perch *Nannoperca australis*: Todd et al. 2017); (2) addressing genetic issues (e.g. pygmy perches: Attard et al. 2016; Brauer et al. 2016); (3) alien fish management (e.g. Eastern Gambusia: Magellan and García-Berthou 2016; Tonkin et al. 2014); and (4) habitat quality management to ensure suitable aquatic plants and food availability for the target threatened fish (Cochran-Biederman et al. 2015).

Further understanding of the reintroduction ecology of Yarra Pygmy Perch and similarly threatened small-bodied freshwater fishes will be critical to aid species recovery and persistence in large floodplain rivers. Reintroductions can then be planned around complex ecological relationships between habitat conditions, food sources, hydrological influences and alien fish to maximise recruitment in the first year of reintroduction and promote population establishment and species recovery.

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