Department of Primary Industries

Young-of-year common carp (*Cyprinus carpio*) nursery sources in and around Koondrook–Perricoota Forest following the 2016 flood

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Cover image: Light trapping Thule Creek, October 2016 (Kate Martin)

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Non-technical summary

Young-of-year common carp (Cyprinus carpio) nursery sources in and around Koondrook–Perricoota following 2016 flood

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Objectives

1. Determine if common carp spawned in Koondrook–Perricoota Forest.
2. Determine if young-of-year common carp dispersed from Koondrook–Perricoota Forest into the Murray River.

Key words

Koondrook–Perricoota Forest, Common Carp, Otolith, Elemental Isotopes.

Summary

Koondrook–Perricoota Forest (KPF) is located on the Murray River floodplain upstream of Barham in NSW. It spans approximately 33,000 hectares and is one of six Living Murray icon sites due to its significant wetland and creek ecosystems. These ecosystems are highly reliant on floodwaters with flooding being historically more common than under current regulated conditions. Recently, a range of environmental works and measures have been completed that enable the KPF to be flooded without the need for an overbank flow. This can potentially have a range of positive and negative outcomes for fish populations (and for other environmental values such as the River Red Gum community in the forest). The creation of shallow wetland habitat may provide ideal spawning conditions for native small-bodied fish. Similar conditions are also favoured by the introduced common carp and there is a risk that inundating a large area of wetland and creek habitat will allow common carp to successfully spawn and recruit. Consequently, KPF could potentially act as a major source population of common carp that could colonise the Murray River following environmental watering.

An earlier study calculated the ages of young-of-year carp collected in KPF following a managed flood event in 2014 and determined that they were from reproductive events that occurred within the forest after connectivity to the Murray River was lost.

Here we expand on that work and use otolith (ear bones) chemistry to determine if common carp originating from KPF colonised the Murray River as young-of-year (YOY) fish following a natural

flood in 2016. Larval fish were sampled from within KPF and in the surrounding rivers in order to determine if the forest had a unique chemical signature that differentiated it from other areas.

Young-of-year carp were sampled during autumn 2017 and the chemical signature from near the core of their otoliths was matched to the larval chemical signatures in order to estimate where they were spawned.

Results clearly showed that common carp YOY collected in the Murray River around Torrumbarry Weir did not originate from reproductive events in KPF. Furthermore, only a single YOY collected inside KPF originated from KPF, all others originated from natal locations outside of the forest. While it is possible that YOY originating from KPF migrated beyond the study zone and were undetected by this study, the findings more strongly suggest that the larval carp within the KPF had a very poor survival rate, most likely due to acidic and anoxic water associated with the flood. Combined results from the 2014 work and this study suggest that regular small-scale managed floods that don't inundate large areas of leaf litter may result in more successful recruitment of common carp than much larger floods that inundate areas that have been dry for several years.

Introduction

The inundation of a river's floodplain is important for the conservation of biotic diversity and the production of plant and animal biomass, including fish (Junk et al. 1989). Riverine fish in the Murray-Darling Basin (MDB) are susceptible to the effects of river regulation as many species utilise off-channel habitat (Conallin *et al.* 2011; Lyon *et al.* 2010). For some species, floodplain inundation provides an opportunity to spawn and then connections to the river allow the juvenile fish to disperse back into the river. Otolith chemistry research is enabling researchers to gain a detailed understanding of the migration history of a fish, including its place of hatch, by analysing the chemical composition of its otolith (Elsdon et al. 2008).

Otoliths (fish ear bones) are found in all teleost fishes and are important for balance and/or hearing (Campana 1999). Otoliths are formed by the daily accumulation of layers of calcium carbonate from before hatch to death and are therefore commonly utilised for ageing fish. Another important characteristic of otoliths is that they are metabolically inert, thus they retain the chemical composition of the environment they were in at the time each layer was deposited (Campana 1999). Therefore, the chemical composition of the otolith reflects the water chemistry at the time that the layer was deposited. It is possible to analyse different layers within the otolith so that a fish's migration history can potentially be determined – if there are differences in water chemistry among sites. For example, otolith chemistry analysis demonstrated that Barmah-Millewa Forest was a key spawning ground for common carp (*Cyprinus carpio*), and, when conditions were suitable, it was the source of most young-of-year common carp captured downstream at Torrumbarry Weir (Crook & Gillanders 2006; Macdonald & Crook 2014).

The Koondrook–Perricoota Forest (KPF) is a large floodplain forest located adjacent to the Murray River in southern New South Wales and is one of The Living Murray (TLM) icon sites, containing significant wetland and creek ecosystems (Figure 1). A range of environmental works and measures have been implemented in order to enable watering of the forest without relying on an overbank flow. There are many fish objectives relating to the flooding and these are described in detail in Duncan et al. (2015). One objective relates specifically to spawning and recruitment of common carp and native fish; Objective 1: the operation of the scheme will not result in dispersal of common carp spawned in KPF to the Murray River.

In 2016, a very wet winter and spring resulted in a natural flood in KPF that commenced in early August and ceased in late November 2016 (Figure 2a). The flow at Torrumbarry in mid-October 2016 exceeded 50,000ML/d and was the largest recorded since December

2010. Given this was a natural flood event that was not supplemented with environmental water, the inlet channel and return channel were closed and all downstream regulators including Thule Creek were fully open in order to pass all flows (Figure 2b). Inflows initially were via Swan Lagoon, but as the event increased in magnitude, water flowed into KPF at multiple points downstream of Swan Lagoon. Outflows at Barber Creek occurred from late August to early December 2016, and outflows also occurred at Thule Creek from mid-August to late December 2016. Thus the 2016 flood event potentially provided over four months of connectivity between KPF and the surrounding rivers. During the flood event, dissolved oxygen levels of water entering KPF decreased as the flood progressed (Figure 2c, Table 1), and the floodwater coming off KPF also declined as the flood progressed (Figures 2d and 1e), potentially impacting the ability of fish to survive within the forest.

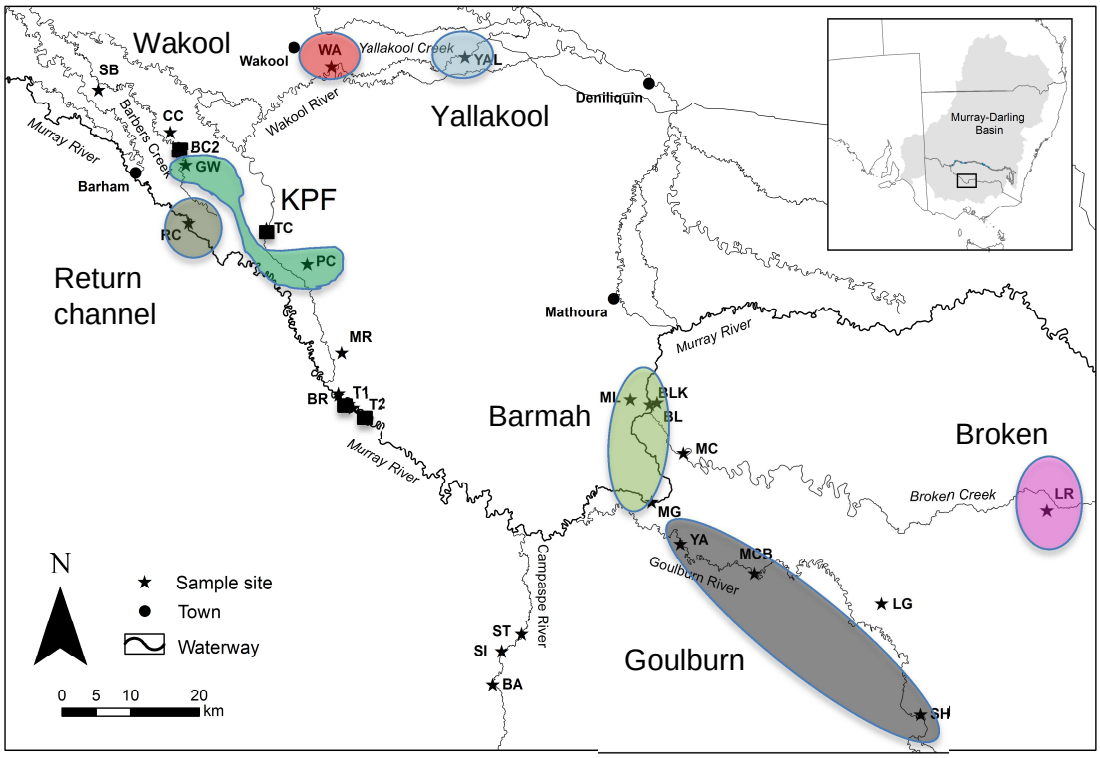


Figure 1. Map of the study area showing the larval sampling sites (stars) and sites where both larvae and young-of-year were sampled (squares). Site codes are given in Table 1. Polygons show the larval zones based on similar otolith chemistry results. Sample sites that aren't included in zones were those where no larval fish were sampled, or where their otoliths were too damaged to analyse (see results).

The movement of adult common carp (44 fish), golden perch (six fish) and silver perch (one fish) were tracked during the flood using acoustic telemetry to determine whether they would enter KPF during a natural flood (Duncan *et al.* 2017). Results indicated that common carp typically moved into KPF while native fish did not enter the forest. Some common carp passed through KPF into other river systems while others remained within the forest throughout the duration of flooding. The movement of common carp into KPF was considered to be a spawning migration based on previous monitoring of a managed environmental flow in 2014 that demonstrated common carp spawned and recruited within KPF. Thus there is concern that regular environmental watering of KPF during late winter and spring could provide ideal conditions for common carp spawning and recruitment, and that juveniles could then colonise the Murray River if connectivity between the Murray River and KPF was maintained for an extended period. Therefore, it is important to determine whether larval carp originating from KPF during the 2016 flood subsequently colonised the Murray River. By better understanding the use of KPF as a breeding habitat for carp, environmental water can be delivered in a way that will minimise the risk of increased carp dispersal into the Murray River. By analysing the elemental composition of a fish's otolith (ear bone) it may possible to identify their natal site, if there is sufficient differentiation in the water chemistry among spawning sites.

If KPF is found to be a 'hotspot' for common carp reproductive events, as well as a significant source population for the species in the Murray River, timing of environmental flows could be managed to avoid coinciding with peak spawning time of the species.

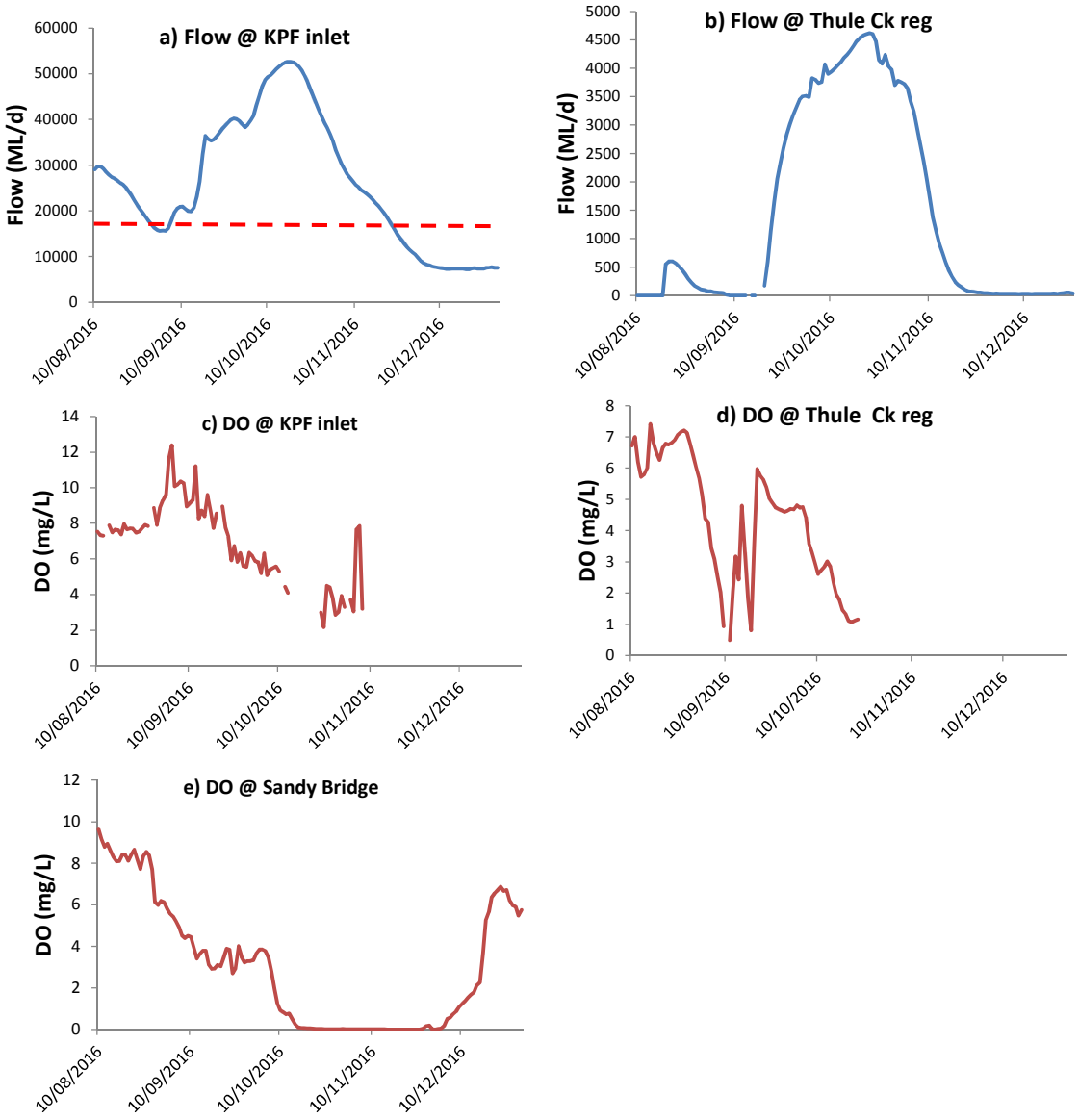


Figure 2. a) Murray River flows at the KPF inlet channel, b) flow at the Thule Creek regulator c) dissolved oxygen at the KPF inlet channel, d) dissolved oxygen at the Thule Creek regulator and e) dissolved oxygen at the Sandy Bridge regulator (Barber Creek) downstream of KPF. The red line indicates the approximate* flow when water begins to enter the Koondrook–Perricoota Forest via Swan Lagoon. * Approximate as the commence-to-flow level at Swan Lagoon is variable as the silt in the mouth shifts around. Note that the monitoring equipment for dissolved oxygen at Sandy Bridge was offline for a few weeks when dissolved oxygen levels were at their lowest point, thus the zero readings may not be correct.

Table 1. Water quality parameters for locations inside Koondrook–Perricoota Forest (KPF) and sites outside KPF during larval sampling.

Site	Location	Date	Time	Depth (m)	Rep No.	Temperature (°C)	pH	Conductivity (mS/cm	Turbidity (FTU)	Dissolved oxygen (mg/l)
Cow Creek	KPF	20/10/2016	14:37	0.2	1	19.07	6.65	0.134	1.8	0
Cow Creek	KPF	21/10/2016	7:40	0.2	2	17.02	6.47	0.132	26.7	0
Marywood Road	KPF	20/10/2016	16:20	0.2	1	20.06	6.59	0.18	25.4	0
Marywood Road	KPF	21/10/2016	9:22	0.2	1	16.38	6.6	0.183	20.9	0
Barber Creek Sample Site	KPF	18/10/2016	12:45	0.2	1	17.2	6.62	0.136	19.4	0
Barber Creek Sample Site	KPF	19/10/2016	9:30	0.2	2	15.66	6.74	0.131	30.6	1.93
Thule Creek	KPF	19/10/2016	16:45	0.2	1	20.45	6.54	0.177	5	0
Thule Creek	KPF	20/10/2016	10:17	0.2	1	16.89	6.84	0.139	25	0
Sandy Bridge Crossing_Barber Creek	Outside KPF	17/10/2016	17:04	0.2	1	18.4	6.36	0.143	10.7	0
Sandy Bridge Crossing_Barber Creek	Outside KPF	18/10/2016	8:50	0.2	2	17.72	6.7	0.148	10.2	0
Torrumbarry Weir_Murray River	Outside KPF	8/11/2016	15:00	0.2	1	19.66	6.33	0.083	28.5	3.77
Torrumbarry Weir_Murray River	Outside KPF	9/11/2016	9:33	0.2	2	19.6	6.36	0.082	59.8	3.55
Return Channel_Murray River	Outside KPF	18/10/2016	16:05	0.2	1	16.98	6.7	0.106	67.9	2.99
Return Channel_Murray River	Outside KPF	19/09/2016	12:35	0.2	2	17.01	6.74	0.104	65.8	2.78

Methods

Fish collection

Sampling for larval common carp was conducted while KPF was flooding. Five one week sampling trips were conducted between 12/09/16 and 11/11/16. We attempted to collect larval common carp (<4 week old) from seven sites within KPF as well as nineteen comparison sites outside of KPF (Table 2). Samples from the Wakool, Yallakool, Murray River (Morning Glory) the Goulburn River were provided by researchers from the Department of Land, Water and Environment and Charles Sturt University. Larval fish were sampled using a combination of drift nets, quatrefoil light traps and boat trawls. No set sampling methodology was used as the goal was to sample a minimum of 15 to 30 larval common carp at each site. Typically, five light traps were randomly set at dusk along the littoral edge at each site and retrieved early the following morning. The entire sample from the light trap was preserved in 100% ethanol in the field and brought back to the laboratory for processing. Fish were identified to species level and classified by developmental stage as either larvae (yolk-sac larva, protolarvae, flexion, post flexion, metalarvae) or juvenile/adults (Serafini & Humphries 2004).

YOY (<150mm FL) common carp were collected during routine condition monitoring in February 2017 using a combination of boat and backpack electrofishing (Duncan & Martin 2017). Fish were preserved in 100% ethanol and brought back to the laboratory for processing.

Table 2. Larval and young-of-year samples collected and used for otolith chemistry analysis.

Stream	Site	Site code	KPF or comparison site	Latitude	Longitude	Larval/juvenile carp collected (number sent for LA ICP-MS analysis)	Number of YOY Carp for LA ICP-MS analysis
Barber Creek	Barbers Creek Sample Site 2	BC2	KPF	-35.603	144.207	136* (2)	0
Barbers Creek	Grasses Waterhole	GW	KPF	-35.621	144.215	40*	10
Broken River Lagoon	Broken River Lagoon	BR	KPF	-35.929	144.451	40*	10
Cow Creek	Cow Creek	CC	KPF	-35.578	144.191	418*	0
Burrumbury Creek	Marywood Road	MR	KPF	-35.875	144.458	0	0
Pothole Creek	Pothole Creek	PC	KPF	-35.755	144.406	40*	10
Thule Creek	Thule Creek	TC	KPF	-35.707	144.342	22 (19)	0
Barber Creek	Sandy Bridge Crossing	SB	Comparison	-35.519	144.078	1	0
Barmah Lake	Barmah Lake	BLK	Comparison	-35.949	144.966	27 (25)	0
Broken Creek	Larissa Road	LR	Comparison	-36.099	145.594	117 (30)	0
Broken Creek	McDonald Road	MC	Comparison	-36.016	145.007	0	0
Campaspe River	Strathallan Bridge	ST	Comparison	-36.252	144.737	0	0
Campaspe River	Singer Road	SI	Comparison	-36.274	144.704	0	0
Campaspe River	Ballendella SSR	BA	Comparison	-36.317	144.688	0	0
Goulburn River	Yambuna	YA	Comparison	-36.135	144.999	10 (10)	0
Goulburn River	Shepparton	SH	Comparison	-36.365	145.383	1	0
Goulburn River	McCOYS Bridge	MCB	Comparison	-36.177	145.118	4 (4)	0
Goulburn River	Loch Garry	LG	Comparison	-36.219	145.323	0	0
Moira Lake	Moira Lake	ML	Comparison	-35.944	144.923	35 (29)	0
Murray River	Return Channel	RC	Comparison	-35.697	144.217	75 (16)	0

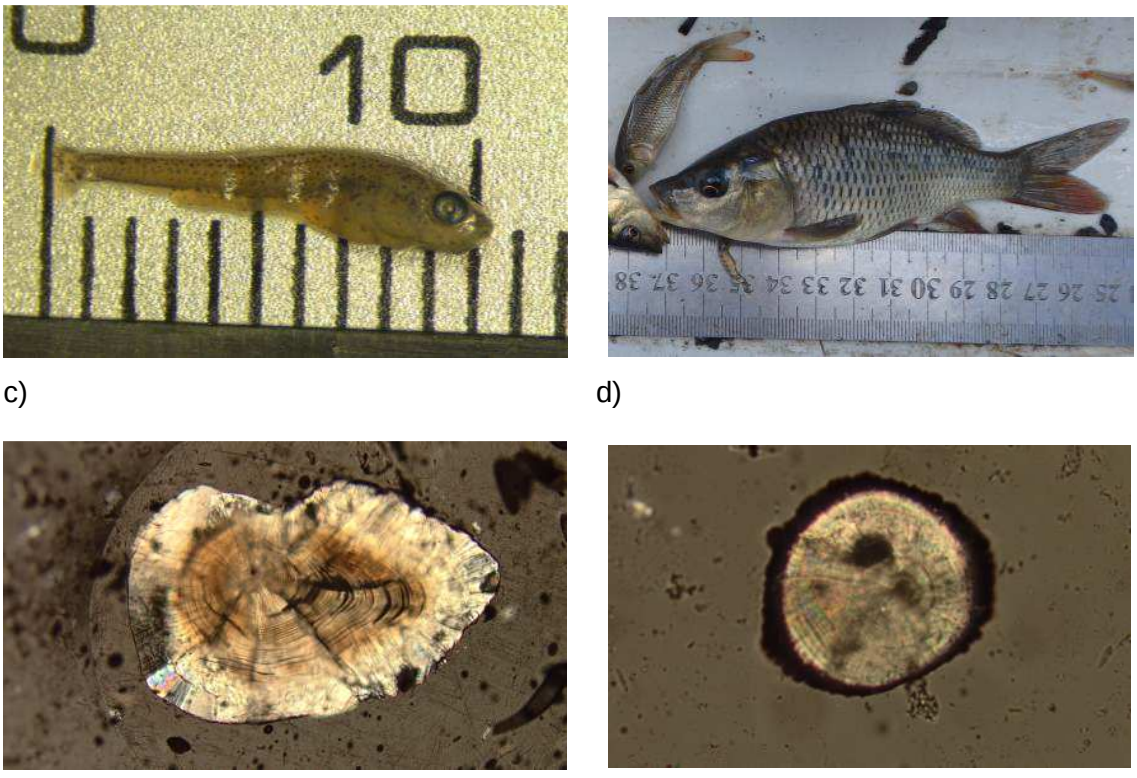
Murray River	Morning Glory	MG	Comparison	-36.079	144.954	8 (8)	0
Murray River	Murray River Barmah Lake	BL	Comparison	-35.9513	144.9543	6 (6)	0
Murray River	D/S Torrumbarry Weir	T1	Comparison	-35.941	144.466	40*	29
Murray River	U/S Torrumbarry Weir	T2	Comparison	-35.946	144.473	570*	29
Wakool River	Edward-Wakool Zone 3-Site 1	WA	Comparison	-35.496	144.453	40 (30)	0
Yallakool River	Edward-Wakool Zone 1_Site 1	YAL	Comparison	-35.488	144.668	26 (26)	0

*These samples had been affected by acid etching and had become brittle and were not suitable for LA ICP-MS analysis. It is not known why some samples were affected and others were not.

Otolith chemistry analysis

The lapillus otoliths were selected for chemical analysis based on previous work that demonstrated that they can be used to discriminate between capture locations (Crook & Gillanders 2006; Macdonald et al. 2012). Lapillus otoliths were extracted from larval and YOY fish (Figures 3a and 3b) under a dissecting microscope, cleaned and rinsed thoroughly with ultrapure water. Ultrapure water was obtained from a Milli-Q system filtered through a Millipore Elix system running at 15MΩ. For each individual, one otolith was mounted and set aside for possible future daily ageing, while the other was used for otolith chemistry analysis. YOY otoliths were mounted whole, proximal surface downwards, on an acid-washed glass slide in an ~200ppm indium-spiked thermoplastic glue (Crystalbond, ProSciTech Pty Ltd; http://Proscitech. com au) (Figure 3c). They were then carefully polished to the primordium using a graded series of wetted lapping films (9 µm and 5µm). Larval otoliths were also mounted whole on an acid washed slide (Figure 3d). These otoliths were mounted with no particular orientation due to the size of the otolith and the difficultly of handling and care was taken to avoid getting crystalbond on the surface of the otolith as this can interfere with chemical analysis.

a) b)



c) d)

Figure 3. a) Larval common carp, b) young-of-year common carp, c) lapillus otolith from a young-of-year common carp and b) lapillus otolith from a larval common carp. Note the daily age rings on both otoliths.

All otoliths were examined and photographed using a fluorescence microscope fitted with a UV light source, digital camera and cellSens image analysis software. The mounted larval otoliths were then cut from the slides using a glass cutter, then carefully mounted onto the analysis slide

using crystalbond. This technique ensures the larval otoliths are aligned as accurately as possible for the laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS) analysis. Samples were arranged randomly to remove any systematic error that may arise during laser-ablation analysis. Each analysis slide held 30 larval or YOY otoliths. The samples were again rinsed in ultrapure water and air dried overnight at room temperature and stored in individual resealable bags.

Otoliths were analysed for trace elements using a LA ICP-MS. The system consisted of a New Wave Research 213 nm high performance (Nd:YAG) ultraviolet laser ablation system coupled to an Agilent 7900 ICP-MS. Two analysis slides with 30 otolith sections on each were placed in a sealed chamber and viewed remotely via an image analysis system. Each otolith was sampled using a 30 µm diameter 'spot' ablation. For larvae, a single sample was collected over the primordia, whilst for young-of-year fish one sample was collected at the primordia (core) and another adjacent to the margin (edge). The sample at the primordia was considered to represent the natal origin and the sample adjacent to the margin represented the period of life immediately preceding capture.

Each otolith spot was sampled using a 30 µm laser beam diameter at a pulse rate of 5 Hz and a fluence of 10 J.cm-2. Otoliths were pre-ablated for three seconds to eliminate any possible surface contamination. Ablation occurred in a helium flushed chamber that was mixed with argon for injection into the plasma. The elemental isotopes chosen for analysis were ^{7}Li, ^{25}Mg, ^{44}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba as well as ^{43}Ca that was measured for use as the internal standard and ^{115}In used as an indicator to discriminate between otolith material and thermoplastic glue. The internal otolith concentration of ^{43}Ca was assumed to be constant at 38.8% by weight (Yoshinaga et al. 2000). Element concentrations were calibrated against the National Institute of Standards (NIST) 612 glass reference pallet (Lahaye et al. 1997). Trace element measurements of the blank sample gases were recorded for 20 seconds prior to each sample ablation of 25 seconds, with the concentration of trace elements recorded every 0.17 seconds. Data reduction, including background subtractions, calculation of minimum detection limits (LOD), and mass count data conversion to concentrations (ppm) was done for each individual sample using Iolite v2.5 software. Otolith elemental concentration data were then converted to molar concentrations and standardised to ^{43}Ca (element:Ca, µmol.mol^{-1}).

Internal precision and accuracy were assessed by analysing the NIST 612 as an unknown sample against the actual concentrations, whilst external precision was assessed by measurements of MACS-3 (United States Geological Survey) calcium carbonate reference material. The NIST 612 standard and MACS-3 pellet were analysed twice at the beginning and end of each sampling session, as well as after every 10 ablations to correct for short-term instrumental drift. Average recovery (%) for the NIST 612 ranged from 99.98% to 100.04% for all elements. Average relative standard deviation (RSD) (%) for NIST 612 was: ^{7}Li 0.7, ^{24}Mg 0.5, ^{25}Mg 0.9, ^{55}Mn 0.5, ^{88}Sr 0.4, ^{138}Ba 0.7. External precision (RSD) (%) assessed by measurements

of the MACS-3 reference material for each element was: ^{7}Li 3.0, ^{24}Mg 3.1, ^{25}Mg 3.5, ^{55}Mn 5.2, ^{88}Sr 2.1, ^{138}Ba 6.2. Average LOD (µmol.mol^{-1}) based on three times the standard deviation of the blank gases adjusted for ablation yield (Lahaye et al. 1997) were: ^{7}Li 0.51, ^{24}Mg 0.20, ^{25}Mg 0.45, ^{55}Mn 0.24, ^{88}Sr 0.01, ^{138}Ba 0.01.

Otolith chemistry statistical analysis

Larvae

Data were analysed in the software packages PRIMER-E (Clarke & Warwick 2001) and PERMANOVA+ (Anderson et al. 2008). Data were log(x+1) transformed and normalised. Euclidian distances were used to generate the dissimilarity matrix, which formed the basis of all subsequent analyses. Patterns in the data were visualised using Principal Coordinates Ordination (PCO). Young-of-year core signatures were included in a PCO with larval fish to determine if their signatures overlapped. Any YOY that were not within the ordination space of the larvae were removed from further analysis as they likely came from an un-sampled natal area. A single-factor PERMANOVA was conducted for each element separately to determine if each was capable of spatially differentiating between sampling locations. Given all elements were significantly different among sites (see results), they were all included in a multivariate single-factor PERMANOVA. Significance of results was calculated using 9999 permutations of the data and a P-value <0.05 was considered significant. Discriminant-based analyses were carried out using canonical analysis of principal coordinates (CAP) to determine if larvae could be allocated to their sampling location based on their core elemental chemistry using CAP analysis. The results of this analysis as well as the PCO analysis were used to allocate sampling sites into zones given some localities had a high overlap of elemental chemistry (see results).

Young-of-year

CAP analysis was conducted to determine if the core elemental signature could be used to estimate the natal zone (see above) of the YOY fish. Larval fish were allocated to their natal zone for the analysis and the YOY (core chemistry only) fish were included as ‘unknown’ samples so they could be classified into one of the natal zones.

Results

Larval common carp were successfully sampled from six of the seven sites within KPF and 14 of the 19 sites outside the forest. However, otoliths from five of the sites within KPF and two Murray River sites were found to be damaged when they were extracted from the fish. The daily increments were not visible on the otoliths and they were extremely brittle and prone to break. This has implications for laser ablation given the core of the otolith is difficult to discern and the chemical signature may have been impacted, which would reduce confidence in the results. Therefore, these affected otoliths were not analysed.

Multi-elemental PCO analysis demonstrated that there was some differentiation among core chemistry signatures by site for the larval fish (Figure 4a). PERMANOVA confirmed that each individual element was significantly different among sites (Table 3), as were the multi-elemental otolith signatures (Table 3). Forty-nine percent (99/202) of larval fish were successfully classified into their sampling location using CAP analysis (Table 4). The classification accuracy varied from 0% (Shepparton) to 90% (Yambuna) (Table 4). Based on these results as well as the PCO, it was clear that some sampling locations should be grouped into zones given they had similar otolith core chemistry. These zones were KPF (Grasses Waterhole and Pothole Creek); Barmah (Barmah Lake, Moira Lake, Murray River at Barmah Lake and Murray River at Morning Glory); Broken (Larissa Road); Goulburn (McCoys Bridge, Shepparton and Yambuna); Return Channel (Return Channel); Wakool (Edward-Wakool Zone 3 site 1) and Yallakool (Edward-Wakool Zone 1 site 1) (Figure 4b). The classification success when the CAP analysis was carried out by zone was more successful with 62% percent (126 out of 202) of larval fish successfully classified into their sampling location (Table 5). Success in classifying larval fish from KPF was reasonably high (76%) and indicates that there is a distinct KPF chemical signature. Classification success was greatest for the Goulburn (80%), Yallakool (73%), and Return Channel (71%) (Table 5).

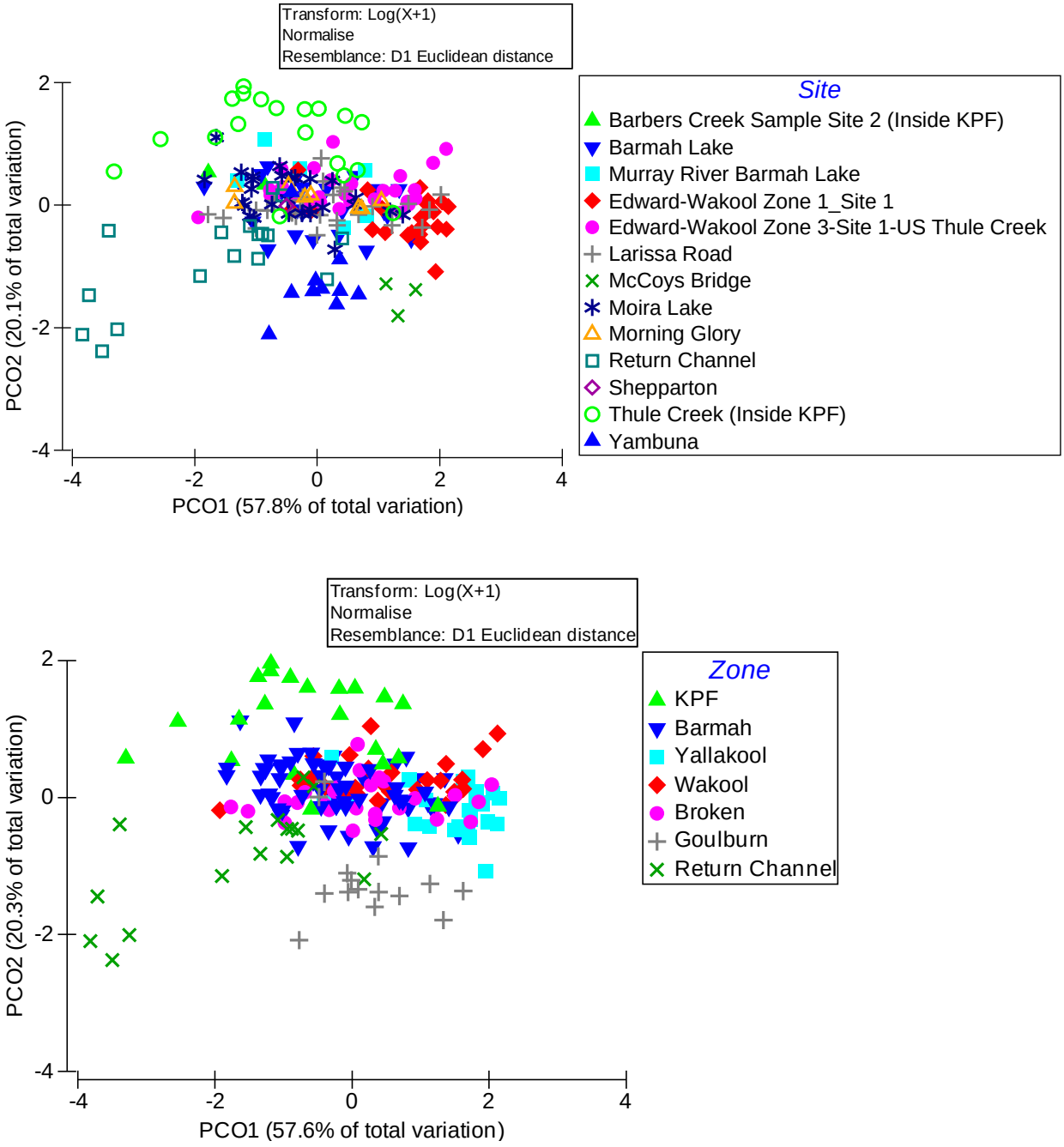


Figure 4. a) Principal Coordinates analysis (PCO) of the larval multi-elemental core signature at each sampling site and b) PCO of the larval multi-elemental core signatures grouped by zone.

Table 3. Mean squares (MS) and significance levels for the single-factor univariate and multivariate PERMANOVA of the larval carp between sites.

Factor	d.f.	MS Li	MS Mg	MS Mn	MS Sr	MS Ba	Multi-elemental
Site	13	3.697*	1.788*	5.687*	1.101*	5.193*	17.467*
Residual	188	0.293	0.265	0.505	0.070	0.333	1.466

*P<0.0001

Table 4. Classification success (number of individuals) for larval fish when grouped by site for CAP analysis. Forty-nine percent of samples were correctly classified to their sampling location.

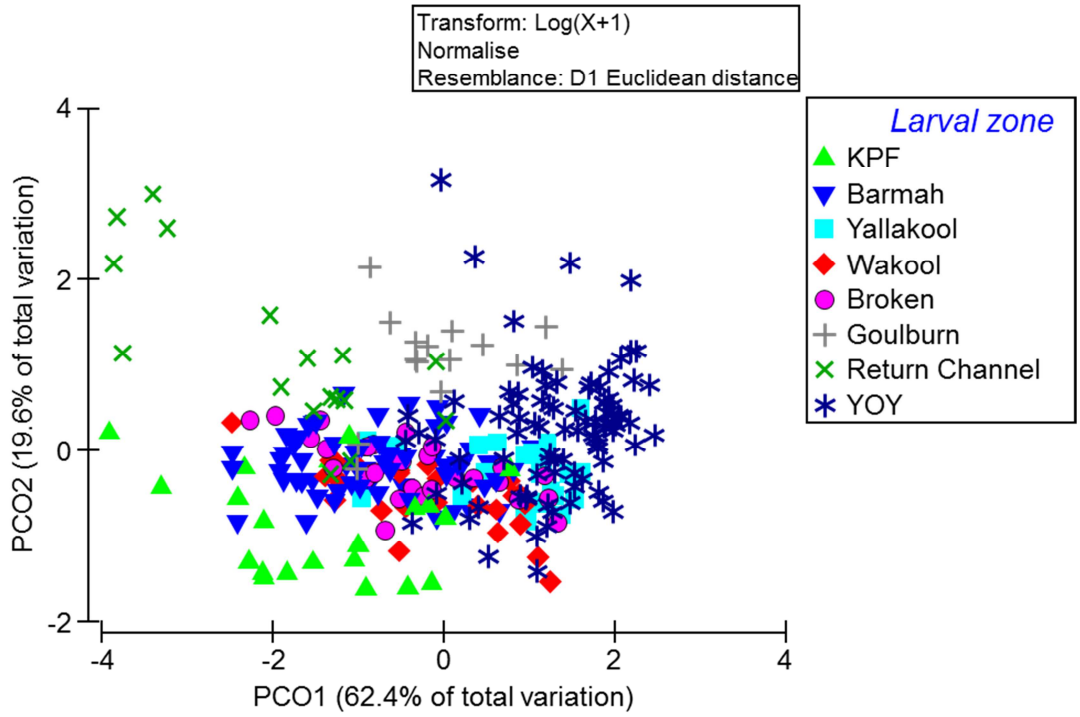
Orig. group	ML	RC	SH	WA	BLK	MG	BC2	LR	YA	BL	YAL	TC	MCB	Total	%correct
Barbers Creek Sample Site 2 (Inside KPF)						1	1							2	50
Barmah Lake	7			3	4	5		2		1	3			25	16
Edward-Wakool Zone 1_Site 1			1	3	1	4					17			26	65
Edward-Wakool Zone 3-Site 1-US Thule Creek	3		1	8	1	2		8			5			28	29
Larissa Road	3			6	1	2		14			2			28	50
McCoys Bridge		1											3	4	75
Moira Lake	12			1	8	1		3		1	2			28	43
Morning Glory	1				1	5					1			8	63
Murray River Barmah Lake										5		1		6	83
Return Channel	1	8	3		1	2			1				1	17	47
Shepparton						1								1	0
Thule Creek (Inside KPF)			1		1	3				1		13		19	68
Yambuna	1								9					10	90

Table 5. Classification success (number of individuals) for larval fish when grouped by natal zone for CAP analysis. Sixty-two percent of samples were correctly classified to their natal zone.

Zone	KPF	Barmah	Yallakool	Wakool	Broken	Goulburn	Return Channel	Total	% correct
KPF	16	5						21	76
Barmah	5	39	9	6	6		1	67	58
Yallakool		6	19		1			26	73
Wakool		2	6	11	9			28	39
Broken		3	3	5	17			28	61
Goulburn		2				12	12	15	80
Return Channel		3		1		1	1	17	71

PCO analysis of YOY core data against larval chemical signatures revealed that 18 fish needed to be removed from the analysis as they fell outside the ordination space of the larval fish (Figure 5a, Appendix 1). Removing these fish resulted in remaining fish falling within or very close to the ordination space of the larval fish (Figure 5b). In contrast, YOY edge chemical signatures were distinct from larval core chemical signatures (Figure 6a) and YOY core and edge signatures were clearly differentiated to one another (Figure 6b). No YOY from outside KPF were assigned to the KPF natal zone at the core of their otoliths. The YOY from the Murray d/s of Torrumbarry and u/s of Torrumbarry were most closely related to the Goulburn (58.3% and 42.1% respectively) and Yallakool (29.2% and 47.4% respectively) zones (Table 6). Only a single YOY collected from KPF (Pothole Creek) was classified into the KPF natal zone (Table 6). No other KPF YOY were classified into the KPF zone at the core of their otolith (Table 6). The core otolith signatures of Grasses Waterhole were most similar to the Yallakool zone (66.7%) while Pothole Creek was most similar to the Yallakool, Barmah and Goulburn zones (40.0%, 20% and 20% respectively). Broken River lagoon was most similar to the Goulburn zone (75%) with some additional contribution from Barmah and Torrumbarry (12.5% each).

a)



b)

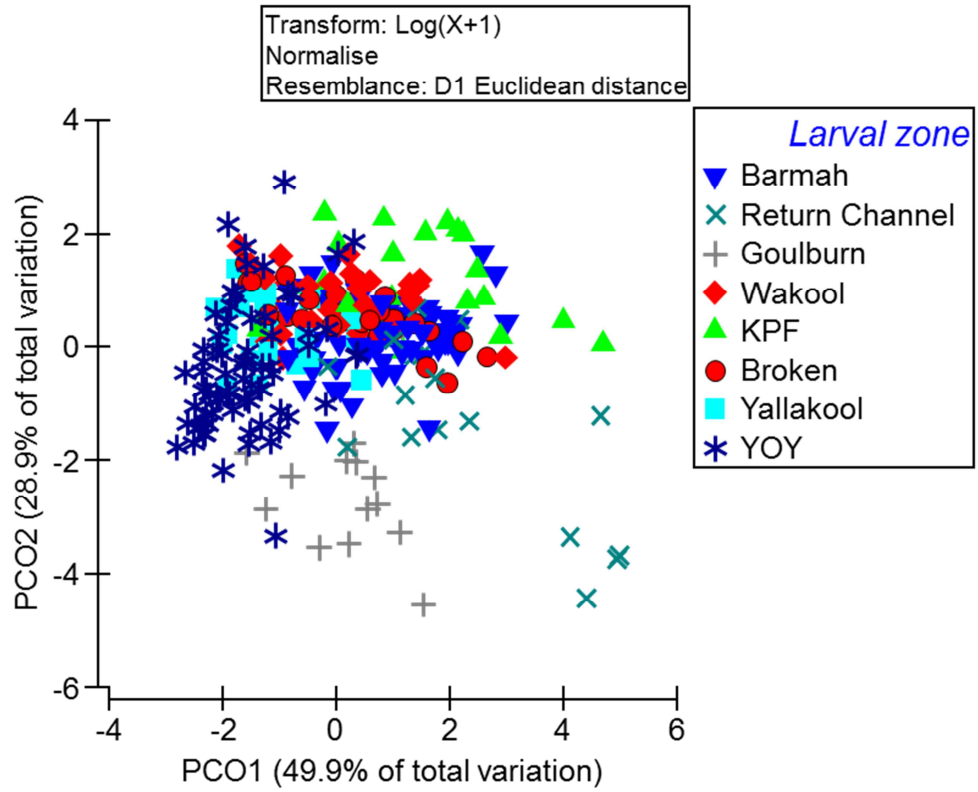
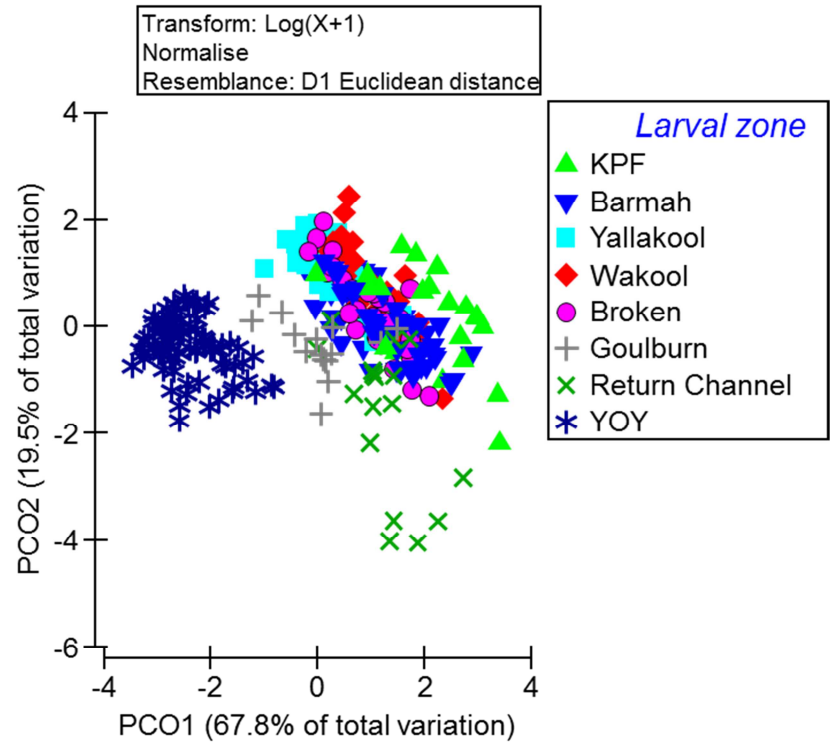


Figure 5. a) Principal Coordinates analysis (PCO) of the larval multi-elemental core signature at each zone with young-of-year fish (core) shown as blue stars, b) PCO of the larval multi-elemental core signature at each zone with 18 young-of-year outliers removed (see results).

a)



b)

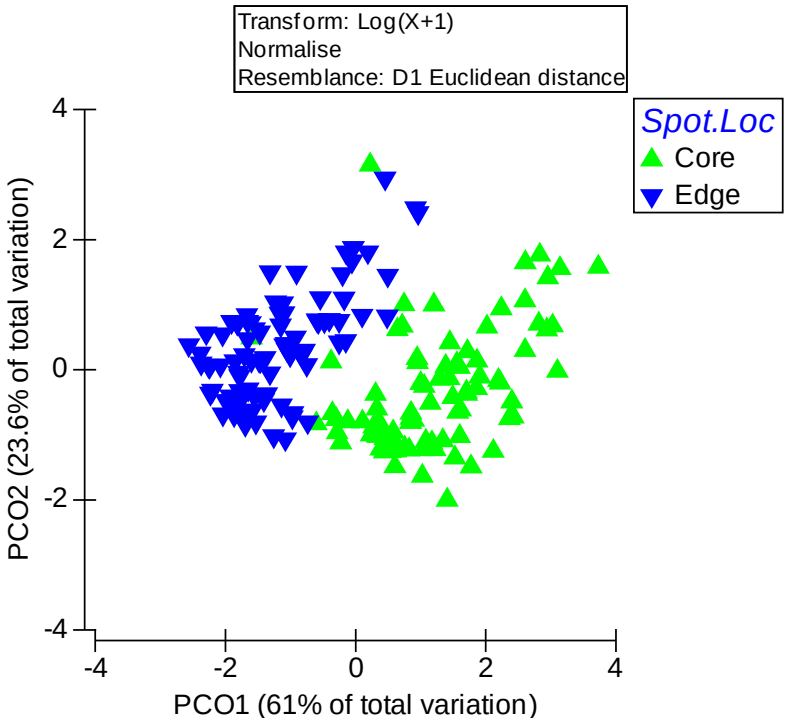


Figure 6. a) Principal Coordinates analysis (PCO) of the larval multi-elemental core signature at each zone with young-of-year fish (edge) shown as unknowns, b) PCO of the YOY core and edge multi-elemental signature. Note that for the latter, two samples were removed (one from u/s of Torrumbarry and one from Broken River Lagoon) because they were outliers and skewed the PCO.

Table 6. Results of the CAP analysis of estimated natal site (% of catch) for young-of-year common carp captured at five locations in early 2017. The actual and estimated % compositions (based on 9999 simulations) of the larval zones in 2016 are given.

Larval fish			Estimated YOY natal site (%)				
			Koondrook–Perricoota Forest			Murray River	
Natal zone	Known (%)	Estimated (%)	Grasses Waterhole (n=9)	Pothole Creek (n=10)	Broken River Lagoon (n=8)	D/S Torrumbarry Weir n=24)	U/S Torrumbarry Weir (n=19)
Barmah	33.2	29.7	22.2	20.0	12.5	8.3	
Broken	13.9	16.3					
Goulburn	7.4	6.9		20.0	75.0	58.3	42.1
Torrumbarry	8.4	6.9					
KPF	10.4	10.4		**10.0**			
Wakool	13.9	11.4	11.1	10.0		4.2	10.5
Yallakool	12.9	18.3	66.7	40.0	12.5	29.2	47.4

Discussion

The ability to identify the source population of common carp YOY is dependent on sampling larval fish from all natal populations and for these populations to have differences in water chemistry. Here we undertook extensive larval sampling in a wide area upstream and downstream of KPF in order to characterise as many natal locations as possible and used previous projects to focus our sampling on areas known to be key natal locations in this area (Crook & Gillanders 2006; Macdonald & Crook 2014). However, flooding was extensive in 2016 and it is possible that some YOY common carp could have originated from outside the study area. This appears to be the case given some YOY core signatures are clearly differentiated from the larval signatures.

Despite this, this study has demonstrated that larval common carp captured from KPF were allocated to the KPF natal zone with 76% accuracy. This is a good allocation success result and indicates that if YOY common carp originated from KPF, there is a good chance that they could be successfully classified to the KPF natal zone. This is also supported by the reasonably high classification success for larval fish outside of KPF to their natal zone, which was comparable to the classification success achieved with common carp in a similar area between 2005 and 2008 (Macdonald & Crook 2014). It is important to note that the exclusion of YOY fish in our study was done by eye rather than using a 95% confidence interval to identify fish that were outside the larval core chemistry ordination space. This can have an impact on the classification accuracy of larval and YOY fish into the natal zone. However, the entire suite of analyses was initially run with the full data set (i.e. no YOY excluded). Results were consistent with those presented here with the same single fish from Pothole Creek being assigned to the KPF zone.

Here, we have shown that only a single YOY common carp out of 70 analysed (once outliers were discarded) were classified to the KPF natal zone. This was an unexpected result, particularly for the YOY collected from within KPF itself given the presence of larval fish within KPF during flooding and the successful recruitment following the 2014 managed flood (Duncan *et al.* 2016). There are four possible explanations for these results; 1) too few larvae from KPF (21 individuals) were analysed resulting in a poorly resolved otolith chemical signature for the forest, 2) YOY fish originating from KPF were present, but in very low numbers and were not sampled, 3) that YOY fish from KPF migrated either downstream or upstream outside of the study area, or 4) that carp mortality at the larval stage within KPF was high. The first explanation is a possibility given the majority of samples that contributed to the KPF zone were from a single location (Thule Creek) within the forest. Consequently, it is possible that the chemical signature was different at the opposite end of the forest. Previous work has shown that the chemical signature of KPF changes over a matter of months when cut off from the river, though there are no data to indicate whether this is also the case with an actively flooding forest (Duncan *et al.* 2016). The second explanation is also possible, but with a sample size of 70 YOY common carp

combined with a KPF natal zone defined with 76% accuracy, we would expect to be able to identify KPF-origin carp even if they were in low abundance. The third explanation is a possibility given previous work has demonstrated that YOY common carp were found downstream of nursery sites (Crook & Gillanders 2006; Macdonald & Crook 2014). Thus there is a chance that YOY originating from KPF are present in sites outside the study zone. This could be tested relatively easily by collecting 1+ aged carp from additional sites in the Murray River and Barber Creek and ageing them to confirm if they were the result of reproductive events during late 2016. Their core otolith chemistry could then be analysed to determine if it matches the KPF signature. The fourth explanation, that larval common carp in KPF had a very high mortality rate, is best supported by the data presented here. We have evidence that the larval fish were affected by acid in the water (low pH) in KPF given so many of the otoliths were brittle and the growth rings weren't visible. Previous work on golden perch (*Macquaria ambigua*) larvae and juveniles held in aquaria containing river red gum (*Eucalyptus camaldulensis)* leaf litter resulted in a reduced pH and was associated with very high mortality rates, particularly when dissolved oxygen was low (Gehrke *et al.* 1993). It is possible that common carp larvae may be similarly affected by combined low pH and low dissolved oxygen conditions that occurred during the 2016 flood during the critical spawning and larval development period (Table 1).

Successful recruitment of common carp was recorded in 2015 following the 2014 managed event (Duncan *et al.* 2016). This managed event was relatively small with approximately 26,000ML of water being diverted into KPF resulting in no floodwater reaching Sandy Bridge (Barber Creek), compared to over five months of flows of up to 5000ML/d at that location following the 2016 flood. We hypothesise that given the 2014 event inundated an area that was more regularly flushed by flooding post December 2010, that it did not inundate as much leaf litter as the 2016 flood resulting in much better water quality (dissolved oxygen levels remained between 4.1 and 8.4mg/l during October and November 2014, NSW DPI unpublished data).

The YOY collected from the three KPF locations were classified into a variety of natal zones. The YOY from Broken River Lagoon primarily originated from the Goulburn zone (75%). Broken River Lagoon is connected to the Murray River and is easily accessible to common carp from the Murray River during high flows. In contrast, Grasses Waterhole and Pothole Creek were dominated by fish originating from the Yallakool zone (70% and 40% respectively) and Barmah zone (22.2% and 20% respectively). The most likely explanation is that juvenile fish migrated into KPF from spawning zones via Thule Creek and the Murray River during the later stages of the flood when water quality was improving.

The YOY fish collected from d/s of Torrumbarry and u/s of Torrumbarry (Murray River) had a mixed origin from the Goulburn (58.3% and 48.1% respectively) and the Yallakool (29.2% and 47.4%) respectively. These results are consistent with previous work. In 2006, YOY collected upstream of Torrumbarry Weir were composed of common carp that originated from Barmah-Millewa Forest (80.8%) and the lower Goulburn River (19.2%) (Macdonald & Crook 2014).

Similarly, in 2003, a study of YOY common carp collected upstream of Torrumbarry estimated that 98% originated from Barmah Lakes (Crook & Gillanders 2006). The former study used similar methods as in this study where larval signatures were compared to core signatures of YOY fish, while the latter study only used YOY fish to infer the natal site. Neither of these studies collected samples from the Yallakool or Wakool systems. If some of the YOY did originate from those rivers, they would have been erroneously assigned to other natal zones. However, given both these studies were carried out during the millennium drought, there was no direct connectivity between the Wakool-Yallakool systems via KPF to the Murray River. It is highly unlikely that that YOY could have made their way to the Murray River upstream of Torrumbarry during those studies. Here, we have shown that under flood conditions, YOY common carp will move extensively and are capable of colonising both wetland and river habitat.

Consequently, the data presented here suggests that the majority of YOY in KPF and the Murray River around Torrumbarry Weir were the result of reproductive events outside of KPF. Connectivity of KPF to surrounding rivers was maintained well into early December (either via Thule Creek, Barber Creek or Swan Lagoon), which would have enabled YOY fish to enter the forest towards the end of the inflows. Dissolved oxygen levels were beginning to increase at this time and may have been sufficient to protect the juvenile fish from the effects of acid water (low pH).

Conclusion

An important limitation of the current study is that YOY were only collected from two sites in the Murray River adjacent to KPF. While the combined results of acidic damage to KPF larval otoliths and near absence of recruitment of common carp in KPF and the Murray River adjacent to KPF suggest this is unlikely, it is possible that YOY originating from KPF dispersed beyond the study area. It is recommended that any future otolith chemistry analysis of common carp belonging to this year class should be collected from a wider area and their core otolith chemistry compared to the larval samples collected in this study to determine if they were potentially of KPF origin.

Otolith chemistry analysis of larval common carp from KPF and surrounding waterways has demonstrated that there are distinct chemical signatures associated with different zones, consistent with previous studies. None of the 58 YOY sampled upstream and downstream of Torrumbarry Weir originated in KPF, and only a single YOY collected within KPF was classified to the KPF natal zone. The results of this study, combined with the results from the 2014 managed event demonstrate that the survival of common carp to YOY stage is most likely dependent on water quality being maintained to an adequate level.

A pattern of small, regular managed flood events is likely to promote greater survival of larval common carp within KPF by minimising the risk of acidic and anoxic blackwater. If connectivity to the Murray River is enabled before the floodplain dries out, these common carp could feasibly

colonise the river. Repeating the current study after a small flood followed by connection to the Murray River would provide additional data on the potential contribution of KPF to the riverine common carp population.

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Appendix

Appendix 1. Young-of-year samples removed from CAP analysis because they were not within the ordination space of the larval fish, indicating they were from natal grounds that were not sampled.

Sample ID	Collection location
X17	Broken River Lagoon
XY24	Broken River Lagoon
Y22	Broken River Lagoon
X23	D/S Torrumbarry Weir
X30	D/S Torrumbarry Weir
XY28	D/S Torrumbarry Weir
XY6	D/S Torrumbarry Weir
Y26	D/S Torrumbarry Weir
Y6	D/S Torrumbarry Weir
Y9	Grasses Waterhole
XY21	U/S Torrumbarry Weir
XY23	U/S Torrumbarry Weir
XY27	U/S Torrumbarry Weir
XY7	U/S Torrumbarry Weir
XY9	U/S Torrumbarry Weir
Y11	U/S Torrumbarry Weir
Y19	U/S Torrumbarry Weir
X17	Broken River Lagoon