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Assessment of stocking effectiveness for Murray cod (*Maccullochella peelii*) and golden perch (*Macquaria ambigua*) in rivers and impoundments of south-eastern Australia

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Abstract. Stock enhancement is a management tool used for fishery recovery worldwide, yet the success of many stocking programs remains unquantified. Murray cod (*Maccullochella peelii*) and golden perch (*Macquaria ambigua*) are important Australian recreational target species that have experienced widespread decline. Stocking of these species has been undertaken for decades, with limited assessment of effectiveness. A batch marking and recapture approach was applied to assess stocked Murray cod and golden perch survival, contributions to wild fisheries, and condition in rivers and impoundments. Stocked fish were marked with calcein. Marked fish were detected during surveys undertaken 3 years and 10 months from initial marking, and it is probable that marks will persist beyond this time. The proportion of calcein marked fish in the population sub-sample whose age was equal to, or less than, the number of years since release, varied by 7–94% for Murray cod, and 9–98% for golden perch. Higher proportions of marked fish were found in impoundments than rivers. Marked Murray cod had significantly steeper length–weight relationships (i.e. higher weight at a given length) to unmarked fish. Our results show that application of methods for discriminating stocked and wild fish provides critical information for the development of adaptive, location-specific stocking strategies.

Additional keywords: calcein, fish stocking, mark retention, otoliths, population enhancement, species decline.

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Introduction

Recovery initiatives to rehabilitate declining fish populations include harvest regulations, harvest quantification, mitigation of stressors, and stock enhancement (Cowx 1994; Molony *et al.* 2003; Halverson 2008). Despite the widespread use of stock enhancement as a fisheries management tool and the development of methods to distinguish stocked and wild fish (e.g. Mohler 1997; Munro *et al.* 2008), there is still little assessment of stocking success worldwide (Lorenzen 2014). Quantifying stocking success is therefore urgently needed to optimise such programs and to maximise outcomes.

In south-eastern Australia's Murray–Darling Basin (MDB), stock enhancement has formed the basis of recovery and ongoing management of Murray cod (*Maccullochella peelii*) and golden perch (*Macquaria ambigua*) for several decades. Murray cod are Australia's largest wholly freshwater fish, recorded to 1800 mm and 113.5 kg (Rowland 2005). Golden perch grow to a maximum of 760 mm and 23 kg (Lake 1967). Both species are important recreational targets (Rowland 2005; Brown 2010). Fisheries within the MDB have declined over the past 100 years (Reid *et al.* 1997) and native fish communities are estimated at 10% of pre-European settlement levels (Murray–Darling Basin Commission 2003). Several factors have contributed to the decline in native fish abundance and distribution, including flow regulation, habitat modification, reduced water quality, in-stream barriers, introduced or alien species, disease, exploitation, translocation and indiscriminate stocking (Murray–Darling Basin Commission 2003).

To prevent further Murray cod and golden perch decline, fishery managers have implemented a range of initiatives that include; closure of the commercial freshwater fishery in all states, except the South Australian golden perch fishery (Ye 2004), introduction of closed seasons and harvest restrictions such as bag and size limits, and implementation of stocking programs with hatchery-reared fish to conserve and recover both species (Lintermans *et al.* 2005). Since 1971, 12.89 million Murray cod (Ingram *et al.* 2011), and over 32 million golden perch (Gillanders *et al.* 2006), produced at both government and private (commercial) fish hatcheries across several states, have been stocked into waterways and impoundments across the MDB to support recreational fishing (Ingram *et al.* 2011) and for conservation efforts (Lintermans *et al.* 2005).

If stocking is to play a major role in the ongoing conservation and rehabilitation of Murray cod and golden perch populations, then assessment of the effectiveness of these stocking programs is required to optimise release strategies and support ecologically and economically defensible decision making. For example, despite closure of the New South Wales (NSW) Murray cod commercial fishery in 2001, and implementation of recreational harvest regulations such as bag and size limits in 1992 (Rowland 2005), stocking of this species continues based on the perception that natural recruitment is insufficient to sustain the fishery. In recognition of the need for evidence regarding the outcomes of fish stocking, an initial study was undertaken between 2002 and 2007 to assess the effectiveness of stocking golden perch in the Edward River, Murrumbidgee River, and Billabong Creek (Crook *et al.* 2016). Crook *et al.* (2016) marked hatchery-reared golden perch with calcein (Billabong Creek) and alizarin complexone (Murrumbidgee and Edward rivers), and demonstrated that stocked golden perch contributed 18–100% to populations. Other less extensive assessments of golden perch stocking in the MDB have been performed (see Harris 2002; Hunt *et al.* 2010), but to our knowledge the outcomes of Murray cod stocking have never been studied in detail. Long-term stocking success studies using chemical marking require a known degree of mark retention and detectability over time (e.g. Mohler 2003; Hill and Quesada 2010). Further research into the long-term retention of calcein marks was highlighted as a priority, so that the limitations of this technique can be understood (Crook *et al.* 2009).

Stocking effectiveness can be affected by hatchery practices that produce variable fish quality with regard to growth and condition. For example, hatchery-reared Atlantic salmon (*Salmo salar*) in Canada were shown to be smaller and in poorer condition than comparable wild fish leading to reduced stocked fish survival (McDonald *et al.* 1998). The release of hatchery fish in poor condition usually results in low survival and has the potential to undermine enhancement and recovery strategies (e.g. Cameron and Baumgartner 2012). Conversely, competition from hatchery-reared fish can lead to replacement of wild fish with stocked fish, particularly if they are released at a larger size than wild recruits of the same yearly cohort (Sweeting *et al.* 2003). Quantification of condition parameters between stocked and wild fish is therefore important for determining the outcomes of fish stocking and to inform stock enhancement strategies.

The present study focuses on the stocking of Murray cod and golden perch in the MDB and aimed to: (1) use a chemical marking technique to identify stocked fish and estimate their contributions to populations in several rivers and impoundments, (2) evaluate the necessity of stocking for fishery augmentation, (3) compare length–weight relationships and condition of stocked (marked) fish with unmarked fish, and (4) to assess the long-term retention of calcein marks in golden perch and Murray cod.

Materials and methods

Study sites

Murray cod and golden perch were sampled from the Murray and Murrumbidgee rivers, Burrinjuck Dam (Murrumbidgee River catchment) and Copeton Dam (Gwydir River catchment) within the MDB (Fig. 1). Reaches of the Murray and Murrumbidgee rivers were selected for inclusion in the study as they formed part of a pre-existing long-term project and both reaches were not previously stocked with calcein marked Murray cod and golden perch. Both reaches support popular recreational fisheries (Rowland 2005). The Murray River reach extends downstream of Yarrawonga Weir (36°00′31″S, 145°59′59″E) to Tocumwal (35°48′51″S, 145°33′23″E). The Murrumbidgee reach extends from Berembed Weir (34°52′47″S, 146°50′13″E) to Euroley Bridge (34°38′21″S, 146°22′25″E; Fig. 1).

Burrinjuck and Copeton dams were selected for inclusion in the study to provide data for Murray cod and golden perch populations from impoundments. The extent of successful, wild recruitment of Murray cod spawned in large, deep impoundments, with extensive water level fluctuations is not known, whereas golden perch are not thought to reproduce effectively in closed waters (King *et al.* 2009). Prior to commencing this study, Burrinjuck and Copeton dams did not contain calcein marked Murray cod and golden perch, but received regular stockings of unmarked hatchery-reared fish (Rowland 1995). Thus, unmarked fish in these dams could be of either stocked or wild origin. Both impoundments contain popular Murray cod and golden perch recreational fisheries (e.g. Rowland 2005; Hall *et al.* 2012).

Study design

To identify stocked Murray cod and golden perch, hatchery-reared fingerlings (30–50-mm total length) were marked with calcein (2,4-bis-[*N*,*N*′–di(carbomethyl)-aminomethyl]fluorescein) using osmotic induction (Crook *et al.* 2006, 2009; Baumgartner *et al.* 2012). Marked Murray cod were usually released in December or January, and golden perch from February to March depending on hatchery availability. Calcein marked hatchery-reared Murray cod and golden perch were released, in equal numbers, at the four sampling sites within each river reach, and released at various access points around Burrinjuck and Copeton dams. The distance between impoundment release and sampling sites varied between 0 and 5 km. It was assumed that released fish dispersed throughout the existing population. Government and private native fish hatcheries produced fish for this study and all fish stocked into the study reach were calcein marked. However, a small number (<1515 per year) of unmarked Murray cod were inadvertently released in the Murray River in 2010 and 2012, and a larger number (between 7374 and 25 111)

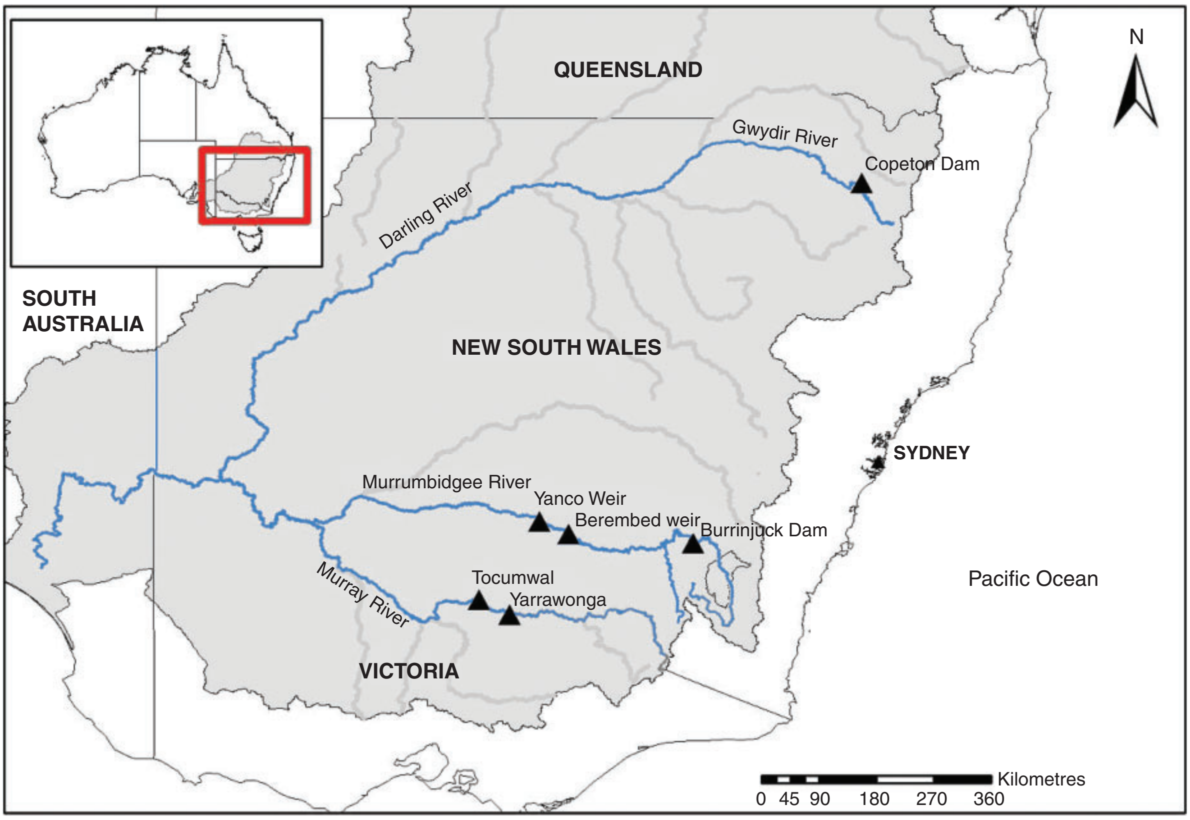

Fig. 1. Study sites for the Murray and Murrumbidgee rivers, Burrinjuck and Copeton dams in New South Wales, Australia. The Murray–Darling Basin is represented by the shaded area.

Table 1. Murray cod and golden perch stocking releases into the Murray and Murrumbidgee rivers, Burrinjuck and Copeton dams
These data are summarised by year of spawning and whether fish were calcein marked

Waterbody	Year of spawning	Murray cod		Golden perch	
		Calcein marked	Unmarked	Calcein marked	Unmarked
Murray River	2009	22 364	1515	23 924	25 211
	2010	18 972		27 118	
	2011	13 377	1509	3636	
	2012	35 740		27 908	
Murrumbidgee River	2009	20 143		65 552	7374
	2010	18 075		50 907	
	2011	12 000		72 481	
	2012	18 888		56 362	
Burrinjuck Dam	2009	60 000		80 000	14 400
	2010	60 000		66 000	
	2011			78 000	
	2012	36 000			
Copeton Dam	2009	60 000		90 000	
	2010	54 000		95 000	
	2011			89 727	
	2012				

of unmarked golden perch were released into the Murray and Murrumbidgee rivers, and Burrinjuck Dam in 2010 (Table 1). Our study was limited by the possibility that some of these unmarked hatchery-reared fish were re-captured, which may have caused underestimates of stocked fish in the affected waterbodies.

The Murray and Murrumbidgee River study sites are constrained by upstream migration barriers (weirs); however, fishways along the Murray River (Barrett 2008), and flooding in both systems during 2010 and 2011 (Whitworth *et al.* 2012), provided opportunity for marked and unmarked fish to migrate in and out of both study reaches. Burrinjuck and Copeton dams offer no fish passage from downstream; however, illegal stocking of unmarked Murray cod and golden perch has been reported in many MDB waterbodies (Anderson *et al.* 1992*b*; Lintermans 2004; Faulks *et al.* 2010), with such activity in the respective upper Copeton and Burrinjuck dam catchments potentially resulting in unmarked fish migrating to the impounded water.

Fish used in this study were also utilised for other research where destructive sampling was required to calculate age and length at maturity (Forbes *et al.* in press). Although non-destructive external detection of calcein marks has been successfully applied for younger age classes (Baumgartner *et al.* 2012; Crook *et al.* 2012), field detection of calcein marks in the current study resulted in many false negatives. Ingram *et al.* (2015) also found that non-destructive field detection methods for calcein were unreliable. As such, we only used otoliths from available collections for age and calcein assessment.

Fish were collected from all sites over 1 week, twice per year (austral seasons autumn and spring), with seasonal data pooled within each year to assess age structure on an annual basis. Fish were collected from the Murray River, Murrumbidgee River, Burrinjuck Dam and Copeton Dam between 2009 and 2013. Sampling generally commenced 1–4 months after initial fish release. The maximum time between fish release and sampling was 3 years 10 months. Boat electrofishing was used to collect a broad size range of fish from river and impoundment sites. Gill netting was used to increase fish numbers collected from impoundments. Electrofishing used a boat fitted with a Smith-Root GPP 7.5 electrofisher unit, operated at 1000 V DC, 120 Hz, 10–30% duty cycle and producing between 3 and 6 amps. Gill netting involved up to 15 nets from 25 to 55 m length with mesh sizes ranging from 40 to 300 mm, and multi-mesh panel nets (5 m of each mesh in the following order: 100 mm, 150 mm, 50 mm, 27 mm, 35 mm, 75 mm). Number, mesh size and net soak time were not consistent among sites, with nets cleared approximately every 2 h.

All Murray cod and golden perch collected were measured (mm), weighed (g), then euthanased (Barker *et al.* 2002). Sagittal otoliths were removed, sectioned and aged (Anderson *et al.* 1992*a*, 1992*b*; Stuart 2006), and assessed for the presence of a calcein mark using a fluorescence dissecting stereomicroscope (Model MZ165FC, Leica, Switzerland) and a 'GFP3' filter set (470-nm excitation filter with 40-nm half bandwidth, 525-nm band pass barrier filter with 50-nm half bandwidth).

Data analysis

Population structure

Calcein marked fish were allocated to year classes based on year of spawning to correlate with the age of natural recruits as per the procedure outlined in Crook *et al.* (2010*b*). The Murray cod and golden perch sub-sample, whose age was equal to, or less than, the number of years since marked fish were released; we refer to hereafter as 'calcein possible'. The proportion of marked fish in the calcein possible population was calculated by dividing the total number of marked fish by the total number of calcein possible fish. These analyses were completed for each of species, waterbody and spawning year.

A log-linear model was used to test whether the proportion of marked fish in the calcein possible population for each species was different between waterbody and spawning year. When the log-linear models were performed using the complete dataset (which included some categories with low counts) poor model convergence was detected, and as such the models were refit including only categories where 10 or more fish were collected. Outputs and interpretations from both models were directly comparable and we present the complete model results.

Mark longevity

Maximum mark longevity was assessed by directly observing calcein marks within sectioned and mounted sagittal otoliths. Hatchery-reared Murray cod and golden perch were given the nominal birthdate of 1 October (Anderson *et al.* 1992*b*; Mallen-Cooper and Stuart 2003; Stuart 2006) to allow back calculation of age for marked fish at the time of capture. Marked fish were pooled for each species and waterbody by year to assess mark longevity. External calcein mark retention and minimum mark longevity were not assessed in this study.

Condition

Of the calcein possible Murray cod and golden perch populations, two indicators of fish condition were used to identify differences that may exist between marked and unmarked fish. Data were pooled for each waterbody and species. The two estimators were:

(1) Condition factor; defined as Fulton's K (e.g. Harris 1987; Ingram 2009; Cameron and Baumgartner 2012), is expressed as:

$$K = 100\left(W/L^3\right)$$

where W is fish weight (g), L is total fish length (cm) and K is fish condition.

(2) Logarithmic length–weight relationship:

$$\log_{10}W = b(\log_{10}L) + \log_{10}a$$

where W equals fish weight (g), L is total fish length (mm), b is the slope (exponent), and a is the y-intercept determined from empirical data. The length–weight relationship is considered isometric when b (regression slope) = 3 (Cameron and Baumgartner 2012).

Two-way analysis of variance (ANOVA) was used to test for differences in condition factor between marked status (calcein marked or unmarked) and waterbody in the calcein possible population for each species. When the two-way interaction was not significant ($P > 0.05$), subsequent tests for differences in condition factor were performed independently for waterbody and marked status. Analysis of covariance (ANCOVA) was used to test for differences between waterbody, $\log_{10}$Length and marked status on weight in the calcein possible population for each species. When the interaction of these three effects was significant, we used separate ANCOVA to test the two-way effects of; waterbody and $\log_{10}$Length; and marked status and $\log_{10}$Length. If the two-way interactions were not significant

Table 2. Number, length (minimum and maximum) and age (minimum and maximum) of Murray cod and golden perch sampled from the Murray and Murrumbidgee rivers, Burrinjuck and Copeton dams in New South Wales, Australia

Calcein possible are those fish whose age was equal to, or less than, the number of years since marked fish were released

Waterbody	Dataset	Murray cod					Golden perch				
		n	Length (mm)		Age (year)		*n*	Length (mm)		Age (year)	
			Min.	Max.	Min.	Max.		Min.	Max.	Min.	Max.
Murray River	All fish	323	59	1270	0	37	195	211	582	2	27
	Calcein possible	101	59	603	0	4	22	244	384	2	3
Murrumbidgee River	All fish	373	71	1170	0	23	228	108	540	0	17
	Calcein possible	105	71	595	0	4	37	110	394	0	3
Burrinjuck Dam	All fish	178	119	1130	0	13	414	85	595	0	23
	Calcein possible	8	119	630	1	3	43	85	410	0	3
Copeton Dam	All fish	219	107	1250	0	19	601	104	640	0	23
	Calcein possible	129	107	610	0	3	120	104	427	0	3

($P > 0.05$), subsequent tests for waterbody, $\log_{10}$Length and marked status were performed independently. The ANCOVA included $\log_{10}$weight as the response variable, $\log_{10}$Length as the covariate and waterbody and marking status as fixed factors. When the ANCOVA or two-way ANOVA showed significant interactions with, or differences between effects, Tukey's HSD test was used *post hoc* to identify groups that were significantly different.

Burrinjuck Dam Murray cod were excluded from condition factor and length–weight relationship analysis because of small sample sizes.

Results

A total of 1093 Murray cod and 1438 golden perch were collected across all sites. Of these, 343 Murray cod and 222 golden perch were calcein possible (fish whose age was equal to, or less than, the number of years since marked fish were released). Murray cod varied in length from 59 to 1270 mm (59–630 mm for the calcein possible fish), whilst age spanned 0–37 years (0–4 years for the calcein possible fish). Golden perch varied in length from 85 to 640 mm (85–427 mm for the calcein possible fish), with an age span of 0–27 years (0–3 years for the calcein possible fish; Table 2).

Murray cod

Population structure

The proportion of marked Murray cod in the calcein possible population was greater in Copeton Dam (94%), than in the Murray River (7%) or the Murrumbidgee River (15%; Table 3). No calcein marked Murray cod were collected from Burrinjuck Dam, and only eight unmarked calcein possible individuals were collected from this impoundment during the study (Table 3). The significant difference in the proportion of marked Murray cod between waterbodies (W) was dependent on spawning year (SY; $\chi^2_{SY \times W} = 307.1$, d.f. = 15, $P < 0.0001$). Of the spawning years where 10 or more fish were collected from Copeton Dam, the percentage of marked fish was always 80% or more, whereas in either river, spawning year classes where 10 or more fish were collected never had more than 17% marked (Table 3).

The maximum possible age that could be determined for marked Murray cod in the Murray and Murrumbidgee rivers and Burrinjuck and Copeton dams was 3 years 10 months (the study duration). Calcein marks were detected in Murray cod sagittal otoliths aged up to 3 years 10 months in the Murrumbidgee River and Copeton Dam, and in fish aged up to 3 years 5 months in the Murray River.

Condition

There was no significant effect between marked and unmarked Murray cod condition factor, either on its own ($F = 0.150$, d.f. = 1, 129, $P = 0.70$) or in the interaction with waterbody ($F = 0.212$, d.f. = 2, 329, $P = 0.81$). Condition factor was significantly different between waterbodies ($F = 21.2$, d.f. = 2, 332, $P < 0.001$). Mean Murray cod condition factor for the Murray River (1.25) and Murrumbidgee River (1.24) populations were significantly lower than in Copeton Dam (1.37; Fig. 2).

The interaction of waterbody, marked status, and length was not significant for Murray cod length–weight relationships ($F = 0.765$, d.f. = 2, 323, $P = 0.47$). However, length–weight relationships were significantly different between marked and unmarked fish ($F = 38.0$, d.f. = 1, 331, $P < 0.0001$). Murray cod length–weight relationships for marked ($b = 3.17$) and unmarked ($b = 2.97$) fish were classified as isometric, but the significantly different regression slopes (b) indicate better condition (with respect to length–weight relationship) for marked than unmarked fish (Fig. 3).

Golden perch

Population structure

Copeton Dam had the largest proportion of marked golden perch (98%), compared with Burrinjuck Dam (23%), Murray River (9%) and the Murrumbidgee River (14%) populations (Table 3). The significant difference in the proportion of marked golden perch in the samples between waterbodies was dependent on spawning year ($\chi^2_{SY \times W} = 209.5$, d.f. = 10, $P < 0.0001$). In the Murrumbidgee River, the proportion of marked golden perch was significantly different between spawning years ($\chi^2_{SY} = 15.2$, d.f. = 2, $P < 0.001$). This appears to be because 3 out of 3 fish in 2012 were marked, compared with 2 out of 26, and 0 out of 8 fish, being marked in 2010 and 2011 respectively (Table 3).

Table 3. Summary of calcein marked and unmarked Murray cod and golden perch sampled from the Murray and Murrumbidgee rivers, Burrinjuck and Copeton dams in New South Wales, Australia

Only those fish aged equal to, or younger than the years since calcein marked fish were first stocked, are included in this table

Waterbody	Spawning year	Murray cod				Golden perch			
		n	Marked	Unmarked	Percentage marked	*n*	Marked	Unmarked	Percentage marked
Murray River		101	7	94	7	22	2	20	9
	2009	17	0	17	0	0	0	0	0
	2010	6	1	5	17	19	1	18	5
	2011	3	0	3	0	3	1	2	33
	2012	58	6	52	10	0	0	0	0
	2013	17	0	17	0	0	0	0	0
Murrumbidgee River		105	16	89	15	37	5	32	14
	2009	21	0	21	0	0	0	0	0
	2010	61	10	51	16	26	2	24	8
	2011	4	3	1	75	8	0	8	0
	2012	12	2	10	17	3	3	0	100
	2013	7	1	6	14	0	0	0	0
Burrinjuck Dam		8	0	8	0	43	10	33	23
	2009	0	0	0	0	0	0	0	0
	2010	8	0	8	0	30	9	21	30
	2011	0	0	0	0	11	0	11	0
	2012	0	0	0	0	2	1	1	50
	2013	0	0	0	0	0	0	0	0
Copeton Dam		129	121	8	94	120	117	3	98
	2009	10	8	2	80	0	0	0	0
	2010	52	52	0	100	51	50	1	98
	2011	65	61	4	94	46	46	0	100
	2012	1	0	1	0	23	21	2	91
	2013	1	0	1	0	0	0	0	0

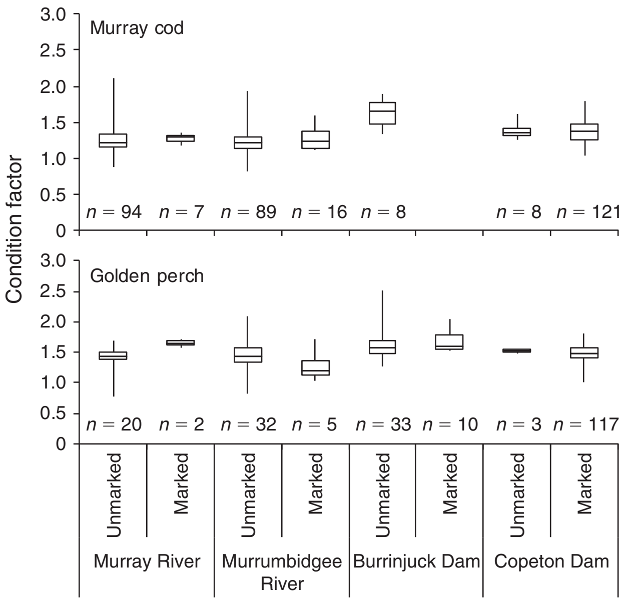

Fig. 2. Box plot of condition factors (Fulton's K) for Murray cod and golden perch sampled from the Murray and Murrumbidgee rivers and Burrinjuck and Copeton dams in New South Wales, Australia. Fish aged equal to, or younger than the years since calcein marked fish were first stocked, are included in this analysis. Box plots are shown with upper and lower quartiles with maximum and minimum outliers.

There were no further significant differences in the proportion of marked fish between spawning year in either of the Murray River, Burrinjuck and Copeton dams ($P > 0.05$). Nevertheless, the proportion of marked golden perch for each spawning year class in Copeton Dam was consistently high, varying between 91% ($n = 23$) for fish spawned in 2012 and 100% ($n = 46$) for the 2011 year class (Table 3).

Calcein marks were detected in golden perch sagittal otoliths aged up to 3 years 10 months in Burrinjuck and Copeton dams, and in fish aged up to 2 years 10 months in the Murray and Murrumbidgee rivers.

Condition

There was no significant effect between marked and unmarked golden perch condition factor, on its own ($F = 0.34$, d.f. = 1, 213, $P > 0.05$), or in the interaction with waterbody ($F = 1.62$, d.f. = 3, 213, $P > 0.05$). There was a significant difference in the condition factor for golden perch among waterbodies ($F = 3.10$, d.f. = 3, 213, $P < 0.05$), with the Murrumbidgee River population having a significantly lower mean condition factor (1.44) than the Burrinjuck Dam population (1.60), but both were not different to the Murray River (1.50) and Copeton Dam (1.50) populations (Fig. 2). The significant length–weight relationship for golden perch ($F = 466.9$, d.f. = 1, 206, $P < 0.0001$) was not influenced by waterbody or marked status (all $P > 0.05$; Fig. 3).

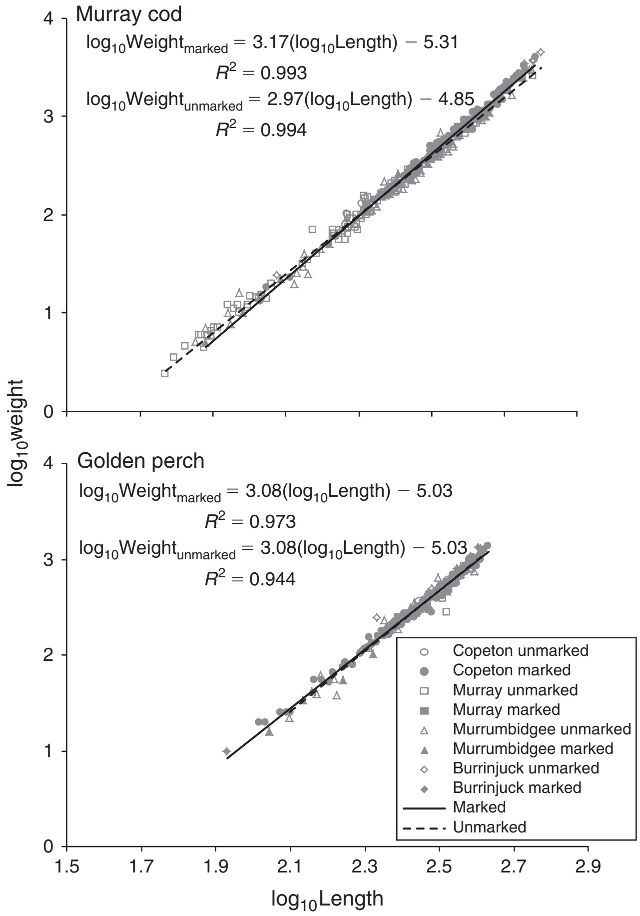

Fig. 3. Scatter plots for log-transformed length–weight relationships for Murray cod and golden perch sampled from the Murray and Murrumbidgee rivers and Burrinjuck and Copeton dams in New South Wales, Australia. Fish aged equal to, or younger than the years since calcein marked fish were first stocked, are included in this analysis.

Discussion

The comparatively low proportion of marked Murray cod and golden perch in the riverine study sites suggests that these populations are primarily self-supporting through natural recruitment, and survival of marked fish is low into these waterbodies. In contrast, marked fish formed a larger proportion of the population within Burrinjuck (golden perch only) and Copeton dams. The virtual absence of unmarked golden perch in Copeton Dam supports observations that this species spawns and recruits in riverine environments (Harris and Gehrke 1994; King *et al.* 2003), rather than standing waters (King *et al.* 2009). We also identified few unmarked Murray cod in Copeton Dam, suggesting that natural recruitment was limited. Murray cod have been observed to spawn in standing waters (Rowland 1983), and larvae have been collected within weir pools (Koehn and Harrington 2005). However, the dominance of stocked fish in the impoundment populations suggests that fisheries in these systems are almost entirely dependent on stocking rather than natural recruitment. Declines in recruitment of golden perch and Murray cod were observed following the construction of several large impoundments throughout the MDB during the 1950s and 1960s (Rowland 1985), with populations established in the new reservoirs following successful stocking programs (Rowland 2005, 2013).

The variable contributions of marked fish demonstrate that adaptive, location-specific fishery enhancement strategies are required to maintain recreational fisheries. For example, our results suggest that in rivers with high levels of natural recruitment, stocking may be inefficient and potentially harmful. However, in some systems the conditions required for successful recruitment may not occur because of river regulation or drought conditions, and stocking may be more effective. Crook *et al.* (2016), for example, found little evidence of natural recruitment of golden perch in Billabong Creek, NSW, and stocking of golden perch fingerlings over successive years resulted in a four-fold increase in catch per unit effort (CPUE). Conversely, Davies *et al.* (1988) identified improved brown trout, *Salmo trutta*, numbers and biomass followed stocking cessation in a Tasmanian river system. Optimal spawning conditions and high juvenile survival were thought to contribute to the sustainability of this fishery (Davies *et al.* 1988). Whether Murray cod and golden perch populations would respond in a similar manner following removal of re-stocking programs is an important hypothesis warranting further investigation.

Riverine stocking reduction or cessation may have minimal effect because wild recruitment in rivers was high. However, river stocking may be important for management strategies designed to maintain sustainability and improve fishery quality. For example, stocking of fish into MDB rivers is thought to assist recovery from fish kills (e.g. Gilligan 2005; Koehn 2005), to enhance recreational fisheries (see Gillanders *et al.* 2006), to re-introduce fish into degraded populations (e.g. Cadwallader and Gooley 1984; Crook *et al.* 2010*b*), or to overcome recruitment bottlenecks (Crook *et al.* 2010*a*). Quantifying the contribution of stocked fish to these populations is vital to measure the relative success of such recovery and enhancement programs. Such efforts are hindered when stocked fish are unable to be accurately identified within a population.

The current study was affected by the release of unmarked hatchery-reared fish in the Murray and Murrumbidgee rivers, and Burrinjuck Dam. As such, the proportion of stocked fish in these populations may be underrepresented. Although the numbers of unmarked hatchery-reared fish released were mostly low in comparison to total fish released, it is an important limitation to acknowledge, and strengthens the requirement to identify all stocked fish, whether by chemical marking or other means. To initiate a large-scale chemical marking study and gather enough data to make conclusive assessments would take several years, and even then it may deliver only information relating to a comparatively young portion of the population. Marking of all hatchery-origin fish, or the ability to discriminate stocked fish using alternative techniques such as genetic identification (e.g. Denson *et al.* 2012), would allow identification of natural recruitment in a population allowing adaptive decision making for fishery managers. Such programs are already in place for salmonid fisheries in North America (see Noakes *et al.* 2000; Rawding *et al.* 2012). Information collected from these programs identified that hatchery fish had replaced wild fish, with management intervention required to protect wild stocks (Noakes *et al.* 2000).

Whilst our study shows that marked fish make a substantial contribution to populations in the receiving ecosystems, there is a range of undesirable outcomes that may be associated with the presence of stocked fish, including replacement of wild with stocked fish (whether by direct stocking, or indirectly through the progeny of previously stocked fish), reduced local genetic heterogeneity, loss of local adaptations, domestication and introduction of infectious diseases and non-target species (Rowland 2013). Continued monitoring of riverine Murray cod and golden perch populations is important to ensure that the potential benefits of stocking (improved fishery sustainability, fishery enhancement, stock recovery) outweigh the potential negative outcomes. These adverse effects are perhaps of less importance in impoundments where natural recruitment may be low and the chance of escapement into rivers is limited. However, overwhelming a wild population with hatchery-reared fish can threaten a species' viability (Phillips 2003).

We found marked Murray cod length–weight relationships were significantly steeper (i.e. higher weight at a given length) than unmarked fish, but whether this difference manifests into distinct biological outcomes is unknown. Should length–weight relationship be a key aspect to juvenile Murray cod survival, hatchery-reared fish may have an advantage over wild fish. Hatchery-reared Murray cod and golden perch are generally released as fingerlings (40–60 mm TL; Ingram *et al.* 2011), at which length they are past the high mortality associated with larval stages. Accordingly, fish stocked in impoundments may have an advantage over wild recruits, or it could be that impoundments offer better conditions for stocked fish survival. Adverse stocking effects may also be present, but less apparent, in riverine populations, with additional research required to evaluate the relationship between stocked and wild fish in both waterbody types.

Murray cod and golden perch have previously been aged to 48 and 26 years respectively (Rowland 1998; Stuart 2006). Such longevity highlights the importance of long-term calcein mark retention for studies that collect data over many years, and also for studies that aim to retrospectively assess populations containing marked fish. Continued mark and release of stocked Murray cod and golden perch would enable this. However, should marks degrade over time, ambiguity in the ability to detect stocked and wild fish may be introduced, thus compromising the integrity of long-term datasets. Crook *et al.* (2009) detected calcein marks in golden perch otoliths over 2 years from the initial marking date, whilst Baumgartner *et al.* (2012) identified externally visible calcein marks in Murray cod up to 57 days post-marking. External calcein marks degrade with exposure to ultraviolet light (Hill and Quesada 2010), but have been detected for up to 1 year 7 months in golden perch (Crook *et al.* 2012), and for up to 3 years in salmonids (Mohler 2003; Negus and Tureson 2004). However, despite degradation of external marks, calcein can still be detected within internal bony structures (Honeyfield *et al.* 2008). For example, calcein marks have been detected in killifish (*Heterandria formosa*) bony structures for up to 6 years (Leips *et al.* 2001). We found calcein marks in golden perch otoliths remained clearly detectable for 3 years 10 months post-marking. Whether these will persist indefinitely remains a priority for further study.

An alternative to destructive sampling for fish ageing and mark detection includes sectioned fish spines (e.g. Koenigs *et al.* 2015). Butler and Rowland (2008) found that dorsal spine sections in eastern cod (*Maccullochella ikei*) revealed no consistent annuli; however, mark detection and age from Murray cod and golden perch spines should be further examined as this could potentially enable future studies to minimise lethal sampling of fish for otolith collection.

In conclusion, our research suggests that stocking programs are vital to support impoundment fisheries where natural recruitment is low, but are of lesser importance to river fisheries where there is substantial natural recruitment. Empirical studies of stocking outcomes improve our understanding of stocked fish survival and the contribution of stocking to populations, thus facilitating informed decisions on the allocation of hatchery-reared fish to achieve maximum benefit.

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