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# Short-term intervention monitoring of a fish community response to an environmental flow in the mid and lower Macquarie River: 2014/2015 watering year 

A report prepared for the Commonwealth Environmental Water Office by NSW Department of Primary Industries - Fisheries

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## Non-technical summary

Short-term monitoring of a fish community response to an environmental flow in the mid and lower Macquarie River: 2014/2015 watering year

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## Objectives

There are obligations under the Basin Plan to report on the contribution of Commonwealth environmental water to environmental objectives. This study was developed to follow guiding principles of the Environmental Outcomes Framework underpinning the management of Commonwealth environmental water. The sampling regime adopted provides an opportunity to examine both short-term responses, as reported here, and long-term outcomes of environmental water delivery. Short-term monitoring of fish communities in association with a single watering season provides information to assess the response of native fish reproduction, recruitment and condition to the delivery of environmental water under the specific antecedent and natural flow conditions.

Although short-term changes in diversity and abundance may be apparent due to immigration and juvenile recruitment of fish, it is more likely that significant changes will manifest in the longer term. Continued long-term monitoring of fish communities in the mid and lower Macquarie River would provide information to assess additional long-term outcomes which align with the Environmental Outcomes Framework, including changes in native fish diversity, abundance and population structure.

## Key words

Abundance; Diversity; Environmental flows; Fish condition; Fish recruitment; Macquarie River; Fish community health; Daily aging

## Summary

During the 2014/2015 watering year 28,483 ML of Commonwealth and NSW environmental water was delivered to the Macquarie River and core areas of the northern and southern marshes from Burrendong Dam from 5 October 2014 until 12 December 2014. The water was delivered to meet the water requirements of the inner floodplain vegetation of the Macquarie Marshes and support native fish in the Macquarie River. Expected short-term responses to the environmental water delivery included increased reproduction and subsequent recruitment of native fish species and an increase in native fish condition.

Recruitment data collected over a single watering year suggests native fish species of the midlower Macquarie River, from opportunistic and equilibrium life-history guilds, benefitted from the provision of environmental water during the spring and early summer of 2014/2015.
The broad aims of this study were to: 1) assess the current health status of the fish community throughout the mid and lower Macquarie River; and 2) examine the fish community response to an environmental water delivery through the mid and lower Macquarie River during the 2014/2015 watering year. These broad aims were addressed more specifically by examining fish species diversity and abundance, juvenile recruitment and fish condition indices over a lotic gradient throughout the Macquarie River system.

Three rounds of standardised fish community sampling captured 5,307 individual fish and decapod crustaceans from 20 sites partitioned into 4 spatially distinct zones throughout the mid and lower Macquarie River. Eighteen species were captured, including 12 native fish species, 4 exotic fish species (plus a hybrid) and 2 native decapod species. In general, native fish species richness was significantly lower within, and downstream of, the Macquarie Marshes compared with sites sampled upstream. The overall condition of the fish community within the Macquarie River declined along a downstream gradient from 'poor' below Burrendong Dam to 'extremely poor' in the Macquarie Marshes and downstream to the Barwon River confluence. State and nationally listed threatened species were present, including the eel-tailed catfish (Tandanus tandanus), silver perch (Bidyanus bidyanus), trout cod (Maccullochella macquariensis) and Murray cod (Maccullochella peelii); however, these species were collected in patchy, low abundances.
Evidence of newly recruited juvenile cohorts following the environmental water release during October to December 2014 (Rounds 2 and 3 of sampling) was apparent for a number of species, including Australian smelt, carp-gudgeon, Murray cod, un-specked hardyhead, flatheaded gudgeon, eel-tailed catfish, Murray-Darling rainbowfish and common carp. To examine recruitment in more detail, seven species representing three reproductive life-history guilds (periodic, equilibrium and opportunistic) were selected for analysis of spatial and temporal variation in recruitment through daily age analysis. The classification of these species to the varying reproductive life-history guilds was based on the expected associations between reproduction and river flows. From daily aging, back-calculated hatch dates of small individuals of each target species were examined in relation to hydrological parameters, including water temperature and river discharge. This was to identify if hatch dates/spawning coincided with a specific feature of the hydrograph (i.e. environmental water releases).
The successful recruitment of Murray cod was almost entirely constrained to Zone 2 (Gin Gin to Warren), where the highest abundances of mature Murray cod were captured. The majority of daily aged Murray cod had back-calculated hatch dates between 28 September and 2 December 2014 when water temperatures ranged from 18 to $25^{\circ} \mathrm{C}$. This approximately coincided with the release of environmental water from Burrendong Dam, though some individuals had hatch dates prior to the arrival of environmental water. While this increase in discharge may not have been required to trigger a spawning event, the increased flow may have boosted primary production that facilitated successful recruitment.

Recruitment data for eel-tailed catfish indicate the primary spawning period was between 27 November 2014 and 31 January 2015. This coincides with water temperatures reaching approximately $20^{\circ} \mathrm{C}$ and moderate discharge ( $\sim 300-1,200 \mathrm{ML} /$ day). It is commonly recognised that flooding is not required to initiate spawning for this species; however, increased flow as a result of the environmental water release may have boosted primary production and subsequently facilitate successful recruitment.
No juvenile golden perch or silver perch were sampled within the Macquarie River during the three rounds of sampling between August 2014 and April 2015. It is recognised that significant flow events are required to trigger spawning within these species. It is likely that there were no
flow events within the Macquarie River during the 2014/2015 study period that were large enough to cue spawning or result in successful recruitment of juveniles.

Juvenile bony bream were primarily caught downstream of the Macquarie Marshes during Round 1 of sampling. The majority of daily aged bony bream had back-calculated hatch dates between 13 June and 7 July 2014. This coincided with water temperatures of approximately $13^{\circ} \mathrm{C}$ and simultaneous flow events within both the Macquarie Marshes and Barwon River. It is hypothesised that juvenile bony bream emigrated from the Macquarie Marshes or Barwon River where they were spawned during a flow event into Zone 4, downstream of the Macquarie Marshes. These findings add significant information to existing literature on the biology of bony bream; extending the known breeding season and highlighting a relationship between flow and recruitment.

Juvenile recruitment of common carp was most prolific within the Macquarie Marshes (Zone 3) and downstream of the Macquarie Marshes (Zone 4). Peaks in hatch date frequency were associated with the leading edge of flow pulses and during sustained periods of increased flow. Hatch dates coincided with water temperatures as low as $10^{\circ} \mathrm{C}$ during periods of increased flow. Rising river levels likely enhanced spawning activity through the inundation of spawning habitat, such as floodplains or riparian vegetation, and/or enhanced primary production that facilitated successful recruitment.

Small-bodied opportunistic species, including Australian smelt, un-specked hardyhead and Murray-Darling rainbowfish, showed that peaks in hatch date frequencies generally occurred during the receding tails of flow events or during sustained periods of increased flow, particularly in association with the spring/early summer environmental water release of 2014. The specific response of each species is discussed in further detail within the report.
A variable offtake curtain was installed on Burrendong Dam in 2014 to help reduce the impacts of cold water pollution downstream of the dam. Data from the present study indicate successful recruitment occurred during the 2014/2015 watering year in a number of native species within Zone 1, directly below Burrendong Dam. However, low abundances and poor recruitment of Murray cod was observed in Zone 1 compared with Zone 2, approximately 150 km downstream. It is likely the fish community response to the cold water mitigation will manifest over a number of years. Therefore, data from the 2014/2015 sampling will contribute to data that should be collected over a larger temporal scale.

This study details important considerations in the design and data interpretation of recruitment monitoring studies. In addition, it will further contribute to the development of adaptive flow management plans and the increasing body of knowledge required in the effective use of environmental water to benefit native fish communities.

## 1 Introduction

The regulation of rivers through the construction of dams, weirs and other diversional structures has altered natural flow regimes and the associated ecological processes for many inland river systems of Australia (Gehrke et al., 1995; Bunn and Arthington, 2002). Typically, the natural flow regimes of dryland rivers are highly variable, with periods of flooding punctuating extended dry periods. The productivity of dryland river and marsh ecosystems is therefore highly dependent on periods of increased flow (Puckridge et al., 1998; Kingsford et al., 1999; Bunn et al., 2006; Arthington and Balcombe, 2011). High flow events and floodplain inundation have been identified as the source and delivery mechanism for basal carbon within riverine ecosystem food webs (Gawne et al., 2007). Flooding also provides habitat, nesting grounds and resources for a range of fauna, including wetland-dependent waterbirds (Kingsford and Johnson 1998), herpetofauna and other aquatic and terrestrial species (Brock, 1998).
Fish are critically dependent on flows. Fish assemblages in dryland rivers have evolved lifehistory strategies attuned to environmental triggers (Sternberg and Kennard, 2013), particularly flow regimes (Bunn and Arthington, 2002). High flow events provide spawning triggers (Reynolds, 1983), boost primary production that facilitates successful recruitment and provide connectivity between habitats (Balcombe et al., 2006, 2007). Temporal changes in native fish abundances have been shown to respond positively to periods of increased flow (Balcombe and Arthington, 2009). Flow largely influences the range of physical habitat available to fish at a number of life-history stages. Larvae, juveniles and adults have been shown to exploit inundated floodplain habitat (Balcombe et al., 2007; Rolls and Wilson, 2010). Enhanced recruitment of native fish has been associated with periods of increased flow (Balcombe and Arthington, 2009). Balcombe and Arthington (2009) attribute this to food-rich ephemeral environments made available as a result of the inundation of backwater and floodplain habitat. Furthermore, within the main river channel, sediment transport and scouring during high flow events is essential to maintenance of deep pools and the input of large woody debris habitat critical for the persistence of native fish species.
Given the extensive literature detailing the importance of river flow to native fish diversity, abundance and recruitment, the restoration of riverine ecosystems is being addressed by providing environmental flows in regulated systems (OEH, 2013). Enhanced native fish spawning and recruitment have resulted from environmental watering events (King et al., 2010). However, the timing, duration and magnitude of the water delivery events should take into account life-history characteristics of the resident native fish species (King et al., 2003; Rolls et al., 2013; Rayner et al., 2015). Hence, the use of adaptive flow management plans that account for species-specific biological traits could optimise outcomes for native fishes (Baumgartner et al. 2014).
Fish are powerful tools used in ecological assessment of aquatic environments (Harris, 1995). Fish have been used as an indicator of aquatic ecosystem health in several large river health monitoring programs in south-eastern Australia (Davies et al., 2010). Advantages associated with this include: i) fish are relatively long-lived and mobile, reflecting both short- and longer-term and local- to catchment-scale processes; ii) they occupy higher trophic levels within aquatic ecosystems and, in turn, express impacts on organisms at lower trophic levels; iii) they are relatively easily and rapidly collected and can be sampled non-destructively; iv) they are typically present in most waterbodies; and v) the biological integrity of fish assemblages can be easily assessed and interpretation of indicators is relatively intuitive. Further, as fish have a high public profile, with significant recreational, economic and social values, they foster substantial public interest (MDBC, 2004).

Flows within the mid Macquarie River, Macquarie Marshes and lower Macquarie River are affected by the flow regulating activities of Burrendong Dam. A number of smaller fish passage barriers also exist on the main river channel (Rayner et al., 2008) and within its floodplains
(Steinfeld and Kingsford, 2013). River regulation has led to a significant reduction in the longterm average annual flows reaching the Macquarie Marshes (Ren and Kingsford, 2011). Furthermore, cold water pollution as a result of a deep-water offtake from Burrendong Dam has significantly affected biological processes of fish for up to $300-400 \mathrm{~km}$ downstream (Astles et al., 2003). During summer, thermal stratification within the dam can exceed $>10^{\circ} \mathrm{C}$ (Astles et al., 2003). The release of this cold, sometimes anoxic hypolimnetic water can affect growth, survival, redistribution, activity and recruitment of native fish species (Astles et al., 2003; Todd et al., 2005) and in some cases can lead to the complete loss of non-tolerant fish species (Todd et al., 2005). Mitigation measures for cold water pollution below Burrendong Dam involved retrofitting a submerged curtain to the existing offtake to increase discharge temperatures. It is likely the fish community response to the cold water mitigation will manifest over a number of years. Therefore, data from the 2014/2015 sampling will contribute to data that should be collected over a larger temporal scale.
During the 2014/2015 water year, 28,483 ML of (Commonwealth and NSW) environmental water was delivered from Burrendong Dam down the Macquarie River to meet the water requirements of the inner floodplain vegetation of the Macquarie Marshes and support native fish in the Macquarie River. In relation to fish, the water was expected to provide access to suitable fish habitat, promote fish movement and provide cues for spawning, recruitment and migration of native fish. The delivery of the water was to be linked to a natural timing trigger ( $5,000 \mathrm{ML}$ or more over a 3-day period at Baroona Gauging Station) between 15 August and 5 October 2014. However, this natural timing trigger did not eventuate. Consequently, the water was released from Burrendong Dam on 5 October 2014. An additional 9,337 ML of Commonwealth environmental water was approved to provide a second peak for native fish if there was a trigger of $3,000 \mathrm{ML}$ over 3 days at Baroona while environmental releases were underway. However, this second trigger did not eventuate so no additional water was provided. In addition to the instream effects, it was expected that the volume of $28,483 \mathrm{ML}$ would support the inundation of approximately 5,000 hectares of semi-permanent wetland vegetation in the South and North Marshes subsystems, including reedbeds, some water couch/mixed marsh areas and some inner river red gum woodlands.

Aspects of native fish diversity, abundance, reproduction, growth, survival and dispersal were identified by Gawne et al. (2013) as priority indicators relevant to monitoring outcomes of environmental flow delivery. Few studies have examined the response of fish to environmental watering within the Macquarie River system (Growns and Gehrke, 2005; Rayner et al., 2009, 2015). In the present study, aspects of these processes were monitored to examine the response of fish to environmental flow delivery in the mid and lower Macquarie River during the 2014/2015 watering year. Short-term expected responses to the environmental water delivery investigated in this study included increased recruitment of native fish species and an increase in native fish condition (Figure 1). Continued monitoring of these fish communities over time will provide information to assess additional long-term outcomes which align with the CEWO Environmental Outcomes Framework (Figure 1).

Figure 1. Environmental Outcomes Framework (CEWO, 2013).

| Basin Plan Objectives | Basin Outcomes |  | 5 year Expected Outcomes | 1 year Expected Outcomes | Related Cause and Effect Diagram (Reference only) <br> Landscape Ecosystem Diversity |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Biodiversity <br> (Basin Plan S. <br> 8.05) | Ecosystem diversity |  |  |  |  |
|  |  |  | - Species diversity |  | Within Ecosystem Diversity |
|  | Species diversity | Vegetation | - Vegetation diversity |  | Landscape Vegetation Diversity |
|  |  |  |  | - Reproduction <br> - Condition | Vegetation Condition and Reproduction |
|  |  |  | - Growth and survival | - Germination <br> - Dispersal | Vegetation Recruitment and Extent |
|  |  | Macroinvertebrates | - Macroinvertebrate diversity |  | Within Ecosystem <br> Macroinvertebrate Diversity |
|  |  | Fish | - Fish diversity |  | Landscape Fish Diversity |
|  |  |  |  | - Condition | Fish Condition |
|  |  |  |  | - Larval abundance <br> - Reproduction | Fish Reproduction |
|  |  |  | - Larval and juvenile recruitment |  | Fish Larval Growth and Survival |
|  |  | Waterbirds | - Waterbird diversity |  | Landscape Waterbird Diversity |
|  |  |  | - Waterbird diversity and population condition (Abundance and Population structure) | - Survival and condition | Waterbird Survival and Condition |
|  |  |  |  | - Chicks | Waterbird Reproduction |
|  |  |  |  | - Fledglings | Waterbird Recruitment and Fledging |
|  |  | Other vertebrate diversity |  | - Young | Other Vertebrate Reproduction |
|  |  |  | - Adult abundance |  | Other Vertebrate Growth and Survival |
| Ecosystem <br> Function <br> (Basin Plan S. <br> 8.06) | Connectivity |  |  | - Hydrological connectivity including end of system flows | Hydrological Connectivity <br> (including end of system flows) |
|  |  |  |  | - Biotic dispersal and movement | Biotic Dispersal |
|  |  |  |  | - Sediment transport | Sediment Transport |
|  | Process |  |  | - Primary productivity (of aquatic ecosystems) | Primary Production |
|  |  |  |  | - Decomposition | Decomposition |
|  |  |  |  | - Nutrient and carbon cycling | Nutrient and Carbon Cycling |
| Resilience <br> (Basin Plan S. <br> 8.07) | Ecosystem resilience |  | - Population condition (individual refuges) | - Individual survival and condition (Individual refuges) | Individual Refuges |
|  |  |  | - Population condition (landscape refuges) |  | Landscape Refuges |
|  |  |  |  | - Individual condition <br> (Ecosystem resistance) | Ecosystem Resistance |
|  |  |  | - Population condition (Ecosystem recovery) |  | Ecosystem Recovery |
| Water quality <br> (Basin Plan S. <br> 9.04) | Chemical |  |  | - Salinity | Salinity |
|  |  |  |  | - Dissolved oxygen | Dissolved Oxygen |
|  |  |  |  | - pH | pH |
|  |  |  |  | - Dissolved organic carbon | Dissolved Organic Carbon |
|  | Biological |  |  | - Algal blooms | Algal Blooms |

## 2 Methods

### 2.1 Study sites

The Macquarie River below Burrendong Dam was partitioned into four spatially, geomorphologically and hydrologically distinct zones at the landscape scale. Analysis of riverine fish assemblages and decapod crustaceans was undertaken within each of the four catchment zones. The delineation of the zones is detailed below and displayed in Figure 2. Five sampling sites within each of the four zones were selected from pre-existing, randomly chosen sampling locations (Astles et al., 2003; Growns and Gehrke, 2005; Davies et al., 2008; Gilligan et al., 2010) or new, randomly selected sampling locations (Appendix 3). Due to difficulties in selecting an appropriate unregulated control river system based on similarity in fish diversity/abundance and abiotic river characteristics, fish community sampling was constrained to the Macquarie River.

## Zone 1: Macquarie River Burrendong Dam to Dubbo

This zone is a $\sim 100 \mathrm{~km}$ stretch of river below Burrendong Dam to Dubbo. Due to earlier cold water pollution from a deep-water offtake in Burrendong Dam, it contains relatively low abundances of a number of native species (Astles et al., 2003). Sampling within this zone presented an opportunity to quantify the benefits of: 1) the environmental water delivery; and 2) mitigation measures of cold water pollution and the effects on the depleted native fish assemblage.

## Zone 2: Macquarie River Gin Gin to Warren

This zone is a $\sim 100 \mathrm{~km}$ stretch of river above the Macquarie Marshes, between Gin Gin and Warren. Native fish species within this zone are in relatively higher abundance than in adjacent sampling zones. However, species diversity and abundance have been negatively impacted by cold water pollution (Astles et al., 2003).

## Zone 3: Macquarie Marshes

This complex wetland system is fundamentally different to the main channel zones. It is dominated by ephemeral wetlands that retain water for limited periods during flow events. The $28,483 \mathrm{ML}$ of environmental water was expected to support the inundation of approximately 5,000 hectares of semi-permanent wetland vegetation in the South and North Marshes subsystems, including reedbeds, some water couch/mixed marsh areas and some inner river red gum woodlands. The five fish community sampling sites within the Macquarie Marshes were constrained to the main Macquarie River channel due to access limitations and water availability of the ephemeral wetlands.

## Zone 4: Lower Macquarie River (below the Macquarie Marshes)

This is a $\sim 100 \mathrm{~km}$ stretch of river below the Macquarie Marshes, between Bells Bridge and the Barwon River confluence. As no substantial tributary inputs accompanied the 5 October environmental water release, the flow did not reach Zone 4. During Round 1 of sampling (September 2014), the Macquarie River downstream of the Marthaguy Creek confluence was flowing at approximately $77 \mathrm{ML} /$ day and provided connectivity to the Barwon River. During Round 2 (December 2014), only small puddles remained at two of the five sampling sites while the remaining sites were completely dry (Appendix 2, Plates 2.16b-2.20b). And during Round 3 (April 2015), the Macquarie River had not flowed since late October 2014 and the pools at each site had been completely dry since January 2015 (J. Ellis and F. Masman, property owners, pers. comm.). However, a 'domestic and livestock' water delivery diverted to Bulgeraga Creek and the North Marsh Channel at Marebone Regulator (see Appendix 7) had recently (approximately $1-2$ days prior) restored flows to 23 ML /day at two of the five study sites (flow
recorded at Carinda Gauging Station), though this flow had not yet reached the lower three sites in Zone 4 during Round 3 of sampling (April 2015).

Figure 2. Location of sample sites within each of the four sampling zones on the Macquarie River.


Note: Sample site coordinates are provided in Appendix 3.

### 2.2 Monitoring timing

Managed environmental flows in 2014/2015 were separated into two events, being:

- a September piggyback flow into the Eastern Marshes of 5,299 ML, delivered in response to a stock and domestic replenishment flow into this subsystem (Figure 3)
- a flow to the Southern and Northern Marshes that was released from Burrendong Dam on 5 October 2014 and reached Marebone on 13 October 2014 in the absence of a prescribed tributary flow trigger. This flow ( $28,483 \mathrm{ML}$ ) continued until 12 December 2014 at Marebone (Figure 3).

Sampling at all sites within Zones 1-3 (five sites per zone) was conducted on three occasions associated with the October 2014 flow event. The first round of sampling (Round 1) was conducted in August/September 2014, prior to the 5 October environmental water release. The second round of sampling (Round 2) was conducted $\sim 60-75$ days after the flow peak had passed through each zone (December 2014/January 2015). Round 2 of sampling was conducted to quantify a recruitment/immigration response of fish to the environmental water release. The final round of sampling (Round 3) was conducted in March/April 2015 to quantify the final outcome of recruitment of the fish assemblages at the end of the breeding season. Sampling within Zone 4 (below Macquarie Marshes) was constrained due to varying water availability during sampling rounds. During Round 1 , all five sites within Zone 4 were sampled; however, during Rounds 2 and 3, only two sites were sampled on each occasion (Appendix 3).

Figure 3. Flows past Marebone Weir from 1 June 2014 to 28 May 2015 by water source.


Note: NSW AEW = New South Wales Annual Environmental Water; CEWO = Commonwealth Environmental Water Office; EWA = Environmental Water Allowance.
Source: Macquarie-Cudgegong Water Resource Area Annual Environmental Watering Plan.

### 2.3 Monitoring protocol

Sampling followed the standard methods for riverine fish as specified by the Sustainable Rivers Audit (SRA) and detailed in Appendix 1. However, given the priority outcomes of the short-term monitoring involved investigating the response of fish condition, reproduction and recruitment to environmental water delivery, further sampling effort was required to achieve minimum sample sizes for target species (see 'Fish recruitment' in Section 2.4). Consequently, the following additional protocols and augmentations were conducted:

- The individual weight and length of all 'large-bodied' species were measured.
- Additional electrofishing effort (outside SRA protocol) was conducted to achieve target sample sizes required for calculation of condition indices (target sample size $\geq 20$ individuals per zone for each large-bodied species). However, individuals captured during the additional sampling did not contribute to the fish assemblage analyses.
- Additional electrofishing effort (outside SRA protocol) and seine netting ( 6 mm mesh) was performed at selected sites within each zone to supplement catches of young-of-year fish and achieve target sample sizes. For large-bodied species, a target of 50 individuals within each zone to a maximum of 130 mm total length (TL) were retained for daily aging. For small-bodied species, 50 individuals within each zone covering the entire size range of the species were retained for daily aging. A pilot aging study was conducted to determine 'maximum length cut-offs' (the approximate maximum fish length for which daily aging was feasible) for juveniles of each species retained for daily aging. Specimens were euthanased and frozen rather than preserved in ethanol to prevent sample shrinkage (Smith and Walker, 2003a).
- Ten replicate baited opera house traps were set as per the SRA unbaited shrimp traps to generate catch per unit effort (CPUE) data on decapod crustaceans (yabbies and shrimps).
- Water quality parameters stratified by depth (surface and 1 m intervals) were collected at each of the sites. Water quality measurements recorded included temperature, dissolved oxygen, pH , turbidity and conductivity. These data were supplemented by New South Wales (NSW) Office of Water gauging station data.


### 2.4 Analytical methodology

## Fish community composition

The catch from each electrofishing operation and bait traps for each sampling event per site was pooled for analysis. Multivariate analyses of the fish assemblage composition were performed in PRIMER 7.0 (Plymouth Marine Laboratory). To compare spatial and temporal variation in the fish community composition, species abundances were transformed to the fourth root with the addition of a 'dummy' variable after shade plot inspection. Similarities between fish assemblages for each sample were calculated using a Bray-Curtis similarity matrix (Bray and Curtis, 1957). Non-metric multi-dimensional scaling (nMDS, PRIMER v7) ordination plots were constructed for visual comparisons of dissimilarity between community composition for factors of zone and sampling event based on the Bray-Curtis similarity matrix.

Permutational analysis of variance (ANOVA)/multivariate ANOVA (MANOVA) (PERMANOVA, PRIMER v7; Anderson, 2001) was used to test for any significant differences in the fish community composition between zones and sampling rounds.

Coherence plots were constructed to group species that were functionally similar between sampling sites. These were constructed using an index of association resemblance measure where a cluster analysis and similarity profile analysis (SIMPROF) test was performed on the variables/species. In addition, a RELATE test was used to examine for linearity in change of the
community composition along a riverine gradient. To do so, multivariate patterns in species abundance from Zone 1 through to Zone 4 were compared against a serial resemblance matrix. A rank correlation was then performed and compared with results from randomly permuted samples.

Using the SRA data analysis methodology described by Robinson (2012), eight fish metrics were derived from the data collected during Round 3 of sampling. These metrics were then aggregated to produce three fish condition indicators. These indicators were then aggregated to derive an overall Fish Condition Index (ndxFS). The SRA-derived indicators were: 1) expectedness (provides a comparison of existing catch composition with historical fish distributions); 2) nativeness (an indicator of the dominance of native versus exotic fish in the assemblage); and 3) recruitment (an indicator of the extent of native fish recruitment within the zone). Indicator scores were scaled between 0 and 100 and were condition rated as 'extremely poor' (0-20), 'very poor' (21-40), 'poor' (41-60), 'moderate' (61-80) and 'good' (81-100).

## Fish health and condition index

Length and weight data collected for Murray cod, golden perch, common carp and bony bream during each sampling event were used in the calculation of a 'condition index' for each fish. Established length/weight relationships for each species (MDBC, 2004; NSW Department of Primary Industries (DPI), unpublished data) were used to estimate the 'expected weight' of individuals based on their length. The relative body condition of each individual was calculated as: recorded weight divided by expected weight

Values $>1$ indicate better-than-average condition, while values $<1$ indicate poorer-than-average condition relative to established length-weight relationships in NSW. Changes in the mean body condition for each species were analysed using parametric univariate ANOVA using zone ( $n=4$ ) and sampling event $(n=3)$ as factors. To examine pairwise comparison a Tukey's HSD (honest significant difference) test was performed.

In addition, using rapid visual assessment for parasites and disease (SRA protocol; see
Appendix 1), the proportion of fish of each species affected by a health condition was calculated.

## Fish recruitment

## Fish sampling

Juvenile specimens for daily age determination were collected during each of the three sampling rounds using the standardised methodology described in Section 2.3 and Appendix 1, and additional measures, as detailed in Section 2.3, for the construction of growth functions. Seven species representing three reproductive life-history guilds (periodic, equilibrium and opportunistic) were selected for analysis of spatial and temporal variation in recruitment during the 2014/2015 watering year. The classification of these species to the varying reproductive lifehistory guilds was based upon the expected associations between reproduction and river discharge (Schiller and Harris, 2001; Growns, 2004; Zeug and Winemiller, 2008). Species from similar reproductive guilds are predicted to respond to environmental variation, including flow variation, in similar ways (Humphries et al., 1999). The current study examined the recruitment response of the following species during the 2014/2015 watering year:

- periodic species—golden perch (Macquaria ambigua), silver perch (Bidyanus bidyanus), bony bream (Nematalosa erebi), common carp (Cyprinus carpio), spangled perch (Leiopotherapon unicolor)
- equilibrium species—Murray cod (Maccullochella peelii) and eel-tailed catfish (Tandanus tandanus)
- opportunistic species—Australian smelt (Retropinna semoni), un-specked hardyhead (Craterocephalus stercusmuscarum fulvus), Murray-Darling rainbowfish (Melanotaenia fluviatilis).

Each of the three reproductive guilds is described in Zeug and Winemiller (2008):

- Periodic strategists have characteristics that are adaptive in environments where resources for larvae and juveniles are patchy in space and time. Species with this strategy usually have contracted breeding seasons synchronised with favourable periods that are relatively predictable between years. Species of this guild are characterised by delayed maturation, high fecundity and large adult size.
- Equilibrium strategists are characterised by greater parental investment per offspring and relatively low inter-annual variation in recruitment. This strategy is proposed to be associated with resource limitation and/or high threat of predation mortality for early life stages.
- Opportunistic strategists have characteristics that allow them to quickly colonise new habitats. Species of this guild are characterised by small adult size, extended breeding seasons and high reproductive output.


## Proportion of recruits

The proportion of new recruits within populations was derived using a similar process to that applied to generate recruitment metrics for the SRA (Robinson, 2012). For large-bodied and generally longer-living species (>3 years), an individual was considered to be a recruit if the body length was less than that of a 1 year old of the same species. For small-bodied and generally short-lived species that reach sexual maturity in less than 1 year, recruits were considered to be those individuals that were less than the species' known average length at sexual maturity. The recruitment length cut-offs used for both large- and small-bodied species were derived from existing length-at-age data from scientific literature (see Appendix 5). The proportion of new recruits collected during each sampling was analysed for factors of zones and sampling round using parametric univariate ANOVA.

## Otolith processing and daily aging

We employed the methodology described in Stocks et al. (2014) to examine and enumerate daily growth rings in otoliths of juvenile specimens. For all target species, with the exception of common carp, the sagittal otoliths (sagittae) were extracted and mounted on a microscope slide using crystalbond mounting adhesive (Structure Probe Inc., West Chester, Pennsylvania, USA). For common carp, the lapilli were extracted and mounted for aging (Vilizzi and Walker, 1999). Otoliths were polished by hand to the core longitudinally from the proximal surface, or from the dorsal and ventral margins for larger specimens (i.e. Murray cod), on $9 \mu \mathrm{~m}$ lapping film followed by $0.5 \mu \mathrm{~m}$ alumina slurry until daily growth increments became visible. A camera (Q-Imaging, MicroPublisher 5.0 RTV, Canada) attached to a compound microscope collected an image of each otolith examined (for example, see Figure 9). Multiple images were collected throughout the polishing process and the images re-stitched prior to aging due to the varying ring visibility with section thickness between primordium and otolith margins. Sectioned otoliths were viewed under a compound microscope using transmitted light at $25 \times$ magnification. Daily rings were counted radially from the primordium to outer otolith edge (see Figure 9) using ImageJ, a digital image analysis program (http://rsb.info.nih.gov/ij/). A $20 \%$ re-read of randomly chosen otoliths was used to calculate a coefficient of variation for the two reads using the equation described in Campana (2001).

## Recruitment and hydrography

For highly abundant species, a random sample of approximately 50 individuals covering the species' size range were aged. Multiple growth functions were fitted to length-at-age data for each species to assess which model provided the best fit. Linear regressions provided the best fit for length-at-age data of juvenile large-bodied species. On the other hand, von Bertalanffy growth functions were fitted to the length-at-age data of small-bodied species using Equation 1:

$$
\begin{equation*}
L_{t}=L_{\infty} \cdot\left(1-e^{-k\left(t-t_{0}\right)}\right) \tag{1}
\end{equation*}
$$

where $L_{t}$ is the length (mm) at age $t$ (days), $L_{\infty}$ is the asymptotic length (mm), $k$ is the rate at which the function approaches $L_{\infty}$ and, $t_{0}$ is the theoretical age (days) at length zero.

For large-bodied species, where high abundances of juveniles were caught during the standardised SRA sampling, and for all small-bodied species, fish ages were estimated from length measurements using the derived species growth model equations. Estimates of fish age were not extrapolated beyond the largest fish aged when constructing the growth functions. However, for species where low abundances of juveniles were captured during standardised sampling (i.e. Murray cod, eel-tailed catfish and bony bream), all captured juveniles were daily aged and their hatch dates plotted as detailed above (i.e. no fish ages were estimated from the growth model). Additional sampling effort was performed to increase catches of juvenile Murray cod and eel-tailed catfish (i.e. 6 mm seine hauls for catfish and additional electrofishing time for Murray cod). Consequently, abundance comparisons of hatch date frequency data for Murray cod and eel-tailed catfish are not comparable to other species due to the additional sampling effort performed outside SRA protocol.
The back-calculated hatch dates of young-of-year target species collected during each round of standardised sampling (SRA protocol) was plotted with water temperature and river discharge to identify if hatch/spawning dates coincided with a specific feature of the hydrograph (i.e. the environmental water releases).

## Water quality

Water quality parameters for each sample were compared between zones and sampling rounds using multivariate analysis. Data were firstly transformed (log transformation applied to skewed distributions), then normalised and used to construct a Eucladian distance dissimilarity matrix. Non-metric multi-dimensional scaling (nMDS, PRIMER v7) ordination plots were constructed for visual comparisons of dissimilarity between water quality parameters of each sampling zone based upon the matrix. A permutational ANOVA/MANOVA (PERMANOVA, PRIMER v7, Anderson 2001) was used to test for any significant differences in environmental variables with zone and sampling event as factors.

## 3 Results

### 3.1 Fish community composition

From three rounds of standardised sampling, 5,307 individual fish and decapod crustaceans were captured from 20 sites on the Macquarie River. In total, 18 species were captured, including 12 native fish species, 4 exotic fish species (plus a hybrid) and 2 native decapod species (Table 1). (Note: freshwater prawns (Macrobrachium) and shrimp (Atyidae spp.) were not differentiated, all individuals were combined under the common name 'long-arm prawn' and counted as a single species). In general, native species richness was significantly lower within, and downstream of, the Macquarie Marshes compared with sites sampled upstream of the Macquarie Marshes. The greatest native species richness occurred within Zone 1, where 10 native fish species were caught. The lowest native species richness occurred within Zone 4, where four native species were caught (Table 1). Exotic species richness was similar between all zones, ranging from three to four species per zone (Table 1). When exotic species richness was examined by site, significant zone ( $F_{3,42}=3.247, P=0.031$ ) and sampling round ( $F_{2,42}=$ $5.314, P=0.009$ ) effects were observed, but the interaction term was non-significant ( $F_{6,42}=0.549, P=0.768$ ). Pairwise comparisons of exotic species richness showed a significant difference between Zone 2 and Zone $4(P=0.030)$ and Round 1 and Round 3 ( $P=0.006$ ). Native species richness varied little within zones between sampling rounds; however, numerous significant differences were observed between zones (Tables 1 and 2 ). When examining native species richness for each sample, a significant zone $\times$ round interaction term was observed ( $F_{6,42}=3.389, P=0.008$ ). Results of pairwise test comparing: 1) native species richness between zones within each round of sampling: and 2) native species richness between sampling rounds within each zone are displayed in Table 2. Total abundance varied between sampling rounds ( $F_{2,51}=4.09, P=0.022$ ). During Round $1,65.55 \pm 6.74$ standard error (SE) individuals were sampled per site; in Round 2, $129.06 \pm 20.76$ individuals were sampled per site; and in Round 3, $106.00 \pm 19.71$ individuals were sampled per site.

The proportion of new recruits throughout the Macquarie River increased after the environmental watering (Figure 4). When the proportion of new recruits collected during each sampling was analysed against the factors of zones and sampling round, a significant zone $\times$ round interaction term was observed ( $F 6,42=4.506, P=0.001$ ). Results of pairwise test comparing: 1) proportion of recruits between zones within each round of sampling; and 2) proportion of recruits between sampling rounds within each zone are displayed in Table 3.

Visual comparison of length frequency compositions within species illustrated variation between zones and sampling rounds (Figure 5). The appearance of newly recruited juvenile cohorts during Rounds 2 and 3 of sampling (post environmental water release) was apparent for the following species: Australian smelt, carp-gudgeon, Murray cod, un-specked hardyhead, flatheaded gudgeon, eel-tailed catfish, Murray-Darling rainbowfish and common carp (Figure 5). The incremental growth in length of dominant cohorts could also be observed between sampling rounds (Figure 5).

Table 1. Total catch by sampling zone and round from standardised fishing (SRA protocol) over the three sampling events.

| Common name | Scientific name | Zone 1 |  |  | Zone 2 |  |  | Zone 3 |  |  | Zone 4 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Round 1 | Round 2 | Round 3 | Round 1 | Round 2 | Round 3 | Round 1 | Round 2 | Round 3 | Round 1 | Round 2 | Round 3 |
| Silver perch | Bidyanus bidyanus |  |  |  | 1 |  |  |  |  |  |  |  |  |
| Goldfish | Carassius auratus | 2 | 4 | 7 | 2 |  | 3 | 26 | 7 | 43 | 7 | 5 | 8 |
| Yabby | Cherax destructor |  | 1 |  |  |  | 1 | 3 | 5 | 8 | 65 | 11 |  |
| Un-specked hardyhead | Craterocephalus stercusmuscarum fulvus | 2 | 32 | 93 | 1 | 16 | 47 |  |  |  |  |  |  |
| Common carp | Cyprinus carpio | 126 | 89 | 80 | 70 | 81 | 88 | 146 | 106 | 84 | 121 | 19 | 5 |
| Eastern gambusia | Gambusia holbrooki | 4 | 159 | 47 |  | 5 | 36 |  | 7 | 14 | 11 | 15 | 3 |
| Carp-goldfish hybrid |  |  |  |  |  |  | 1 |  |  |  |  |  |  |
| Carp-gudgeon spp. | Hypseleotris spp. | 4 | 102 | 6 | 1 | 5 | 2 | 9 | 18 | 17 | 33 | 4 |  |
| Spangled perch | Leiopotherapon unicolor |  |  |  |  |  |  |  |  | 1 | 1 | 29 | 3 |
| Golden perch | Macquaria ambigua | 16 | 22 | 18 | 19 | 18 | 30 | 13 | 3 | 4 | 3 | 3 |  |
| Long-arm prawn | Macrobrachium australiense \& Atyidae spp. | 9 | 137 | 98 | 3 | 273 | 76 | 67 | 205 | 99 | 52 | 23 | 1 |
| Trout cod | Maccullochella macquariensis | 1 |  |  |  |  |  |  |  |  |  |  |  |
| Murray cod | Maccullochella peelii | 2 | 1 | 9 | 54 | 79 | 81 | 5 | 10 | 4 |  |  |  |
| Murray-Darling rainbowfish | Melanotaenia fluviatilis | 51 | 134 | 324 | 56 | 35 | 113 |  |  | 14 |  |  |  |
| Bony bream | Nematalosa erebi | 1 | 1 |  |  |  | 3 | 18 | 2 | 24 | 48 | 15 | 10 |
| Redfin perch | Perca fluviatilis |  |  | 1 |  |  |  |  |  |  |  |  |  |
| Flatheaded gudgeon | Philypnodon grandiceps | 60 | 278 | 92 |  |  |  |  |  |  |  |  |  |
| Australian smelt | Retropinna semoni | 90 | 181 | 172 | 102 | 49 | 16 |  |  |  |  |  |  |
| Eel-tailed catfish | Tandanus tandanus | 5 | 4 | 15 | 1 | 1 | 1 |  |  |  |  |  |  |
| Total |  | 373 | 1145 | 962 | 310 | 562 | 498 | 287 | 363 | 312 | 341 | 124 | 30 |

Note: In Zone 4 during Rounds 2 and 3 of sampling, only 2 of 5 sites were sampled on each occasion due to the river being dry (see Appendix 3 )

Table 2. Results of Tukey's HSD pairwise comparisons of native species richness between:
a) zones within each round of sampling; and b) between sampling rounds within each zone.
a)

| Round 1 |  |  |  |  |  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |
| :--- | :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |  |  |
| Zone 1 |  |  |  |  |  |  |  |  |  |
| Zone 2 |  |  |  |  |  |  |  |  |  |
| Zone 3 |  |  |  |  |  |  |  |  |  |
| Zone 4 |  |  |  |  |  |  |  |  |  |


| Round 2 |  |  |  |  |  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |
| :--- | :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: | :---: |
|  |  |  | $* * *$ |  |  |  |  |  |  |
| Zone 1 |  |  | $*$ |  |  |  |  |  |  |
| Zone 2 |  |  |  |  |  |  |  |  |  |
| Zone 3 |  |  |  |  |  |  |  |  |  |
| Zone 4 |  |  |  |  |  |  |  |  |  |


| Round 3 |  |  |  |  |  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |
| :--- | :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: | :---: |
| Zone 1 |  |  | $*$ | ** |  |  |  |  |  |
| Zone 2 |  |  | $*$ | $* *$ |  |  |  |  |  |
| Zone 3 |  |  |  |  |  |  |  |  |  |
| Zone 4 |  |  |  |  |  |  |  |  |  |

b)

| Zone 1 |  |  |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: | :---: |
|  | Round 1 | Round 2 | Round 3 |  |  |  |  |  |
| Round 1 |  | * |  |  |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |  |

Zone 3

| Zone 3 |  | Round 1 | Round 2 |
| :--- | :--- | :--- | :--- |
| Round 3 |  |  |  |
| Round 1 |  |  |  |
| Round 2 |  |  |  |
| Round 3 |  |  |  |

Zone 4

|  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- |
| Round 1 |  |  |  |
| Round 2 |  |  |  |
| Round 3 |  |  |  |

Note: ${ }^{* * *}=P<0.0001 ;{ }^{* *}=P<0.001 ;{ }^{*}=P<0.05$; blank $=P>0.05$; shaded $=$ not applicable.

Figure 4. Box-and-whisker plot illustrating proportion of juvenile recruits captured from standardised fishing (SRA protocol) during each sampling round.


Note: Individuals were classified as new recruits using length thresholds detailed in Appendix 5.

Table 3. Results of Tukey's HSD pairwise comparisons of proportion of recruits between: a) zones within each round of sampling; and b) between sampling rounds within each zone.
a)

| Round 1 |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: |
|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |  |
| Zone 1 |  |  |  | $* *$ |  |
| Zone 2 |  |  |  | $* *$ |  |
| Zone 3 |  |  |  | $* *$ |  |
| Zone 4 |  |  |  |  |  |

## Round 2

|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |
| :--- | :--- | :--- | :--- | :--- |
| Zone 1 |  |  |  |  |
| Zone 2 |  |  |  |  |
| Zone 3 |  |  |  |  |
| Zone 4 |  |  |  |  |


| Round 3 |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: |
|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |  |
| Zone 1 |  |  |  | $*$ |  |
| Zone 2 |  |  | $*$ |  |  |
| Zone 3 |  |  |  |  |  |
| Zone 4 |  |  |  |  |  |

b)

| Zone 1 |  |  |  |  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |
| Round 1 |  |  |  |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |


| Zone 3 |  |  |  |  |
| :--- | :--- | :--- | :--- | :---: |
|  | Round 1 | Round 2 | Round 3 |  |
| Round 1 |  |  | ** |  |
| Round 2 |  |  |  |  |
| Round 3 |  |  |  |  |


| Zone 2 |  |  |  |  |
| :--- | :--- | :--- | :--- | :---: |
|  | Round 1 | Round 2 | Round 3 |  |
| Round 1 |  |  |  |  |
| Round 2 |  |  |  |  |
| Round 3 |  |  |  |  |


| Zone 4 |  |  |  |  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: |
| Round 1 |  |  |  |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |

Note: ${ }^{* * *}=P<0.0001 ;{ }^{* *}=P<0.001 ;{ }^{*}=P<0.05 ;$ blank $=P>0.05$; shaded $=$ not applicable.

Figure 5. Length frequency composition from standardised fishing (SRA protocol) of each species within four sampling zones over three sampling rounds. Lengths are fork length for forked tailed fish and total length for all other species.

Key: Blue histogram = Round 1 of sampling; Red histogram = Round 2; and Green histogram = Round 3.


Figure 5 (cont'd). Length frequency composition from standardised fishing (SRA protocol) of each species within four sampling zones over three sampling rounds. Lengths are fork length for forked tailed fish and total length for all other species.


Figure 5 (cont'd). Length frequency composition from standardised fishing (SRA protocol) of each species within four sampling zones over three sampling rounds. Lengths are fork length for forked tailed fish and total length for all other species.
Key: Blue histogram = Round 1 of sampling; Red histogram = Round 2; and Green histogram = Round 3.


Figure 5 (cont'd). Length frequency composition from standardised fishing (SRA protocol) of each species within four sampling zones over three sampling rounds. Lengths are fork length for forked tailed fish and total length for all other species.
Key: Blue histogram = Round 1 of sampling; Red histogram = Round 2; and Green histogram = Round 3.


Length (mm)

The highest score of an SRA indicator occurred within Zone 1 and Zone 2 where 'nativeness' was characterised as 'moderate'. Within all other zones, all indicator scores ranged from 'extremely poor' to 'poor' (Tables 4 and 5). After aggregation of the 'expectedness', 'recruitment' and 'nativeness' indicators, the SRA fish condition index characterised the fish assemblages of Zone 1 and Zone 2 as in 'poor' condition and Zone 3 and Zone 4 as 'extremely poor' condition when benchmarked across the Macquarie catchment management area (Tables 2.4 and 2.5).

Table 4. SRA indicators and overall Fish Condition Index (ndxFS) scores calculated from Round 3 of sampling within the Macquarie River for each sampling site.

| Site | Zone | Recruitment | Nativeness | Expectedness | ndxFS |
| :--- | :--- | ---: | ---: | ---: | ---: | ---: |
| Dubbo Boat Ramp | Zone 1 | 46.2 | 67.6 | 60.2 | 47.5 |
| Peach Tree Reserve | Zone 1 | 46.2 | 37.4 | 60.2 | 39.4 |
| Brilbral | Zone 1 | 46.2 | 77.6 | 46.6 | 44.3 |
| Boral TSR | Zone 1 | 46.2 | 70.3 | 65.0 | 51.0 |
| Bonada | Zone 1 | 46.2 | 61.9 | 46.6 | 39.8 |
| Mullah | Zone 2 | 37.6 | 87.5 | 46.6 | 40.4 |
| Gin Gin Bridge | Zone 2 | 37.6 | 81.9 | 53.1 | 43.6 |
| Ellengerah | Zone 2 | 37.6 | 66.1 | 46.6 | 36.8 |
| Wambool | Zone 2 | 37.6 | 42.1 | 46.6 | 32.6 |
| DS Gin Gin Weir | Zone 2 | 37.6 | 71.8 | 60.2 | 44.5 |
| Willancorah | Zone 3 | 4.2 | 28.3 | 22.0 | 3.9 |
| Pillicawarrina | Zone 3 | 4.2 | 12.1 | 14.7 | 0.5 |
| Marebone | Zone 3 | 4.2 | 76.4 | 22.0 | 11.4 |
| Groggans | Zone 3 | 4.2 | 45.0 | 33.2 | 11.6 |
| Old Oxley | Zone 3 | 4.2 | 17.6 | 33.2 | 5.9 |
| Marthaguy Junction | Zone 4 | 0.0 | 67.0 | 3.6 | 8.3 |
| Yanda | Zone 4 | 0.0 | 0.0 | 0.1 | 0.0 |

Note: Three sites in Zone 4 were not sampled during Round 3 because they were dry (see Appendix 3).

Table 5. Summary table displaying SRA indicators and overall fish index scores calculated from Round 3 of sampling for each zone.

| Zone | Recruitment | Nativeness | Expectedness | ndxFS |
| :--- | :--- | :--- | :--- | :--- |
| Zone 1 | Poor $(46.2 \pm 0)$ | Moderate $(62.96 \pm 6.87)$ | Poor $(55.72 \pm 3.82)$ | Poor $(44.4 \pm 2.23)$ |
| Zone 2 | Very poor $(37.6 \pm 0)$ | Moderate $(69.88 \pm 7.89)$ | Poor $(50.62 \pm 2.71)$ | Poor $(39.58 \pm 2.21)$ |
| Zone 3 | Extremely poor $(4.2 \pm 0)$ | Poor $(35.88 \pm 11.58)$ | Very poor $(25.02 \pm 3.60)$ | Extremely poor $(6.66 \pm 2.16)$ |
| Zone 4 | Extremely poor $(0.0 \pm 0)$ | Poor $(33.50 \pm 33.50)$ | Extremely poor $(1.85 \pm 1.75)$ | Extremely poor $(4.15 \pm 4.15)$ |

Note: Categorisation of Sustainable Rivers Audit (SRA) indicator scores are also presented ('extremely poor' $=0-20$; 'very poor' $=21-40$; 'poor' $=41-60$; 'moderate' = 61-80; 'good' = 81-100); values are presented as averages $\pm$ standard error (SE).

Multivariate analysis of fish community composition suggested no significant interaction between sampling round $\times$ zone (Table 6a). However, there was a significant main effect for sampling round and zone (Table 6a). Pairwise comparisons indicated the fish community was significantly different between each of the sampling zones and each of the sampling rounds (Tables 6b and 6c, respectively). For visual comparison, nMDS plots display dissimilarity in the fish community composition between sampling rounds and sampling zones (Figures 6 and 7, respectively).

Table 6. Results of a) PERMANOVA test examining fish community composition; b) pairwise comparison of main effect 'round'; and c) pairwise comparison of main effect 'zone'.
a)

| Source | df | SS | MS | Pseudo-F | P(perm) | Unique perms |
| :--- | :---: | ---: | ---: | ---: | ---: | :---: |
| Zone | 2 | 29,420 | $9,806.70$ | 17.638 | 0.0001 | 9,918 |
| Round | 3 | 6,144 | $3,072.00$ | 5.5252 | 0.0001 | 9,938 |
| Zone $\times$ round | 6 | $2,838.9$ | 473.15 | 0.8510 | 0.6729 | 9,915 |
| Residual | 42 | 23,352 | 556.00 |  |  |  |
| Total | 53 | 63,318 |  |  |  |  |

b)

| Groups | $\mathbf{t}$ | $\mathbf{P}($ perm $)$ | Unique perms |
| :--- | :---: | :---: | :---: |
| Round 1, Round 2 | 2.5157 | 0.0003 | 9,961 |
| Round 1, Round 3 | 2.3673 | 0.0001 | 9,955 |
| Round 2, Round 3 | 2.0620 | 0.0018 | 9,958 |

c)

| Groups | $\mathbf{t}$ | $\mathbf{P ( p e r m )}$ | Unique perms |
| :--- | :---: | :---: | :---: |
| Zone 1, Zone 2 | 3.4172 | 0.0001 | 9,949 |
| Zone 1, Zone 3 | 4.1919 | 0.0001 | 9,941 |
| Zone 1, Zone 4 | 4.4195 | 0.0001 | 9,962 |
| Zone 2, Zone 3 | 4.8241 | 0.0001 | 9,962 |
| Zone 2, Zone 4 | 6.6195 | 0.0001 | 9,945 |
| Zone 3, Zone 4 | 1.7949 | 0.0158 | 9,968 |

Note: PERMANOVA = permutational analysis of variance (ANOVA)/multivariate ANOVA (MANOVA); df = degrees of freedom; SS = sum-of-squares; MS= mean squares

Figure 6. Non-metric multi-dimensional scaling (nMDS) plot illustrating dissimilarity in community composition between sampling rounds.


Figure 7. Non-metric multi-dimensional scaling (nMDS) plot illustrating dissimilarity in community composition between sampling zones.


Coherence plots were constructed to distinguish species which were functionally similar between samples. When rare species were excluded, the SIMPROF test identified five groups of functionally similar species, including: 1) goldfish, yabby, spangled perch and bony bream; 2) un-specked hardyhead, Murray-Darling rainbowfish and eel-tailed catfish; 3) flatheaded gudgeon and Australian smelt; 4) common carp, golden perch, long-arm prawn and Murray cod; and 5) eastern gambusia and carp-gudgeon (Figure 8). The RELATE test
(Primer v7) found seriation in the fish community composition along a riverine gradient from Zone 1 through to Zone 4 ( $R h o=0.333, p=0.001$, permutations $=999$ ).

Figure 8. Coherence plots grouping species that exhibited similar patterns in presence and abundance between samples.

(continued)

Figure 8 (cont'd). Coherence plots grouping species that exhibited similar patterns in presence and abundance between samples.


(continued)

Figure 8 (cont'd). Coherence plots grouping species that exhibited similar patterns in presence and abundance between samples.


### 3.2 Recruitment and daily aging

## Murray cod

All juvenile Murray cod ( $n=40,<113 \mathrm{~mm} \mathrm{TL}$ ) collected in Rounds 2 and 3 of the fish community sampling within Zone 2 were daily aged from transverse sections of a sagittal otolith (Figure 9). A 20\% re-read of Murray cod otoliths by a second observer yielded a coefficient of variation of $2.12 \%$. A linear growth function fitted to juvenile Murray cod length-at-age data provided the best fit and is detailed in Figure 10. The majority of daily aged fish had back-calculated hatch dates between 28 September and 2 December 2014 with water temperatures ranging from 18 to $25^{\circ} \mathrm{C}$ (Figure 11). This approximately coincided with the environmental water reaching Zone 2 on 9 October, though numerous juveniles had backcalculated hatch dates prior to the arrival of the environmental water (Figure 11). Almost all juvenile Murray cod were sampled within Zone 2 during Rounds 2 and 3 of sampling. No juvenile Murray cod were sampled within Zones 3 and 4, while only one juvenile cod ( 107 cm TL ) was caught in Zone 1 during Round 3 of sampling (Figure 5).

Figure 9. Daily aged otolith of a 55 mm total length (TL) Murray cod.


Note: White dots indicate daily growth rings; estimated age of 102 days.

Figure 10. Length-at-age data for Murray cod with growth function fitted.


Figure 11. Frequency of back-calculated hatch dates for daily aged juvenile Murray cod sampled from Zone 2 of the Macquarie River over three sampling events.


Note: Round 1 = September 2014; Round 2 = January 2015; Round 3 = March 2015; additional sampling effort was performed outside Sustainable Rivers Audit (SRA) protocol. Hydrology and water temperature are displayed (hydrograph data: Gin Gin Gauging Station; water temperature: Baroona Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event, based upon the maximum size/age to which daily aging was possible. A larger focus period could likely be assigned to Murray cod however no juvenile recruits >153 days $\mathrm{old} / 113 \mathrm{~mm}$ TL were sampled during the present study.

## Eel-tailed catfish

All juvenile eel-tailed catfish ( $n=31,<115 \mathrm{~mm} \mathrm{TL}$ ) collected in Round 3 of the fish community sampling within Zone 1 were daily aged from longitudinal sections of a sagittal otolith (Figure 12). A 20\% re-read of eel-tailed catfish otoliths by a second observer yielded a coefficient of variation of $2.02 \%$. A linear growth function fitted to juvenile eel-tailed catfish length-at-age data provided the best fit and is detailed in Figure 13. The majority of daily aged fish had back-calculated hatch dates between 27 November 2014 and 31 January 2015. This approximately coincided with water temperatures within Zone 1 reaching $20^{\circ} \mathrm{C}$ (Figure 14). Juvenile eel-tailed catfish were exclusively caught within Zone 1, primarily during Round 3 of sampling (Figure 5).

Figure 12. Daily aged otolith of a 74 mm total length (TL) eel-tailed catfish.


Note: White dots indicate daily growth rings; estimated age of 90 days.

Figure 13. Length-at-age data for eel-tailed catfish with growth function fitted.


Figure 14. Frequency of back-calculated hatch dates for daily aged juvenile eel-tailed catfish sampled from Zone 1 of the Macquarie River over three sampling events.


Note: Round 1 = August 2014; Round 2 = January 2015; Round 3 = March 2015; additional sampling effort was performed outside Sustainable Rivers Audit (SRA) protocol. Hydrology and water temperature collected at the 'DS Burrendong' Gauging Station are displayed. Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).

## Bony bream

All juvenile bony bream ( $n=32,<73 \mathrm{~mm}$ fork length; FL) collected in Round 1 of the fish community sampling within Zone 4 were daily aged from longitudinal sections of a sagittal otolith (Figure 15). A 20\% re-read of bony bream otoliths by a second observer yielded a coefficient of variation of $0.59 \%$. A linear growth function fitted to juvenile bony bream length-at-age data provided the best fit and is detailed in Figure 16. High variability in the growth rates of juveniles was observed, as indicated by the low global $R$ value ( $R_{2}=0.323$ ). This was of little concern as no fish ages were estimated from lengths using the growth model. Rather all captured juveniles were aged and these data used to construct Figure 17.
The majority of Juvenile bony bream were caught within Zone 4 during Round 1 of fish community sampling (Figure 5). The majority of daily aged fish had back-calculated hatch dates between 13 June and 7 July 2014 (Figure 17). This coincided with water temperatures of approximately $13^{\circ} \mathrm{C}$ and a late June 2014 flow pulse in the Macquarie Marshes that was significantly reduced by the time it passed through the Macquarie Marshes and reached Zone 4 (Figure 17). Catches of juvenile bony bream within Zone 4 may therefore be attributed to the downstream dispersal of juveniles from the Macquarie Marshes (Zone 3). Alternatively, the recruitment observed may be associated with the receding tail of a flow event within the Barwon River and a small flow event within the lower Macquarie River that created connectivity between the two river systems (Figure 17).

Figure 15. Daily aged otolith of a 56 mm fork length (FL) bony bream.


Note: White dots indicate daily growth rings; estimated age of 82 days.

Figure 16. Length-at-age data for bony bream with growth function fitted.


Figure 17. Frequency of back-calculated hatch dates for daily aged juvenile bony bream sampled during standardised fish community sampling (SRA protocol) within Zone 4 of the Macquarie River over three sampling events.


Note: Round 1 = September 2014; Round 2 = December 2014; Round 3 = April 2015; hydrology and water temperature are displayed (hydrograph data for the Macquarie River Zone 3, Macquarie River Zone 4 and Barwon River were collected at Pillicawarrina, Carinda and Geera gauging stations, respectively; water temperature is for Macquarie River at the Carinda Gauging Station. Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).

## Australian smelt

A subsample of 50 Australian smelt ( $<52 \mathrm{~mm} \mathrm{FL}$ ) collected from Zones 1 and 2 during Round 2 of the fish community sampling were daily aged from longitudinal sections of a sagittal otolith (Figure 18). A 20\% re-read of Australian smelt otoliths by a second observer yielded a coefficient of variation of $3.30 \%$. A von Bertalanffy growth function fitted to length-at-age data provided the best fit and is detailed in Figure 19. Back-calculated hatch dates occurred throughout the majority of the study period; however, peaks in hatch dates generally occurred during the receding tails of flow events or during sustained periods of high flow after substantial increases in flow (Figure 20). Australian smelt were caught within Zones 1 and 2 during each of the three rounds of sampling (Table 1 and Figure 5).

Figure 18. Daily aged otolith of a 27 mm fork length (FL) Australian smelt.


Note: White dots indicate daily growth rings; estimated age of 78 days.

Figure 19. Length-at-age data for Australian smelt with von Bertalanffy growth function fitted.


Figure 20. Frequency of back-calculated hatch dates for daily aged juvenile Australian smelt sampled during standardised fish community sampling (SRA protocol) from: a) Zone 1; and b) Zone 2 of the Macquarie River over three sampling events.


Note: Round 1 = August 2014; Round 2 = January 2015; Round 3 = March 2015; hydrology and water temperature are displayed (hydrograph data: a) Wellington Gauging Station, b) Gin Gin Gauging Station; water temperature: a) DS Burrendong Gauging Station, b) Baroona Gauging Station. Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).
(continued)

Figure 20 (cont'd). Frequency of back-calculated hatch dates for daily aged juvenile Australian smelt sampled during standardised fish community sampling (SRA protocol) from: a) Zone 1; and b) Zone 2 of the Macquarie River over three sampling events.
b)


Note: Round 1 = September 2014; Round 2 = January 2015; Round 3 = March 2015; hydrology and water temperature are displayed (hydrograph data: a) Wellington Gauging Station, b) Gin Gin Gauging Station; water temperature: a) DS Burrendong Gauging Station, b) Baroona Gauging Station. Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).

## Un-specked hardyhead

A subsample of 49 un-specked hardyhead ( $<60 \mathrm{~mm}$ FL) collected from Zones 1 and 2 during Round 2 of the fish community sampling were daily aged from longitudinal sections of a sagittal otolith (Figure 21). A 20\% re-read of un-specked hardyhead otoliths by a second observer yielded a coefficient of variation of $2.11 \%$. A von Bertalanffy growth function fitted to length-at-age data provided the best fit and is detailed in Figure 22. Back-calculated hatch dates occurred throughout the majority of the focus period; however, peaks in hatch dates generally occurred during the receding tails of flow events or during sustained periods of increased flow when water temperatures reached $20^{\circ} \mathrm{C}$ (Figure 23). Un-specked hardyhead were only sampled within Zones 1 and 2, with very few un-specked hardyhead caught during Round 1 of sampling (Table 1 and Figure 5).

Figure 21. Daily aged otolith of a 19 mm fork length (FL) un-specked hardyhead.


Note: White dots indicate daily growth rings; estimated age of 46 days.

Figure 22. Length-at-age data for un-specked hardyhead with von Bertalanffy growth function fitted.


Figure 23. Frequency of back-calculated hatch dates for daily aged juvenile un-specked hardyhead sampled during standardised fish community sampling (SRA protocol) from: a) Zone 1; and b) Zone 2 of the Macquarie River over three sampling events.
a)


Note: Round 1 = August 2014; Round 2 = January 2015; Round 3 = March 2015; hydrology and water temperature are displayed (hydrograph data: a) Wellington Gauging Station, b) Gin Gin Gauging Station; water temperature: a) DS Burrendong Gauging Station, b) Baroona Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).
(continued)

Figure 23 (cont'd). Frequency of back-calculated hatch dates for daily aged juvenile un-specked hardyhead sampled during standardised fish community sampling (SRA protocol) from: a) Zone 1; and b) Zone 2 of the Macquarie River over three sampling events.
b)


Note: Round 1 = September 2014; Round 2 = January 2015; Round 3 = March 2015; hydrology and water temperature are displayed (hydrograph data: a) Wellington Gauging Station, b) Gin Gin Gauging Station; water temperature: a) DS Burrendong Gauging Station, b) Baroona Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).

## Common carp

A subsample of 36 common carp ( $\leq 112 \mathrm{~mm}$ FL) collected from Zones 3 and 4 during Rounds 2 and 3 of the fish community sampling were daily aged from longitudinal sections of a lapilli otolith (Figure 24). The 'focus period' of common carp was 80 days (approx. 112 mm FL). The interpretability of daily growth increments was not reliable for fish older than 80 days. Similarly, Vilizzi (1998) noted age interpretation was difficult when $>80-90$ increments were present. This was attributed to morphological change in the lapilli by increased curvature of the sagittal plane (Vilizzi, 1998). A 20\% re-read of common carp otoliths by a second observer yielded a coefficient of variation of $1.11 \%$. A linear growth function fitted to length-at-age data provided the best fit and is detailed in Figure 25. Peaks in hatch date frequency were associated with the leading edge of a flow pulse and during sustained periods of increased flow (Figure 26). Hatch dates coincided with water temperatures as low as $10^{\circ} \mathrm{C}$ (Figure 26). Juvenile recruitment of common carp was most prolific within and downstream of the Macquarie Marshes (Zones 3 and 4) (Figure 5).

Figure 24. Daily aged otolith of a 52 mm fork length (FL) common carp.


Note: White dots indicate daily growth rings. Estimated age of 51 days.

Figure 25. Length-at-age data for common carp with growth function fitted.


Figure 26. Frequency of back-calculated hatch dates for daily aged juvenile common carp sampled during standardised fish community sampling (SRA protocol) from: a) Zone 3; and b) Zone 4 of the Macquarie River over three sampling events.
a)


Note: Round 1 = September 2014; Round 2 = December 2014; Round 3 = April 2015; hydrology and water temperature are also displayed (hydrograph data: a) Macquarie River Pillicawarrina Gauging Station and Barwon River Geera Gauging Station, b) Carinda Gauging Station and Barwon River Geera Gauging Station; water temperature: a) Marebone Wier Gauging Station, b) Carinda Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).
(continued)

Figure 26 (cont'd). Frequency of back-calculated hatch dates for daily aged juvenile common carp sampled during standardised fish community sampling (SRA protocol) from:
a) Zone 3; and b) Zone 4 of the Macquarie River over three sampling events.
b)


Note: Round 1 = September 2014; Round 2 = December 2014; Round 3 = April 2015; hydrology and water temperature are also displayed (hydrograph data: a) Macquarie River Pillicawarrina Gauging Station and Barwon River Geera Gauging Station, b) Carinda Gauging Station and Barwon River Geera Gauging Station; water temperature: a) Marebone Wier Gauging Station, b) Carinda Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).

## Murray-Darling rainbowfish

A subsample of 33 Murray-Darling rainbowfish ( $<43 \mathrm{~mm} \mathrm{FL}$ ) collected from Zones 1 and 2 during Rounds 1 and 2 of the fish community sampling were daily aged from longitudinal sections of a sagittal otolith (Figure 27). A 20\% re-read of Murray-Darling rainbowfish otoliths by a second observer yielded a coefficient of variation of 5.90\%. A von Bertalanffy growth function fitted to length-at-age data provided the best fit and is detailed in Figure 28. Back-calculated hatch dates occurred throughout the majority of the study period; however, peaks in hatch date frequencies generally occurred during the receding tails of flow events or during sustained periods of increased flow (Figure 29). Fish spawned over a wide temperature range, from as low as $10^{\circ} \mathrm{C}$ to as high as $25^{\circ} \mathrm{C}$ (Figure 29). The majority of Murray-Darling rainbowfish were caught within Zones 1 and 2, with none caught within Zone 4 (Table 1 and Figure 5).

Figure 27. Daily aged otolith of a 14 mm fork length (FL) Murray-Darling rainbowfish.


Note: White dots indicate daily growth rings. Estimated age of 55 days.

Figure 28. Length-at-age data for Murray-Darling rainbowfish with von Bertalanffy growth function fitted.


Figure 29. Frequency of back-calculated hatch dates for daily aged juvenile Murray-Darling rainbowfish sampled during standardised fish community sampling (SRA protocol) from: a) Zone 1; and b) Zone 2 of the Macquarie River over three sampling events.


Note: Round 1 = August 2014; Round 2 = January 2015; Round 3 = March 2015; hydrology and water temperature are displayed (hydrograph data: a) Dubbo Gauging Station, b) Gin Gin Gauging Station; water temperature: a) Baroona Gauging Station, b) Baroona Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).
(continued)

Figure 29 (cont'd). Frequency of back-calculated hatch dates for daily aged juvenile Murray-Darling rainbowfish sampled during standardised fish community sampling (SRA protocol) from: a) Zone 1; and b) Zone 2 of the Macquarie River over three sampling events.
b)


Note: Round 1 = September 2014; Round 2 = January 2015; Round 3 = March 2015; hydrology and water temperature are displayed (hydrograph data: a) Dubbo Gauging Station, b) Gin Gin Gauging Station; water temperature: a) Baroona Gauging Station, b) Baroona Gauging Station). Coloured arrows indicate the 'focus period' for each sample. The 'focus period' extends to earliest date to which a hatch date could be assigned to fish from each sampling event (based upon the maximum size/age to which daily aging was possible).

### 3.3 Fish health and fish condition index

## Common carp condition index

Analysis of common carp condition indices (Table 7) showed a significant interaction between sampling round and zone ( $F=20.30, P<0.001$ ). Results of pairwise tests comparing: 1) fish condition between zones within each round of sampling; and 2) fish condition between rounds within each zone are displayed in Tables 8 a and 8b, respectively.

## Murray cod condition index

Analysis of Murray cod condition indices (Table 7) showed a significant interaction between sampling round and zone ( $F=4.464, P=0.002$ ). Results of pairwise tests comparing: 1) fish condition between zones within each round of sampling; and 2) fish condition between rounds within each zone are displayed in Tables 8a and 8b, respectively.

## Golden perch condition index

Analysis of golden perch condition indices (Table 7) showed no significant interaction between sampling round and zone ( $F=0.503, P=0.774$ ). No significant difference was observed between sampling rounds ( $F=1.810, P=0.167$ ). A significant difference in fish
condition was observed between zones ( $F=27.976, P<0.001$ ), where Zone 1 was significantly greater than Zones 2, 3 and 4 (Table 7).

## Bony bream condition index

Analysis of bony bream condition indices showed no significant interaction between sampling round and zone ( $F=1.457, P=0.233$ ). No significant difference was observed between sampling rounds ( $F=0.236, P=0.791$ ). A significant difference in fish condition was observed between zones ( $F=5.664, P=0.002$ ), where Zone 3 was significantly greater than Zone 4 (Table 7).

Table 7. Fish condition indices for large-bodied species by sampling round and zone.

| Common carp |  |  |  |
| :--- | :---: | :---: | :---: |$|$ Round 1 $\quad$ Round 2 $\quad$ Round 3 \(~\left(\begin{array}{llll|}\hline Zone 1 \& 1.044 \pm 0.109 \& 0.922 \pm 0.088 \& 0.915 \pm 0.09 <br>

\hline Zone 2 \& 1.005 \pm 0.087 \& 0.944 \pm 0.084 \& 0.932 \pm 0.081 <br>
\hline Zone 3 \& 0.834 \pm 0.085 \& 0.872 \pm 0.108 \& 0.871 \pm 0.074 <br>
\hline Zone 4 \& 0.928 \pm 0.123 \& 0.861 \pm 0.063 \& <br>
\hline\end{array}\right.\)

| Murray cod |  |  |  |
| :---: | :---: | :---: | :---: |
|  | Round 1 | Round 2 | Round 3 |
| Zone 1 | $1.249 \pm 0.015$ |  | $1.062 \pm 0.071$ |
| Zone 2 | $0.986 \pm 0.095$ | $0.998 \pm 0.118$ | $0.963 \pm 0.071$ |
| Zone 3 | $1.207 \pm 0.127$ | $1.075 \pm 0.08$ | $0.972 \pm 0.116$ |

## Golden perch

|  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- |
| Zone 1 | $1.045 \pm 0.119$ | $0.995 \pm 0.143$ | $0.958 \pm 0.175$ |
| Zone 2 | $0.826 \pm 0.113$ | $0.841 \pm 0.117$ | $0.796 \pm 0.125$ |
| Zone 3 | $0.818 \pm 0.104$ | $0.77 \pm 0.127$ | $0.8 \pm 0.089$ |
| Zone 4 | $0.795 \pm 0.027$ | $0.721 \pm 0.119$ |  |


| Bony bream |  |  |  |
| :---: | :---: | :---: | :---: |
|  | Round 1 | Round 2 | Round 3 |
| Zone 2 |  |  | $1.008 \pm 0.079$ |
| Zone 3 | $0.966 \pm 0.161$ | $1.019 \pm 0.027$ | $0.972 \pm 0.141$ |
| Zone 4 | $0.889 \pm 0.119$ | $0.771 \pm 0.073$ | $0.813 \pm 0.104$ |

Note: Values are mean $\pm$ standard deviation (SD).

Table 8. Results of Tukey's HSD pairwise comparisons of condition index for common carp and Murray cod between: a) zones within each round of sampling; and b) between sampling rounds within each zone.
a)

Common carp

| Round 1 |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: |
|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |  |
| Zone 1 |  |  | $* * *$ | $* * *$ |  |
| Zone 2 |  |  | $* * *$ | $* * *$ |  |
| Zone 3 |  |  |  | $* * *$ |  |
| Zone 4 |  |  |  |  |  |


| Round 2 |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: |
|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |  |
| Zone 1 |  |  | $* * *$ | $* * *$ |  |
| Zone 2 |  |  | $* * *$ | $* * *$ |  |
| Zone 3 |  |  |  |  |  |
| Zone 4 |  |  |  |  |  |

Murray cod

| Round 1 |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: |
|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |  |
| Zone 1 |  | $* *$ |  |  |  |
| Zone 2 |  |  | $* * *$ |  |  |
| Zone 3 |  |  |  |  |  |
| Zone 4 |  |  |  |  |  |

Round 2

|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |
| :---: | :--- | :--- | :--- | :--- |
| Zone 1 |  |  |  |  |
| Zone 2 |  |  |  |  |
| Zone 3 |  |  |  |  |
| Zone 4 |  |  |  |  |

## Round 3

|  | Zone 1 | Zone 2 | Zone 3 | Zone 4 |
| :--- | :--- | :--- | :--- | :--- |
| Zone 1 |  |  |  |  |
| Zone 2 |  |  |  |  |
| Zone 3 |  |  |  |  |
| Zone 4 |  |  |  |  |

Note: *** $=P<0.0001 ;{ }^{* *}=P<0.001 ;^{*}=P<0.05$; blank $=P>0.05$; shaded $=$ not applicable.
(continued)

Table 8 (cont'd). Results of Tukey's HSD pairwise comparisons of condition index for common carp and Murray cod between: a) zones within each round of sampling; and b) between sampling rounds within each zone.
b)

Common carp

| Zone 1 |  |  |  |  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: |
|  |  | $* * *$ | $* * *$ |  |  |  |  |
| Round 1 |  |  |  |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |


| Zone 2 |  |  |  |  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: |
| Round 1 |  | $* *$ | $* * *$ |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |


| Zone 3 |  |  |  |  |
| :--- | :--- | :--- | :--- | :---: |
|  | Round 1 | Round 2 | Round 3 |  |
| Round 1 |  |  |  |  |
| Round 2 |  |  |  |  |
| Round 3 |  |  |  |  |

## Murray cod

| Zone 1 |  |  |  |  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |
| Round 1 |  |  |  |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |


| Zone 2 |  |  |  |  |
| :--- | :--- | :--- | :--- | :---: |
|  | Round 1 | Round 2 | Round 3 |  |
| Round 1 |  |  |  |  |
| Round 2 |  |  |  |  |
| Round 3 |  |  |  |  |


| Zone 3 |  |  |  |  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: | :---: |
|  |  |  | * |  |  |  |  |
| Round 1 |  |  |  |  |  |  |  |
| Round 2 |  |  |  |  |  |  |  |
| Round 3 |  |  |  |  |  |  |  |

## Zone 4

|  | Round 1 | Round 2 | Round 3 |
| :--- | :--- | :--- | :--- |
| Round 1 |  | * |  |
| Round 2 |  |  |  |
| Round 3 |  |  |  |

Note: *** $=P<0.0001$; ** $=P<0.001$; ${ }^{*}=P<0.05$; blank $=P>0.05$; shaded $=$ not applicable; Zone 4 not applicable to Murray cod as none were caught in that zone

## Proportion of catch suffering a health condition

The most prevalent health conditions observed over three rounds of fish community sampling throughout the Macquarie River during the 2014/2015 watering year were anchor worms (Lernaea spp.), affecting $8.53 \%$ of individuals, and wounds, affecting $0.50 \%$ of individuals. All other symptoms affected $<0.50 \%$ of the population (Figure 30). The proportion of the population affected by anchor worms varied between sampling rounds. During Round $1,5.71 \%$ of the population was affected by anchor worms; this increased to $10.59 \%$ in Round 2 and remained relatively stable into Round 3 at $9.40 \%$ (Figure 30). The species of fish most heavily affected by symptoms of disease and parasites were Murray cod and golden perch (Figure 31). For each of these two species, symptoms were most prevalent during Round 2 of sampling (Figure 31).

Figure 30. The percentage of individuals affected by a range of symptoms of disease or parasites for each round of sampling throughout the Macquarie River during the 2014/2015 watering year.


Figure 31. The percentage of individuals of each species affected by symptoms of disease or parasites for each round of sampling throughout the Macquarie River during the 2014/2015 watering year.


### 3.4 Water quality

In most instances, water quality parameters measured throughout the Macquarie River fell within relevant Australia and New Zealand Environment and Conservation Council (ANZECC) guidelines for NSW upland and lowland rivers (ANZECC and ARMCANZ, 2000). However, at a number of locations, water quality parameters were outside the ANZECC trigger values (Table 9). Multivariate analysis of water quality variables illustrated a significant interaction between sampling zone $\times$ round ( $F_{6,42}=5.677$ ). Pairwise comparisons of the interaction term indicated water quality variables were significantly different between each of the sampling zones within rounds, with the exception of Zone 2 and Zone 3 in Round 1 of sampling. Each of the sampling rounds was also different within each zone, with the exception of Round 2 and Round 3 within Zone 4. Dissimilarity of water quality parameters between sampling rounds and sampling zones are displayed by nMDS plots in Figures 32 and 33, respectively.

Table 9. Average ( $n=2$ ) water quality measurement at $0.2 m$ depth for each sampling site during each round of sampling. Shaded values indicate water quality measurements outside ANZECC guidelines.

|  |  |  |  |  | und 1 |  |  |  |  |  | nd 2 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Site name | Zone | Water temp. ( ${ }^{\circ} \mathrm{C}$ ) | pH | Conductivity (mS.cm ${ }^{-1}$ ) | Dissoled oxygen (\% saturation) | Turbidity | Secchi depth (m) | Water temp. ( ${ }^{\circ} \mathrm{C}$ ) | pH | Conductivity (mS.cm ${ }^{-1}$ ) | Dissoled oxygen (\% saturation) | Turbidity | Secchi depth (m) |
| Bonada | 1 | 12.95 | 7.89 | 0.468 |  | 0.1 | 1.76 | 25.53 | 8.03 | 0.403 |  | 0 | 1.52 |
| Boral TSR | 1 | 14.44 | 9.195 | 0.4595 |  | 10.4 | 0.99 | 25.92 | 8.32 | 0.4135 |  | 4.65 | 1.3 |
| Peach Tree Reserve | 1 | 14.875 | 8.055 | 0.565 | 81.68 | 3.95 | 0.83 | 28.15 | 8.485 | 0.4175 | 96.94 | 7.9 | 0.9 |
| Brilbral | 1 | 15.17 | 8.165 | 0.5345 | 95.22 | 5.15 | 0.7 | 28.35 | 8.42 | 0.416 | 99.13 | 6.8 | 1.04 |
| Dubbo Boat Ramp | 1 | 15.255 | 8.105 | 0.593 | 85.97 | 11.95 | 0.52 | 29.365 | 8.39 | 0.4255 | 81.66 | 12.5 | 0.77 |
| Wambool | 2 | 13.58 | 8.575 | 0.6505 |  | 66.75 |  | 29.305 | 8.435 | 0.4415 | 88.33 | 44.85 | 0.51 |
| DS Gin Gin Weir | 2 | 16.39 | 8.27 | 0.76 | 96.71 | 24.2 | 0.4 | 27.355 | 8.38 | 0.4905 | 80.83 | 30.9 | 0.44 |
| Gin Gin Bridge | 2 | 16.36 | 8.22 | 0.76 | 97.93 | 24.1 |  | 29.175 | 8.46 | 0.452 | 73.71 | 24.55 | 0.55 |
| Mullah | 2 | 14.99 | 7.67 | 0.5665 |  | 28.25 | 0.41 | 27.395 | 8.34 | 0.455 | 73.63 | 42.45 | 0.5 |
| Ellengerah | 2 | 15.925 | 7.97 | 0.561 |  | 34.2 | 0.32 | 27.01 | 8.65 | 0.451 | 81.31 | 50.65 | 0.34 |
| Marebone | 3 | 16.145 | 7.715 | 0.7425 | 92.61 | 26.35 | 0.36 | 27.42 | 8.2 | 0.449 | 67.35 | 60 | 0.3 |
| Groggans | 3 | 18.685 | 8.08 | 0.742 | 86.69 | 32.41 | 0.24 | 28.775 | 8.31 | 0.483 | 79.83 | 101.8 | 0.21 |
| Old Oxley | 3 | 17.32 | 7.99 | 0.7365 | 87.35 | 42.8 | 0.19 | 26.37 | 8.26 | 0.4905 | 69.93 | 145 | 0.13 |
| Willancorah | 3 | 14.795 | 7.72 | 0.782 | 65.34 | 48.5 | 0.15 | 24.695 | 7.835 | 0.564 | 47.01 | 233 | 0.15 |
| Pillicawarrina | 3 | 18.555 | 8.21 | 0.761 |  | 51 | 0.14 | 24.24 | 8.215 | 0.5565 | 78.58 | 142.5 | 0.15 |
| Yanda | 4 | 16.86 | 8.505 | 0.81 | 95.31 | 83.5 | 0.18 | River dry |  |  |  |  |  |
| Marthaguy Junction | 4 | 18.635 | 8.195 | 0.8015 | 86.8 | 59.15 | 0.23 |  |  |  |  |  |  |
| Brewon | 4 | 20.435 | 8.365 | 0.775 | 101.06 | 64.5 | 0.1 |  |  |  |  |  |  |
| Binghi | 4 | 20.985 | 8.25 | 0.7695 | 96.83 | 227 | 0.1 | 32.705 | 8.89 | 2.22 |  | 741.5 | 0.14 |
| Glenacre | 4 | 19.565 | 8.125 | 0.77 | 95.41 | 117.5 | 0.14 | 25 | 8.785 | 1.605 | 64.43 | 387 |  |

Table 9 (Cont'd). Average water quality measurement at 0.2 m depth for each sampling site during each round of sampling. Shaded values indicate water quality measurements outside ANZECC guidelines.

|  |  | Round 3 |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Site name | Zone | Water temp. ( ${ }^{\circ} \mathrm{C}$ ) | pH | Conductivity (mS.cm ${ }^{-1}$ ) | Dissoled oxygen (\% saturation) | Turbidity | Secchi depth (m) |
| Bonada | 1 | 23.66 | 8.295 | 0.431 | 97.22 | 0 | 2.42 |
| Boral TSR | 1 | 24.165 | 8.19 | 0.442 | 106.61 | 0 | 1.65 |
| Peach Tree Reserve | 1 | 23.67 | 8.21 | 0.442 | 95.1 | 0 | 1.4 |
| Brilbral | 1 | 27.2 | 8.19 | 0.4455 | 100.65 | 0 | 1.4 |
| Dubbo Boat Ramp | 1 | 24.58 | 7.985 | 0.4505 | 97.57 | 3.85 | 0.92 |
| Wambool | 2 | 24.035 | 8.225 | 0.49 | 98.85 | 7.15 | 0.61 |
| DS Gin Gin Weir | 2 | 24.775 | 8.12 | 0.49 | 97.69 | 3.9 | 0.87 |
| Gin Gin Bridge | 2 | 25.02 | 8.505 | 0.499 | 107.17 | 6 | 0.7 |
| Mullah | 2 | 24.655 | 8.235 | 0.4955 | 105.75 | 6.8 | 0.6 |
| Ellengerah | 2 | 23.08 | 8.155 | 0.5 | 97.59 | 16.4 | 0.63 |
| Marebone | 3 | 20.225 | 7.955 | 0.5035 | 82.19 | 37.5 | 0.37 |
| Groggans | 3 | 19.745 | 8.335 | 0.5085 | 101.48 | 95.35 | 0.2 |
| Old Oxley | 3 | 17.785 | 8.16 | 0.5205 | 82.48 | 46.495 | 0.2 |
| Willancorah | 3 | 18.61 | 8.305 | 0.9975 | 87.32 | 154 |  |
| Pillicawarrina | 3 | 17.28 | 8.49 | 0.521 | 101.42 | 190 | 0.15 |
| Yanda | 4 | 20.47 | 7.525 | 0.5945 | 47.59 | 0 | 1.12 |
| Marthaguy Junction | 4 | 20.225 | 8.24 | 0.693 | 76.54 | 7.2 | 0.6 |
| Brewon | 4 | River dry |  |  |  |  |  |
| Binghi | 4 |  |  |  |  |  |  |
| Glenacre | 4 |  |  |  |  |  |  |

Figure 32. Non-metric multi-dimensional scaling (nMDS) plot illustrating dissimilarity of water quality variables between sampling rounds.

Non-metric MDS
Normalise
Resemblance: D1 Euclidean distance


Figure 33. Non-metric multi-dimensional scaling (nMDS) plot illustrating dissimilarity of water quality variables between sampling zones.

Non-metric MDS
Normalise
Resemblance: D1 Euclidean distance


## 4 Discussion

### 4.1 The role of environmental flows in the recruitment of fish

Typically, the natural flow regimes of dryland rivers are highly variable, with periods of flooding punctuating extended dry periods. The productivity of dryland river and marsh ecosystems is therefore highly dependent on periods of high flow (Puckridge et al., 1998; Kingsford et al., 1999; Bunn et al., 2006; Arthington and Balcombe, 2011). High flow events and floodplain inundation have been identified as the source and delivery mechanism for basal carbon within riverine ecosystem food webs (Gawne et al., 2007). In addition, fish assemblages in dryland rivers have evolved life-history strategies attuned to environmental triggers (Sternberg and Kennard, 2013), particularly flow regimes (Bunn and Arthington, 2002). Three primary mechanisms associated with high flow events within lotic systems have been proposed to facilitate enhanced native fish recruitment: 1) high flow events provide spawning triggers; 2) high flow events boost primary production that facilitates successful recruitment; and 3) during high flow events, additional habitat and food-rich ephemeral environments are made available as a result of the inundation of backwaters and floodplains (Reynolds, 1983; Junk et al., 1989; Harris and Gehrke, 1994; Balcombe et al., 2006, 2007;Balcombe and Arthington, 2009; King et al., 2009). However, the timing, duration and magnitude of such flow events are of critical importance if they are to significantly enhance native fish recruitment. To further complicate the matter, different species have varying life-history characteristics that may respond to different hydrological conditions (King et al., 2003; Rolls et al., 2013; Rayner et al., 2015).
When there is synchrony between the breeding season of a species and conditions optimal for spawning and successful recruitment, strong cohorts can be observed within a population. This is described by the 'window of opportunity hypothesis' (WOOH), which states that in any one year, if optimal conditions for recruitment vary temporally, fish that spawn over a protracted period have a recruitment advantage over those that spawn only for a brief period (Humphries et al., 2013). This is because the probability that a proportion of the larvae of protracted spawning species will encounter a period when conditions are optimal for recruitment is greater than for larvae with only a brief spawning period (Humphries et al., 2013). Humphries et al. (2013) indicated that the recruitment of Australian smelt supports this hypothesis. Findings from the present study indicate that the recruitment of other native fish species is also likely to follow this principle, and hence indicates the importance of flow events in the recruitment native fish species.

The following subsections detail the recruitment patterns of a number of species sampled within the Macquarie River during the 2014/2015 watering year. The recruitment of each species is examined in relation to water temperature and hydrological characteristics and compared with that of existing literature when available.

## Golden perch

No juvenile golden perch were sampled within the Macquarie River during the three rounds of sampling between August 2014 and April 2015. The smallest fish sampled were 120-145 mm TL $(n=3)$ in September 2014 within Zone 4. Existing literature details golden perch require increased river discharge to cue spawning (Lake, 1967; Mackay, 1973), and there is speculation over studies that suggest otherwise (Mallen-Cooper and Stuart, 2003) on the basis of the location of spawning (King et al., 2009). For golden perch, spawning is believed to be preceded by a substantial upstream migration (Reynolds, 1983; Mallen-Cooper et al., 1995). In the southern Murray-Darling Basin, the spawning of golden perch occurs primarily from October to December, coinciding with rising and descending limbs of within-channel and overbank flows at water temperatures of $17-25^{\circ} \mathrm{C}$ (Rowland, 1983b; Mallen-Cooper and Stuart, 2003; King et al., 2009; Zampatti and Leigh, 2013; Zampatti et al., 2015). The absence of juvenile golden perch
throughout the Macquarie River during the 2014/2015 sampling may be associated with: 1) sampling biases (i.e. juvenile golden perch may be less susceptible to electrofishing); or, more probably, 2) no flow event was large enough to cue spawning or result in successful recruitment of juveniles. The latter hypothesis is supported by studies of King et al. (2009) who observed limited recruitment response to smaller flow peaks, both in-channel and small inundation events.

Because golden perch are able to maintain gonadal maturity for an extended period, and do not reabsorb oocytes until February or March (Lake, 1967; Rowland, 1983b; Battaglene, 1991), significant environmental water releases (more than the 28,483 ML of the 2014/2015 watering year) from October to February, when water temperatures are between 17 and $25^{\circ} \mathrm{C}$, may be beneficial for juvenile recruitment.

## Murray cod

The majority of daily aged Murray cod had back-calculated hatch dates between 28 September and 2 December 2014 with water temperatures in the range of $18-25^{\circ} \mathrm{C}$. This approximately coincided with the release of environmental water reaching Zone 2 on 9 October, though numerous juveniles had back-calculated hatch dates earlier than the arrival of the environmental water. Existing literature details Murray cod are a temperature-cued spawner, spawning at water temperatures of $16-20^{\circ} \mathrm{C}$ and do not require an increase in discharge to trigger a spawning event (Lake, 1967; Rowland, 1983a, 1998; Humphries, 2005; King et al., 2009). Day length or some other undefined consistent inter-annual variable has also been suggested as a spawning cue (King et al., 2009). Multiple juvenile Murray cod had back-calculated hatch dates that coincided with water temperatures up to $25^{\circ} \mathrm{C}$, considerably greater than the upper limit of $20^{\circ} \mathrm{C}$ cited in existing literature (Lake, 1967; Rowland, 1983a, 1998). The apparent higher upper limit of water temperature at which hatching occurred may be a result of: 1) Murray cod stocking during late January 2014 (Appendix 8); or 2) variation in the method of recording water temperature between studies (i.e. surface temperatures versus at depth).

While an increase in discharge may not be required to trigger a spawning event, high flow events may increase primary production that facilitates successful recruitment. This is supported by King et al. (2009) who found that Murray cod did not appear to increase their spawning activity in a flood year, but their recruitment was increased, likely through the availability of abundant food resources. Under the 'window of opportunity hypothesis', the successful recruitment of cod is restricted to a narrow window of opportunity due to the brief spawning period.

## Bony bream

The majority of juvenile bony bream were captured within Zone 4 during Round 1 of fish community sampling. The majority of daily aged bony bream had back-calculated hatch dates between 13 June and 7 July 2014. This coincided with water temperatures of approximately $13^{\circ} \mathrm{C}$ and a late June 2014 flow pulse in the Macquarie Marshes that was significantly reduced by the time it passed through the Macquarie Marshes and reached Zone 4 (Figure 17). Catches of juvenile bony bream within Zone 4 may therefore be attributed to the downstream dispersal of juveniles from the Macquarie Marshes (Zone 3).

A second hypothesis for the strong recruitment of juvenile bony bream within Zone 4 during Round 1 of sampling may be associated with the receding tail of a flow event within the Barwon River and a small flow event within the Macquarie River that created connectivity between the two river systems. It is possible that juveniles may have emigrated from the Barwon River to the Macquarie River when the systems were connected. In support of this hypothesis, bony bream as small as 22 mm have been recorded conducting upstream migrations (Baumgartner, 2003). It should be noted that the 130 day 'focus period' associated with Round 1 of sampling does not incorporate the leading edge of the April 2014 flow event within the Barwon River and it is therefore likely the hatch date frequency data only capture the tail-end of this earlier recruitment
pulse. Analyses of otolith chemistry may provide insight into the recruitment source of juvenile bony bream and potentially reinforce the importance of hydrological connectivity between river systems and/or the importance of wetland ecosystems in the recruitment of bony bream.

Findings of the present study add significant information to existing literature on the biology of bony bream. Earlier studies found that bony bream spawn independently of flooding in December-January in the lower Murray and October-December in NSW at temperatures of 21$23^{\circ} \mathrm{C}$ (Llewellyn, 1983; Puckridge and Walker, 1990). This study shows that the spawning window for bony bream is significantly larger than previously thought, and flow events may play a key role in spawning and the subsequent recruitment of the species. A similar study conducted within the Gwydir River system found hatch dates of bony bream coincided with a period of declining flow levels throughout February and March, although peaked at the timing of a brief late February flow pulse (Southwell et al., 2015). In further support of these findings, Gehrke et al. (1995) observed bony bream within the Paroo and Darling catchment to be more abundant after flooding.

## Eel-tailed catfish

Recruitment data for eel-tailed catfish indicate the primary spawning period was between 27 November 2014 and 31 January 2015. This coincides with water temperatures reaching approximately $20^{\circ} \mathrm{C}$ and moderate discharge ( $\sim 300-1,200 \mathrm{ML} /$ day). These findings are supported by earlier studies that have observed courtship in October at water temperatures of $20^{\circ} \mathrm{C}$ in south-eastern Queensland (Merrick and Midgley, 1981), though it is significantly lower than the $24^{\circ} \mathrm{C}$ that Davis (1977) and Lake (1967) suggest is required to initiate spawning from January to March. It is commonly recognised that flooding is not required to initiate spawning for this species (Davis, 1977). However, as is the case for all species, high flow events may boost primary production that facilitates successful recruitment.

## Australian smelt

Hatches date frequencies and recruitment data from the present study indicate the recruitment of Australian smelt occurred throughout the majority of the study period at water temperatures as low as $10^{\circ} \mathrm{C}$. However, peaks in hatch date frequencies generally occurred during the receding tails of flow events or during sustained periods of increased flow when temperatures were $\geq 15^{\circ} \mathrm{C}$. A peak in hatch date frequency within Zone 1 during Round 1 of sampling did not coincide with a flow event within Zone 1; however, it did coincide with a flow event within Zone 2 (i.e. tributary inflow downstream of the Zone 1 gauging station). This suggests that a proportion of juvenile Australian smelt may have conducted an upstream migration from Zone 2 to Zone 1. These findings are supported by earlier studies that suggest Australian smelt have a protracted spawning period (August-April) and begin to breed in winter at water temperatures exceeding $15^{\circ} \mathrm{C}$ (Milton and Arthington, 1985; Humphries et al., 2013). Humphries et al. (2013) indicated that the recruitment of Australian smelt supports the 'window of opportunity hypothesis', where although the species has a protracted spawning period, temperature and prey density (responding to river-specific interactions between temperature and discharge) play a key role in the timing and magnitude of successful recruitment.

## Un-specked hardyhead

Peaks in the hatch date of un-specked hardyhead commenced in early November, during the receding tails of flow events or during sustained periods of increased flow when water temperatures reached $20^{\circ} \mathrm{C}$. Observations from the present study are supported by existing literature that details un-specked hardyhead have an extended breeding season from midOctober to mid-February; however, earlier studies suggest breeding commences at higher water temperatures ( $24^{\circ} \mathrm{C}$; Llewellyn, 1979) than observed in this study. Existing literature does not
link spawning to increases in discharge; however, evidence from the present study does identify increased recruitment following minor flow events.

## Murray-Darling rainbowfish

Back-calculated hatch dates of Murray-Darling rainbowfish occurred throughout the majority of the study period; however, peaks in hatch date frequencies generally occurred during the receding tails of flow events and during sustained periods of increased flow. The present study indicates that Murray-Darling rainbowfish spawned at temperatures as low as $10^{\circ} \mathrm{C}$ and as high as $25^{\circ} \mathrm{C}$. Existing literature suggests Murray-Darling rainbowfish are a seasonal breeder, spawning from spring to summer when water temperatures exceed $20^{\circ} \mathrm{C}$-significantly higher than that observed in the present study. Existing literature details rising water levels are not a prerequisite for spawning (Lintermans, 2007, Backhouse and Frusher, 1980); however, evidence from the present study and those of Gilligan et al. (2009) did identify increased recruitment following flow events.

## Spangled perch

Very few juvenile spangled perch were sampled within the Macquarie River during the three rounds of sampling between August 2014 and April 2015. One 45 mm spangled perch was sampled in September 2014 (Round 1) while all other fish sampled in December 2014 (Round 2) were $>75 \mathrm{~mm}$ FL. Existing literature details spangled perch commence spawning around November when surface temperatures reach $26^{\circ} \mathrm{C}$ and bottom temperatures $20^{\circ} \mathrm{C}$. Spawning may occur as late as mid-February if temperatures are suitable (Llewellyn, 1973). An increase in water level, although not essential, triggers spawning if temperatures are suitable (Lake, 1967). Within the Paroo and Darling catchment, Gehrke et al. (1995) found spangled perch to be more abundant after flooding.
In the present study, spangled perch were found almost exclusively in Zone 4. Zone 4 experienced loss of most refugia pools during extended periods of no flow from October 2014 to April 2015. It is therefore expected that recruitment would be limited within the lower Macquarie River and population maintenance is possibly supplemented by emigration from the Barwon River.

## Common carp

Recruitment of common carp was most prolific within the Macquarie Marshes (Zone 3) and downstream of the Macquarie Marshes (Zone 4) (Figure 5). Peaks in hatch date frequency were associated with the leading edge of flow pulses and during sustained periods of increased flow (Figure 26). Hatch dates coincided with water temperature as low as $10^{\circ} \mathrm{C}$ (Figure 26). A large peak in hatch date frequency during the 2014/2015 watering year was associated with the arrival of environmental water in the Macquarie Marshes. Further minor recruitment within Zone 3 during February 2015 was not associated with a flow pulse but rather a sustained period of moderate flow within the Macquarie Marshes (Figure 26). Alternatively, this recruitment may be attributed to a flow pulse in the Barwon River and the subsequent upstream migration of juveniles to the Macquarie Marshes (Figure 26). The absence of common carp recruitment within Zone 3 during Round 1 of sampling, associated with the late June 2014 flow pulse (Figure 26a), may be attributed to the downstream dispersal of juveniles into Zone 4. A peak in hatch date frequencies downstream of the Macquarie Marshes (Zone 4) directly aligns with this aforementioned flow pulse in the Macquarie Marshes (Zone 3). Little of this flow pulse passed through the Macquarie Marshes and reached Zone 4 (Figure 26b). A second hypothesis for the strong recruitment of juvenile carp within Zone 4 during Round 1 of sampling associated the recruitment with the receding tail of a large flow event within the Barwon River (Figure 26) and the subsequent emigration of juveniles from the Barwon into the lower Macquarie. The 80 day 'focus period' associated with Round 1 of sampling does not incorporate the leading edge of the

July 2014 flow events within the Barwon River and Macquarie River and therefore it is possible the hatch date frequency data only capture the tail end of this earlier recruitment pulse. No juvenile recruitment of common carp was observed downstream of the Macquarie Marshes during Round 2 of sampling. This is likely attributed to the fact that the Macquarie River downstream of the Macquarie Marshes ceased flowing, with only a small number of refuge pools remaining.

Existing literature on the reproductive biology of common carp is supported by the findings of the present study. Published data indicate common carp have an extended spawning season, generally peaking in spring and continuing into summer when water temperatures are $17-25^{\circ} \mathrm{C}$ (Brown et al., 2005; Lintermans, 2007). Rising river levels are not essential for spawning; however, these conditions can enhance spawning activity through the inundation of spawning habitat, such as floodplains or riparian vegetation. Previous studies have indicated common carp recruitment is greatest when high spring flows inundate floodplains and floodplain lakes (Gehrke et al., 1995; Brown et al., 2005; Gilligan et al., 2010).

## Evaluation of the October to December 2014 Commonwealth and NSW environmental water release on native fish recruitment

Recruitment data collected over a single watering year suggests native fish species of the midlower Macquarie River, from opportunistic and equilibrium life-history guilds, benefitted from the provision of (Commonwealth and NSW) environmental water during the spring and early summer of 2014-2015. Opportunistic small bodied species including Australian smelt, unspecked hardyhead and Murray-Darling rainbowfish, showed peaks in hatch date frequencies during the receding tails of flow events or during sustained periods of increased flow, particularly in association with the October to December 2014 environmental water release.

Peaks in the recruitment of equilibrium species, known to be temperature queued spawners, including Murray cod and eel-tailed catfish occurred during the 2014 Commonwealth and NSW environmental water release. The majority of juvenile Murray cod had hatch dates that coincided with the arrival of environmental water. While this increase in discharge may not have been required to trigger a spawning event, the increased flow may have boosted primary production that facilitated successful recruitment. Recruitment data for eel-tailed catfish indicated the primary spawning period coincided with water temperatures reaching approximately $20^{\circ} \mathrm{C}$ and moderate discharge ( $\sim 300-1,200 \mathrm{ML} /$ day $)$. It is commonly recognised that flooding is not required to initiate spawning for this species; however, increased flow as a result of the 2014 environmental water release may have boosted primary production and subsequently facilitate successful recruitment. Due to difficulties in selecting an appropriate unregulated control river system based on similarity in fish diversity/abundance and abiotic river characteristics, fish community sampling was confined to the Macquarie River. Because of the absence of a control, it is difficult to demonstrate cause and effect and attribute peaks in fish recruitment to the environmental water release. Although the data strongly indicates an association between the recruitment of a number of native fish species and the environmental water release, to fully attribute the successful recruitment to the environmental water release, it is critical that recruitment monitoring be repeated during a year where there is no environmental watering during the breeding season. This additional monitoring would act as a 'control' to bring further validity to the results obtained from the present study.

The collection of juvenile bony bream was constrained to the Macquarie River below the Macquarie Marshes. Hatch dates of these juveniles were back-calculated to between 13 June and 7 July 2014. This coincided with water temperatures of approximately $13^{\circ} \mathrm{C}$; a flow pulse within the Macquarie Marshes; the receding tail of a flow event within the Barwon River, and; a small flow event within the lower Macquarie River that created connectivity between the two river
systems. Although the recruitment of bony bream was not related to the October 2014 Commonwealth and NSW environmental water release, (due to the absence of flows passing through the Macquarie Marshes and reaching Zone 4) recruitment data highlights the relationship between flows and the recruitment of bony bream and the potential importance of connectivity between the Macquarie and Barwon River. Future environmental water releases within the Macquarie River should aim to create connectivity between the Macquarie River and Barwon River particularly in synchrony with flow events occurring in the Barwon River. This may facilitate immigration of native fish species to the Macquarie River.

No juvenile golden perch, silver perch and spangled perch were sampled within the Macquarie River during the three rounds of sampling between August 2014 and April 2015. Discharges greater than those delivered as part of the October to December 2014 environmental water release ( $\sim 1,000 \mathrm{ML}$ /day at Baroona) are likely required to cue the spawning of these periodic/flow-dependent species. However, the timing of such flow events must align with the breeding season and spawning temperature thresholds of these native fish species (Rayner et al. 2015).

### 4.2 Variation in fish community composition over a lotic gradient

## SRA indicators

The Murray-Darling Basin Authority's (MDBA's) Sustainable Rivers Audit (SRA) program (Davies et al., 2010) established a set of fish assemblage metrics reflecting aspects of fish community 'health' within the Murray-Darling Basin. These metrics can be aggregated into themed indicators (expectedness, recruitment and nativeness) and an overall Fish Condition Index (SRA ndxFS). In general, the overall condition of the fish community of the Macquarie River assessed in the present study declined along a downstream gradient from 'poor' below Burrendong Dam to 'extremely poor' in the Macquarie Marshes and downstream to the Barwon River confluence. The Sustainable Rivers Audit 1, conducted from 2004 to 2007, classified the Macquarie 'Slopes' (incorporating Zone 1 and Zone 2) as 'very poor' and 'Lowland' (incorporating Zone 2 and Zone 3) as 'poor' (MDBC, 2008). More recently, the Sustainable Rivers Audit 2, conducted from 2008 to 2010, classified both the Macquarie 'Slopes' and 'Lowland' as 'extremely poor' (MDBA, 2011). Findings from the present study suggest an improvement in the fish community of Macquarie 'Slopes' from 'extremely poor' to 'poor'; however, such a comparison is not entirely appropriate as this study was confined to the Macquarie River channel, whereas the SRA operates at a catchment scale.

## Burrendong Dam thermal curtain

Cold water pollution as a result of a deep-water offtake from Burrendong Dam has significantly affected biological processes of fish for up to 300-400 km downstream (Astles et al., 2003). During summer, thermal stratification within the dam can exceed $>10^{\circ} \mathrm{C}$ (Astles et al., 2003). The release of this cold, sometimes anoxic hypolimnetic water can affect growth, survival, redistribution, activity and recruitment (Astles et al., 2003; Todd et al., 2005) of native fish species and in some cases can lead to the complete loss of non-tolerant fish species (Todd et al., 2005).

Mitigation measures for cold water pollution below Burrendong Dam involved retrofitting a submerged curtain to the existing offtake to increase discharge temperatures (State Water, 2015). Preliminary findings indicate that cold water pollution is being reduced, though not eliminated, under the current operation of the thermal curtain. A $3^{\circ} \mathrm{C}$ difference between November 2013 and November 2014 water temperatures below Burrendong Dam has been recorded (Gray, 2015). These data were collected with the curtain set at 7 m below the dam
surface. It has been suggested the curtain be raised to 5 m to further reduce the impacts of cold water pollution downstream (Gray, 2015).

Data from the present study indicate that successful recruitment occurred during the 2014/2015 watering year in a number of native species within Zone 1, directly below Burrendong Dam. However, within Zone 1, poor abundance and recruitment of Murray cod was observed compared with Zone 2, approximately 150 km downstream. Similar results were observed by Astles et al. (2003) who investigated relationships between fish community composition and temperature regime within the Macquarie River. Astles et al. (2003) attributed the poor abundances of Murray cod to cold water pollution. It is likely the fish community response to the cold water mitigation will manifest over a number of years. Therefore, data from the 2014/2015 sampling would contribute to data that should be collected over a longer temporal scale.

### 4.3 Fish recruitment in the Macquarie Marshes

Limited evidence of native fish recruitment was observed within the main channel system of the Macquarie Marshes during the three rounds of fish community sampling throughout the 2014/2015 watering year. However, the alignment of bony bream hatch dates, caught downstream of the Macquarie Marshes, with a flow pulse within the Macquarie Marshes illustrates the possibility that the Macquarie Marshes was a recruitment source of juvenile bony bream.

Although native species recruitment was low within the Macquarie Marshes, high levels of common carp recruitment were observed, particularly after flow events. Similarly, strong recruitment of common carp was observed in the Macquarie Marshes after monitoring of a small 20,952 ML environmental flow event in February 2008 (Rayner et al., 2009). Rayner et al. (2015) monitored recruitment of native and exotic species in association with widespread flooding of the Macquarie Marshes between July 2010 and February 2011. Recruitment of native species, including bony bream and spangled perch, was observed; however, exotic species (common carp, goldfish and eastern gambusia) had a longer spawning window and recruited earlier than native species due to their lower spawning threshold temperatures (Rayner et al., 2015). From that study, Rayner et al. (2015) identified the importance of the alignment in the timing of environmental water deliveries and spawning temperature thresholds of native species present in the Macquarie Marshes.
It is known that common carp alter ecosystems through both bottom-up and top-down ecological processes with flow-on indirect impacts at multiple trophic levels (Weber and Brown, 2009; Badiou et al., 2011; Weber and Traunspurger, 2014). Primary impacts relate to interspecies competition for resources and aquatic habitat alteration through benthic foraging by adult carp. Adult carp compete with native benthic foraging fishes and both adult and juvenile carp compete for zooplankton with planktivorous fish and the larvae of all native fish species (Fletcher, 1986; Schiller and Harris, 2001). Other direct impacts include potential predation on the eggs and larvae of native fishes (Curtis, 1942; Lachner et al., 1970; Page and Burr, 1997; Schiller and Harris, 2001). Such mechanisms may be responsible for the low abundances and poor recruitment of native species within the Macquarie Marshes; however, as detailed by Gilligan and Rayner (2007) and Hume et al. (1983), the specific impacts of carp are complex and difficult to isolate from other interrelated anthropogenic changes to ecosystems.

### 4.4 Fish condition

It is generally accepted that fish with a high body condition index are typically more resistant to negative environmental factors and have a greater reproductive potential. However, a number of biases exist when comparing condition indices at a short temporal scale when the objective is to assess the impacts of environmental watering events. Changes in condition are likely to be attributed to an increase in the species' gonado-somatic index (GSI) (i.e. gonad development).

For most native large-bodied species, an increase in the GSI is seasonal, irrespective of hydrological conditions. However, a further increase in GSI may result from oocyte hydration as a result of favourable hydrological conditions (Lake, 1967; Rowland, 1983b; Battaglene, 1991). To examine temporal change in fish condition with validity, sampling must be repeated over multiple years both during and outside the species' reproductive period.

### 4.5 Considerations in the design of recruitment response monitoring

In an ideal scenario larval sampling should be conducted in addition to recruitment monitoring to examine 'relative recruitment strength'. This would provide insight into whether peaks in recruitment are due to increased spawning activity or increased survival of larval fish due to favourable environmental conditions. Moreover, it should be noted that 'relative recruitment strength' can only be calculated for species where abundances of eggs and/or early larval phase can be quantified (i.e. species with drifting eggs and/or early larval phase). However, when conducted in isolation, recruitment monitoring as employed here has numerous advantages over reproduction monitoring that uses traditional egg and larval sampling methods. Firstly, although fish may spawn, not all spawning events may be successful due to the prevailing environmental conditions necessary for larval survival and subsequent recruitment. Additionally, most mortality occurs during early life-history stages because larvae are likely to be more sensitive to environmental factors than older life stages (Childs et al., 1998; Koehn and Harrington, 2006). As a result, sampling juveniles rather than larval fish and eggs gives a better picture of what is actually surviving and contributing to the population.

Despite the advantages associated with recruitment monitoring, obtaining and interpreting useful data are largely dependent upon the initial design of the monitoring project. The following subsections detail important considerations in the design and interpretation of recruitment monitoring studies with reference to the current study.

## Fish stocking

The stocking of native fishes, including Murray cod and golden perch, is conducted by recreational fishing groups within the Macquarie River. Up to now, these stocked fish have not been marked for later identification, creating difficulties in discriminating between stocked and naturally recruited fish. However, stocking records and hatch dates of stocked fishes are maintained by NSW Fisheries and independent hatcheries. This information was used to determine the possibility that captured juveniles were stocked fish. Approximately 4,544 Murray cod, $45-50 \mathrm{~mm}$ in length, were stocked on 22 January 2015 within the vicinity of Zone 2 (Appendix 8). The collection of juvenile Murray cod during Round 2 of sampling preceded this stocking event. It is therefore assumed all juveniles caught during Round 2 were naturally recruited. Round 3 of sampling was conducted after the stocking event. Hatchery data indicate these stocked fish were hatched on 10 November 2014. A peak in back-calculated hatch dates of daily aged Murray cod caught during Round 3 of sampling coincides with the hatch date of stocked Murray cod. It is therefore possible that a number of juvenile Murray cod sampled during Round 3 were hatchery-reared fish. In addition, 21,818 golden perch, 40 mm in length, were stocked on the same date at a number of locations within the vicinity of Zone 2 (Appendix 8); however, no juvenile golden perch were caught during sampling throughout the entirety of the Macquarie River. In the future, all native fish stocked into river systems will be batch-marked with calcien (a fluorescent marker) in the hatchery prior to release to facilitate a more accurate assessment of the implications of stocked fish within studies of fish recruitment in the field.

## Sampling interval

Fish age estimates used in the construction of hatch date frequency figures (Section 3.2) were not extrapolated beyond the largest fish aged in the species' size-at-age plot (i.e. daily aging becomes difficult and less accurate as a species gets older). Consequently, interpretation of
hatch dates for each sample is confined to the period defined by arrows in each hatch date frequency histogram, defined as the 'focus period'. When designing recruitment monitoring projects, multiple sampling events should be conducted at appropriate intervals around the watering event. The sampling intervals should be less than the shortest 'focus period' of all target species' to prevents gaps in hatch date frequency figures. Additionally, the sampling interval should be minimised to reduce biases introduced by mortality. Hatch date frequency figures do not account for changes in exposure time to the chance of mortality. As a result, skewed hatch date frequency graphs, with increased frequency of hatch dates closer to the sampling event, may not be a result of increased recruitment success, but rather, older fish recruited earlier in the focus period have been susceptible to mortality for longer periods and have subsequently been reduced in numbers at the time of sampling. To reduce mortality bias and account for the maximum age that can accurately be assigned to a species, sampling in the present study was conducted on three occasions around the watering event with a 2 -month interval between sampling rounds.

## Experimental control

Due to difficulties in selecting an appropriate unregulated control river system based on similarity in fish diversity/abundance and abiotic river characteristics, fish community sampling was confined to the Macquarie River. Because of the absence of a control, it is difficult to demonstrate cause and effect and attribute peaks in fish recruitment to the environmental water release. This is particularly the case for Murray cod which are known to spawn irrespective of flow between October and December at water temperatures between 16 and $20^{\circ} \mathrm{C}$ (Lake, 1967; Rowland, 1983a, 1998; Humphries, 2005; King et al., 2009). The release of environmental water in October 2014 therefore coincided with the breeding season of Murray cod. While an increase in discharge may not have been required to trigger a spawning event, the high flow event may have boosted primary production that facilitated successful recruitment. To attribute the successful recruitment of Murray cod (and other species) in 2014 to the environmental water release, it is critical that recruitment monitoring be repeated during a year where there is no environmental watering during the breeding season. This additional monitoring would act as a 'control' to bring further validity to the results obtained from the present study.

## Daily age validation and first ring formation

An essential component of daily aging studies is the validation of the deposition of daily growth rings, and knowledge of the timing of the formation of the first growth ring. A number of the species investigated in the present study have had daily growth ring formation validated; however, age validation studies are yet to be conducted for a number of species (Table 10). Species can deposit their first major increment at different early life-history events, such as hatching or first exogenous feeding (Geffen, 1992). Consequently, age is expressed as 'estimated age' throughout this report. Nevertheless, aging error margins as a result of the timing of the first ring formation would be minimal given the short intervals between hatching, yolk absorption and exogenous feeding.

Table 10. Species of the Macquarie River system and details of the associated daily age validation studies.

| Species | Daily aging <br> validated? | Reference |
| :--- | :---: | :--- |
| Silver perch (Bidyanus bidyanus) | $\times$ |  |
| Un-specked hardyhead <br> (Craterocephalus stercusmuscarum fulvus) <br> Common carp (Cyprinus carpio) | $\times$ |  |
| Carp-gudgeon (Hypseleotris spp.) | $\checkmark$ | (Vilizzi, 1998; Smith and Walker, 2003b) |
| Spangled perch (Leiopotherapon unicolor) | $\times$ |  |
| Trout cod (Maccullochella macquariensis) | $\times$ |  |
| Murray cod (Maccullochella peelii) | $\times$ |  |
| Golden perch (Macquaria ambigua) | $\checkmark$ | (Humphries, 2005) |
| Murray-Darling rainbowfish (Melanotaenia | $\times$ | (Brown and Wooden, 2007) |
| fluviatilis) | $\times$ |  |
| Bony bream (Nematalosa erebi) | $\checkmark$ | (Kristensen et al., 2008) |
| Redfin perch (Perca fluviatilis) | $\times$ |  |
| Flatheaded gudgeon (Philypnodon grandiceps) | $\checkmark$ | (Tonkin et al., 2008) |
| Australian smelt (Retropinna semoni) | $\times$ |  |
| Eel-tailed catfish (Tandanus tandanus) |  |  |

## Spawning, hatch dates and abiotic conditions

With knowledge of the incubation period of each species, and the assumption that the first daily growth increment is deposited at the time of hatching, spawning dates in addition to hatch dates can be matched with water temperature and hydrological conditions. The incubation period of each species sampled in the Macquarie River is detailed in Table 11. It should be noted that there are a number of factors that may result in a minor mismatch between estimated hatch dates for each species and the associated abiotic conditions. These include:

- Gauging location: river discharge and water temperature were measured at one gauging station within each zone. On average, it takes approximately 5 days for a flow pulse to travel through a zone. Therefore, there may be up to $\pm 5$ days between the alignment of hatch date and river discharge/temperature.
- Fish migration: fish capture locations may be different to the hatch location as a result of fish moving upstream or downstream.
- Aging error: the ageing coefficient of variation calculation for each species fell within the expected reference point of $5 \%$ (Campana, 2001). Only minor variation was observed between the two readers' age assignments, particularly in older fish.

Table 11. Egg incubation period for fish species sampled in the Macquarie River.

| Species | Incubation <br> period | Reference |
| :--- | :--- | :--- |
| Silver perch (Bidyanus bidyanus) | $1-2$ days | (Lake, 1967; Rowland, 1984, 1996; Neira et al., 1998) |
| Goldfish (Carassius auratus) | 7 days | (Lintermans, 2007) |
| Un-specked hardyhead <br> (Craterocephalus stercusmuscarum fulvus) <br> Common carp (Cyprinus carpio) | $4-7$ days | (Lintermans, 2007) |
| Carp-gudgeon (Hypseleotris spp.) | $2-6$ days | (Winfield and Nelson, 2012) |
| Spangled perch (Leiopotherapon unicolor) | 2 days | (Lintermans, 2007) |
| Murray cod (Maccullochella peelii) | 2 days | (Llewellyn, 1973; Lintermans, 2007) |
| Golden perch (Macquaria ambigua) | $5-13$ days | (Lake, 1967; Lintermans, 2007) |
| Murray-Darling rainbowfish (Melanotaenia fluviatilis) | $7-9$ days | (Backhouse and Frusher, 1980; Reid and Holdway, 1995; Lintermans, 2007) |
| Trout cod (Maccullochella macquariensis) | $5-10$ days | (Ingrim and Rimmer, 1993) |
| Bony bream (Nematalosa erebi) | - |  |
| Redfin perch (Perca fluviatilis) | $7-14$ days | (Lintermans, 2007) |
| Flatheaded gudgeon (Philypnodon grandiceps) | $4-8$ days | (Llewellyn, 2007) |
| Australian smelt (Retropinna semoni) | $9-10$ days | (Milton and Arthington, 1985; Lintermans, 2007) |
| Eel-tailed catfish (Tandanus tandanus) | 7 days | (Lintermans, 2007) |

## 5 Conclusions and recommendations

- Recruitment data collected over a single watering year suggests native fish species of the mid-lower Macquarie River, from opportunistic and equilibrium life-history guilds, benefitted from the provision of Commonwealth and NSW environmental water during the spring and early summer of 2014-2015.
- Small-bodied opportunistic species, including Australian smelt, un-specked hardyhead and Murray-Darling rainbowfish, showed peaks in hatch date frequencies that occurred during the receding tails of flow events or during sustained periods of increased flow, particularly in association with the October to December 2014 environmental water release.
- The majority of juvenile Murray cod had hatch dates coincided with the arrival of environmental water from Burrendong Dam during the spring and early summer of 20142015. While this increase in discharge may not have been required to trigger a spawning event, the increased flow may have boosted primary production that facilitated successful recruitment.
- Recruitment data for eel-tailed catfish indicated the primary spawning period coincided with water temperatures reaching approximately $20^{\circ} \mathrm{C}$ and moderate discharge ( $\sim 300-$ $1,200 \mathrm{ML} /$ day). It is commonly recognised that flooding is not required to initiate spawning for this species; however, increased flow as a result of the environmental water release may have boosted primary production and subsequently facilitate successful recruitment.
- Recruitment data collected from daily aged bony bream adds significant information to existing literature on the biology of bony bream; extending the known breeding season and highlighting a relationship between flow and recruitment.
- Future environmental water releases within the Macquarie River should aim to create connectivity between the Macquarie River and Barwon River particularly in synchrony with flow events occurring in the Barwon River. This may facilitate immigration of native fish species to the Macquarie River.
- Repeated sampling should be conducted over multiple watering years to examine native fish response to a variety of natural and regulated flow scenarios.
- For environmental water deliveries to benefit native fish recruitment, the release of environmental water must align with the breeding season and spawning temperature thresholds of native species targeted by the flow event.
- A small environmental water release (i.e. $28,483 \mathrm{ML}$ ) delivered at the appropriate time does likely benefit the recruitment of some equilibrium and opportunistic species within the mid and lower Macquarie River; however, higher discharges (>1,000 ML/day) are likely required to cue the spawning of periodic/flow-dependent species such as golden perch and silver perch.
- The Environmental Outcomes Framework (CEWO, 2013) identifies connectivity in regards to biotic dispersal and movement as an outcome to environmental water delivery (Figure 1). An acoustic telemetry component in future monitoring and evaluation projects on the Macquarie River will provide a means to examine the movement response of fish to the delivery of environmental flows. More specifically, it would demonstrate how environmental water delivery may provide: 1) connectivity between habitats; and 2) a trigger for spawning migrations.
- Analysis of otolith chemistry may provide more certainty about which river system juveniles recruited from; i.e. do flow events that create connectivity between the Barwon River and Macquarie River allow for the emigration of juveniles into the Macquarie River?
- Larval sampling should be conducted in addition to recruitment monitoring to examine 'relative recruitment strength'. This will provide insight into whether peaks in recruitment are due to increased spawning activity or increased survival of larval fish due to favourable environmental conditions.
- Daily age validation should be conducted for species not previously validated.
- Fish community monitoring should continue over a large temporal scale to investigate the efficacy of the variable offtake curtain in mitigating cold water pollution from Burrendong Dam.


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## Appendices

## Appendix 1 - Murray Darling Basin Authority - Sustainable Rivers Audit Protocol for Fish Theme sampling - Implementation Period 8: 2012-13

## Introduction

This protocol applies to the sampling of SRA sites as part of the Fish Theme. It was developed by the MDBA's SRA team in consultation with the SRA Joint Venture Committee, Independent Sustainable Rivers Audit Group, the Jurisdictional Managing Agencies (JMAs) and the Fish Taskforce.

## Prerequisites

The following prerequisites must be met before applying this protocol:

- a current sampling plan has been received from the Authority office
- the site has been validated in accordance with the Site Validation protocol
- field staff meet the requirements of their respective JMAs and agencies in applying this protocol, and
- field staff must comply with the requirements of the Australian Code of Electrofishing Practice.


## Sampling principles

The staff undertaking sampling must adhere to following principles, which are fundamental to the application of this protocol:

- Sampling is to take place between 1 November and 30 April. In the North of the Basin (specifically the Paroo, Warrego and Condamine) the sampling period may extend to the end of May if temperatures earlier in the season are too high to work.
- Sampling is not to take place during periods of high flow, either natural or from impoundment releases. High flows are those which in the judgement of the field operators would pose an occupation health and safety risk or compromise catch efficiency to unacceptable levels.
- The Authority office must be notified as soon as practicable by the sampling agency or the JMA of any change to the application of this protocol or methods deployed at a site during a site visit in the event of equipment malfunction, site abandonment or disruption to a sampling event.
- Each major habitat type present at a site must be sampled at least once and then remaining sampling effort should occur in the most abundant habitat types. Recognised habitat types are pool edges, middle portions of pools, runs and riffles, slow-flowing back waters, emergent vegetation, submerged vegetation, large woody debris and debris dams.
- Sites must be sampled using the most appropriate methods, as listed in Tables A1.1 and A1.2. It is likely that both backpack and boat electrofishing will be used at most sites.
- Data are only to be recorded for fish greater than 15 mm total length.
- All caught fish are released live, except where State noxious or alien fish policies require otherwise or where samples are required as voucher specimens (see Table 5 Voucher specimens).


## Deployment of sampling methods

Fish sampling will be conducted by electrofishing (boat and backpack) and by use of baittraps and opera-house traps. Given the wide variety of site conditions in the Basin (from small upland streams to large lowland rivers) the teams will need to make a site-specific assessment of conditions. This assessment will need to identify the type of habitats present and their relative abundance, after which a decision can be made on the appropriate mix of sampling methods. In particular, the site should be assessed to identify all habitats that can be electrofished by boat, and/or backpack methods, and the proportion suitable for each method should be determined. Guidance on the selection and deployment of fish sampling methods is provided in the following tables.

Table A1.1: Choice of fish sampling method under varying site characteristics.

| Site character | Appropriate methods |
| :---: | :---: |
| Large river sites: <br> $>15 \mathrm{~m}$ wetted channel width (as estimated by sampling teams) | Adopt large boat electrofishing. <br> Include backpack shots as necessary to sample each wadeable habitat type at least once. <br> Deploy 10 bait-traps. |
| Small river sites: <15 m wetted channel width (as estimated by sampling teams) | Adopt small boat electrofishing Include backpack shots as necessary to sample each wadeable habitat type at least once Deploy 10 bait-traps. |
| Wadeable habitats | Adopt backpack electrofishing. However, bank-mounted electrofishers can be used instead of backpack electrofishers for sites with electrical conductivity levels between $1500 \mu \mathrm{~S} / \mathrm{cm}$ and the detection limits of the bank-mounted unit, provided agencies accept their use. <br> Deploy 10 bait-traps. |

Table A1.2: Application of fish sampling methods.

| Method name | Application |
| :---: | :---: |
| Boat electrofishing | - Deploy 12 shots. <br> - Note: A shot is 90 seconds of accumulated power-on time. <br> - In portions of streams >15 m wetted channel width (as estimated by sampling teams), adopt alternate shots alongside both banks. <br> - In portions of streams <15 m wetted channel width (as estimated by sampling teams), adopt zigzag coverage of sampled area. <br> - Deploy two mid-channel shots when mid-channel water depth $<4 \mathrm{~m}$. |
| Backpack (and Bank-mount) electrofishing | - Deploy 8 shots. <br> - Note: A shot is 150 seconds of accumulated power-on time. <br> - In portions of streams <10 m wetted channel width (as estimated by sampling teams), adopt zigzag coverage of sampled area. <br> - In streams >10 m wetted channel width (as estimated by sampling teams), adopt alternate shots alongside both banks. <br> - Where electrical conductivity is $>1,500 \mu \mathrm{~S} / \mathrm{cm}$ and where agencies accept their use, bank-mounted electrofishers can be used in place of backpack electrofishers using the same procedures. |
| Bait-trap | - Deploy 10 bait-traps for 2 hours ( $\pm 1 / 2$ hour depending on duration of electrofishing effort). <br> - Only deploy at locations with depth $<1 \mathrm{~m}$. <br> - Do not use bait or chemical light sticks in the traps. <br> - Set traps in slow-flowing or backwater areas independent of electrofishing sites. <br> - Pool the catch from all traps and record as a single event. |

Table A1.3: Number of electrofishing shots required with each type of electrofishing gear.

| Method | Proportion of site |  |  |  |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $<\mathbf{1 / 8}$ | $\mathbf{1 / 8}$ | $\mathbf{1 / 4}$ | $\mathbf{3 / 8}$ | $\mathbf{1 / 2}$ | $\mathbf{5 / 8}$ | $\mathbf{3 / 4}$ | $\mathbf{7 / 8}$ | All |  |  |
| Boat | 0 | 0 | 4 | 5 | 6 | 8 | 9 | 11 | 12 |  |  |
| Backpack | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 8 |  |  |

## Bait-trap placement

## Sampling practices guided by Table A1.2above.

## Measurement of individuals and subsampling

Fish smaller than 15 mm may be recorded but should not be reported as core SRA data as they will not be used in the analysis. A subsample of 50 individuals per species captured by each method (ie boat electrofishing, backpack electrofishing and bait-traps) used at each site should be measured for the listed attributes below. The subsample begins with the first individual of each species collected from each method and continues until the $50^{\text {th }}$ individual. During electrofishing operations, in the shot/replicate where the $50^{\text {th }}$ fish is found, all individuals present should be measured and recorded in order to avoid any bias in the size
of fish selected for the subsample. For bait-traps the catch is pooled prior to counting and measuring, each fish is identified, and the first 50 of each species are measured. An effort should be made to reduce bias in sub-sampling the pooled catch, e.g. use a small aquarium dip-net to net the pooled catch. (Note: NSW DPI prefers to collect the data for each trap independently and then, using random sampling of the data, generate a compliant pooled data return for MDBA.) If the number of individuals captured by each sampling method is less than 50, all of them should be measured for the attributes listed below.

Data to be recorded for each individual are outlined below.

1) Length: Caudal fork length for species with a forked tail and total length for species with round tails are to be measured. Approximate length should be estimated for damaged fish and this will be considered equivalent to a measured length during data analysis. Length should be measured to the nearest mm using a measuring board.
2) Weight: If weight is to be recorded for that species (refer to Table A1.4), then each individual fish should be blotted dry and measured on a balance with a suitable range for individual being measured:
a. for fish $>50 \mathrm{~g}$, record weight to nearest gram, or
b. for fish 50 g or less, record weight to nearest 0.1 g .
3) Health and condition: For data requirements see section: Fish health and condition.
4) Voucher specimens: These are to be collected if that species requires a voucher specimen (refer to Table A1.4and section: Voucher specimens).

## Species list

The species list is a combination of those species thought to have occurred in the Basin under reference conditions, those species caught during previous sampling events and those alien species expected to be caught in future sampling.

The list may be updated from time-to-time where new species are identified, taxonomy revised or data collection requirements change. Those species for which voucher specimens should be collected or weight recorded are indicated in this list. Requirements for voucher specimens are detailed below.

When identifying alien species such as carp and goldfish, field teams should be aware that there is the possibility of hybridisation. Jurisdictions may decide whether or not they wish to record the presence of hybrids. However, in doing so they should be aware that when the data are received by the MDBA, any hybrids will be assigned to a single parent taxon (e.g. carp or goldfish) based on which parent stock the body dimensions of the hybrid most resemble. This will therefore require that jurisdiction field teams either record hybrids as a single parent taxa (e.g. carp or goldfish) immediately in the field, OR record in the field as hybrids and take appropriate notes to allow matching to either parent taxa prior to transmission of data to MDBA.

Table A1.4: Murray-Darling Basin SRA fish taxa, including whether they require voucher specimen collection and/or weighing.

| Taxa code | Scientific name | Common name | Get voucher specimen | Record weight |
| :---: | :---: | :---: | :---: | :---: |
| ACAFLA | Acanthogobius flavimanus | Yellowfin goby | YES |  |
| ACABUT | Acanthopagrus butcheri | Black bream |  |  |
| AFUTAM | Afurcagobius tamarensis | Tamar River goby | YES |  |
| ALDFOR | Aldrichetta forsteri | Yellow-eyed mullet | YES |  |
| AMBAGA | Ambassis agassizii | Olive perchlet | YES <br> photo only | YES |
| AMOBIF | Amoya bifrenatus | Bridled goby | YES |  |
| ANGAUS | Anguilla australis | Short-finned eel |  |  |
| ANGREI | Anguilla reinhardtii | Long-finned eel | YES |  |
| ARGHOL | Argyrosomus hololepidotus | Mulloway |  |  |
| ATHMIC | Atherinosoma microstoma | Small-mouthed unspecked hardyhead | YES |  |
| BIDBID | Bidyanus bidyanus | Silver perch | YES ${ }^{\text {a }}$ | YES |
| BIDWEL | Bidyanus welchi | Welch's grunter | YES ${ }^{\text {a }}$ |  |
| CARAUR | Carassius auratus | Goldfish |  |  |
| CARCAR | Carassius carassius | Crucian carp | YES | YES |
| CRAAMN | Craterocephalus amniculus | Darling River unspecked hardyhead | YES | YES |
| CRAFLU | Craterocephalus fluviatilis | Murray un-specked hardyhead | YES | YES |
| CRASTE | Craterocephalus stercusmuscarum fulvus | Unspecked un-specked hardyhead | YES |  |
| CYPCAR | Cyprinus carpio | Common carp |  |  |
| GADBIS | Gadopsis bispinosus | Two-spined blackfish |  |  |
| GADMAR | Gadopsis marmoratus | River blackfish |  |  |
| GALBRE | Galaxias brevipinnis | Climbing galaxias | YES | YES |
| GALMAC | Galaxias maculatus | Common galaxias | YES |  |
| GALFUS | Galaxias fuscus | Barred galaxias | YES |  |
| GALOLI | Galaxias olidus | Mountain galaxias | YES |  |
| GALROS | Galaxias rostratus | Flat-headed galaxias | YES | YES |
| GALSP1 | Galaxias sp1 | Obscure galaxias | YES |  |
| GALSP2 | Galaxias sp2 | Riffle galaxias | YES | YES |
| GALTRU | Galaxias truttaceus | Spotted galaxias | YES | YES |
| GAMHOL | Gambusia holbrooki | Gambusia |  |  |
| GEOAUS | Geotria australis | Pouched lamprey | YES | YES |
| (continued) |  |  |  |  |

Table A1.4 (cont'd): Murray-Darling Basin SRA fish taxa, including whether they require voucher specimen collection and/or weighing.

| Taxa code | Scientific name | Common name | Get voucher specimen | Record weight |
| :---: | :---: | :---: | :---: | :---: |
| HYPGAL | Hypseleotris galii | Firetail gudgeon | YES ${ }^{\text {b }}$ |  |
| HYPSPP | Hypseleotris spp. | Carp gudgeons (lumped) | YES ${ }^{\text {b }}$ |  |
| LEIUNI | Leiopotherapon unicolor | Spangled perch |  |  |
| LIZARG | Liza argentea | Flat-tailed mullet | YES |  |
| MACAMB | Macquaria ambigua ambigua | Golden perch |  |  |
| MACAUS | Macquaria australasica | Macquarie perch |  |  |
| MACCOL | Macquaria colonorum | Estuary perch |  | YES |
| MACMAC | Maccullochella macquariensis | Trout cod / Bluenose cod | YES ${ }^{\text {c }}$ |  |
| MACPEE | Maccullochella peelii peelii | Murray cod | YES ${ }^{\text {d }}$ |  |
| MELFLU | Melanotaenia fluviatilis | Murray-Darling rainbowfish | YES ${ }^{\text {e }}$ |  |
| MELSPL | Melanotaenia splendida tatei | Desert rainbowfish | YES ${ }^{\text {e }}$ | YES |
| MISANG | Misgurnus anguillicaudatus | Oriental weatherloach | YES |  |
| MOGADS | Mogurnda adspersa | Southern purplespotted gudgeon | YES <br> photo only |  |
| MORMOR | Mordacia mordax | Short-headed lamprey | YES |  |
| MUGCEP | Mugil cephalus | Sea mullet | YES |  |
| MYXELO | Myxus elongatus | Sand mullet | YES |  |
| NANAUS | Nannoperca australis | Southern pygmy perch |  |  |
| NANOBS | Nannoperca obscura | Yarra pygmy perch |  | YES |
| NEMERE | Nematalosa erebi | Bony herring |  |  |
| NEOHYR | Neosilurus hyrtlii | Hyrtl's tandan |  |  |
| ONCMYK | Oncorhynchus mykiss | Rainbow trout | $\begin{aligned} & \hline \text { YES }^{f} \\ & <100 \mathrm{~mm} \end{aligned}$ |  |
| PERFLU | Perca fluviatilis | Redfin perch |  |  |
| PHIGRA | Philypnodon grandiceps | Flathead gudgeon | YES |  |
| PHIMAC | Philypnodon macrostomus | Dwarf flathead gudgeon | YES | YES |
| PORREN | Porochilus rendahli | Rendahl's tandan |  |  |
| PSEOLO | Pseudogobius olorum | Blue-spot goby |  |  |
| PSEURV | Pseudaphritis urvillii | Congolli |  |  |
| REDMAC | Redigobius macrostoma | Large-mouthed goby | YES |  |
| RETSEM | Retropinna semoni | Australian smelt |  |  |
| RUTRUT | Rutilus rutilus | Roach | YES | YES |

(continued)

Table A1.4 (cont'd): Murray-Darling Basin SRA fish taxa, including whether they require voucher specimen collection and/or weighing.

| Taxa code | Scientific name | Common name | Get voucher <br> specimen | Record <br> weight |
| :--- | :--- | :--- | :--- | :--- |
| SALTRU | Salmo trutta | Brown trout | $\mathrm{YES}^{\dagger}$ |  |
| TANTAN | Tandanus tandanus | Freshwater catfish |  |  |
| TASLAS | Tasmanogobius lasti | Lagoon goby |  |  |
| TINTIN | Tinca tinca | Tench |  | YES |

${ }^{\text {a }}$ Bidyanus bidyanus and Bidyanus welchi may both be in the Paroo and voucher specimens and/or colour images are required for confirmation.
${ }^{\mathrm{b}}$ Hypseleotris species should be collected in Queensland valleys to determine if Hypseleotris galii is present. For all other valleys the genus is lumped.
${ }^{c}$ Maccullochella macquariensis to be photographed when individual $>120 \mathrm{~mm}$ and a voucher specimen collected when $<100 \mathrm{~mm}$.
${ }^{\text {d }}$ Maccullochella peelii peelii to have voucher specimen collected for small fish ( $<120 \mathrm{~mm}$ ) in regions where Maccullochella macquariensis occurs.
${ }^{e}$ Melanotaenia fluviatilis and Melanotaenia splendida tatei may both be in the Paroo, Warrego and Middle to Upper Darling and voucher specimens and/or colour images are required for confirmation.
${ }^{\dagger}$ Small trout ( $<100 \mathrm{~mm}$ ) should have voucher specimens collected to ensure Salmo trutta and Oncorhynchus mykiss are distinguished.

## Voucher specimens

Voucher specimens are to be collected for rare species, uncertain species and for notable range extensions of any species. Required species are shown in the Get Voucher column of Table A1.4. Note the following recommendations for voucher collections.

- Collect at least three specimens where possible, covering a range of size and colouration.
- Preserve specimens in 90-100\% alcohol.
- Label each voucher sample with the following information: SRA site ID, State, river name, date collected and collector's name.
- Use a container of adequate size so that fish are not bent or crammed in, and ensure adequate preservative concentration is maintained.
- Obtain good quality digital images of all live specimens depicting body colouration and fin shape.
- Note: Good quality digital images of live specimens depicting body colouration and fin shape are an acceptable alternative when specimens are too large to be effectively preserved and for those species identified in Table 4 above.

Identification of the specimen is a three-stage process.

1. Return specimens to the sampling team's laboratory and use collective knowledge and identification aids to confirm identification.
2. If uncertainty remains, use a known specialist in that particular taxon to confirm the specimen's identification.
3. If a specialist is not available or any uncertainty remains, send the specimen to the following fish taxonomist, who will confirm identification or, in conjunction with the Authority, determine a process to have the specimen identified.

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## Fish health and condition

The presence of any abnormality sighted on any part of a measured fish should be recorded on the field sheet. At least one side of the fish should be checked completely for abnormalities. Abnormalities are to be assessed only on the measured subsample of each species.

Those fish abnormalities that are considered reportable are listed in Table A1.5. The fact that a handled fish exhibits one or more abnormalities must be indicated by listing the health code against attribute 'HealthCode' in the data return. A 'HealthCode' entry is recorded as a continuous sequence of the codes available in Table A1.5. Examples of valid entries include 'D', 'DWLP' and 'PLWD'. The codes 'yes' or 'true' can be used when an abnormality is observed but the descriptive health code has been misplaced, lost or forgotten. This ensures that the presence of all abnormalities is recorded even if the type of abnormality is lost.

Table A1.5: Reportable abnormalities exhibited by handled fish.

| Health code | Abnormality description |
| :--- | :--- |
| D | Deformity (skeleton deformities, blindness, fin deformities, asymmetical, etc) |
| F | Fin condition poor (broken, eroded fins) |
| S | Lesions (skin abnormality with raised and/or discoloured scales) |
| U | Ulcers (skin is broken, crater like, redness) |
| T | Tumour (localised abnormal growth) |
| W | Wounds (eg bird strikes, hook wounds) |
| G | Fungus |
| L | Lernaea spp. (but only where a notable number are present. This is defined as: If fish <br> <100 $m$ total length, report any Lernaea sp., If fish >100 mm total length, report if <br> more than 3 Lernaea sp.) |
| P | Other visible parasites |
| O | Other: the abnormality must be photographed and described and/or a specimen <br> preserved |
| Yes | Either of these codes can be used when an abnormality is observed but the above list <br> has been misplaced, lost or the health code has been forgotten |
| No |  |

## Appendix 2 - Sample site photographs

Plate A2.1: Bonada (Zone 1),
Round 1 sampling event.


Plate A2.2: Boral TSR (Zone 1),
Round 1 sampling event.


Plate A2.3: Peach Tree Reserve (Zone 1),
Round 1 sampling event.


Plate A2.4: Brilbral (Zone 1),
Round 1 sampling event.


Plate A2.5: Dubbo Boat Ramp (Zone 1), Round 1 sampling event.


Plate A2.6: Wambool (Zone 2),
Round 1 sampling event.


Plate A2.7: Gin Gin Bridge (Zone 2),
Round 1 sampling event.


Plate A2.8: Mullah (Zone 2),
Round 1 sampling event.


Plate A2.9: Ellengerah (Zone 2),
Round 1 sampling event.


Plate A2.10: DS Gin Gin Weir (Zone 2),
Round 1 sampling event.


Plate A2.11: Marebone (Zone 3),
Round 1 sampling event.


Plate A2.12: Groggans (Zone 3),
Round 1 sampling event.


Plate A2.13: Old Oxley (Zone 3),
Round 1 sampling event.


Plate A2.14: Willancorah (Zone 3),
Round 1 sampling event.


Plate A2.15: Pillicawarrina (Zone 3),
Round 1 sampling event.


Plate A2.16: Yanda (Zone 4), Round 1 (left) and Round 2 (right) sampling events.


Plate A2.17: Brewon (Zone 4), Round 1 (left) and Round 2 (right) sampling events.


Plate A2.18: Marthaguy Junction (Zone 4), Round 1 (left) and Round 2 (right) sampling events.


Plate A2.19: Binghi (Zone 4), Round 1 (left) and Round 2 (right) sampling events.


Plate A2.20: Glenacre (Zone 4), Round 1 (left) and Round 2 (right) sampling events.


## Appendix 3 - Sample site coordinates

Table A3.1. Sites sampled during each sampling round.

| Site name | Zone | Latitude | Longitude | Sampled (yes/no) |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Round 1 | Round 2 | Round 3 |
| Bonada | 1 | -32.6264 | 149.0727 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Peach Tree Reserve | 1 | -32.4462 | 148.7747 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Brilbral | 1 | -32.4218 | 148.7282 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Dubbo Boat Ramp | 1 | -32.2472 | 148.5985 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Boral TSR | 1 | -32.5019 | 148.9078 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Wambool | 2 | -31.9863 | 148.2105 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| DS Gin Gin Weir | 2 | -31.9295 | 148.1400 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Gin Gin Bridge | 2 | -31.9101 | 148.0919 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Mullah | 2 | -31.8893 | 148.0434 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Ellengerah | 2 | -31.8039 | 147.9632 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Marebone | 3 | -31.3923 | 147.6984 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Old Oxley | 3 | -31.1265 | 147.5788 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Pillicawarrina | 3 | -30.7947 | 147.5203 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Groggans | 3 | -31.2087 | 147.6402 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Willancorah | 3 | -30.9003 | 147.5074 | $\checkmark$ | $\checkmark$ | $\checkmark$ |
| Yanda | 4 | -30.4187 | 147.5480 | $\checkmark$ | $\times$ | $\checkmark$ |
| Brewon | 4 | -30.2343 | 147.5422 | $\checkmark$ | $\times$ | $x$ |
| Binghi | 4 | -30.1801 | 147.5110 | $\checkmark$ | $\checkmark$ | $\times$ |
| Marthaguy Junction | 4 | -30.2663 | 147.5790 | $\checkmark$ | $\times$ | $\checkmark$ |
| Glenacre | 4 | -30.1695 | 147.4764 | $\checkmark$ | $\checkmark$ | $\times$ |

Note: Sites not sampled were dry during the sampling period.

## Appendix 4 - Captured species photographs

Plate A4.1: Mature (left) and juvenile (right) eel-tailed catfish sampled from Zone 1 on the Macquarie River.


Plate A4.2: Mature trout cod sampled from
Zone 1 on the Macquarie River.


Plate A2.3: Mature (left) and juvenile Murray cod (right) sampled from Zone 1 on the Macquarie River.


Plate A4.4: Mature silver perch sampled from Zone 2 on the Macquarie River.


Plate A4.5: Mature bony bream sampled from Zone 4 on the Macquarie River.


## Appendix 5 - Recruitment length cut-offs

Table A5.1: Size limits used to distinguish new recruits for each species.

| Species | Estimated size at 1 year old or at sexual maturity (fork or <br> total length) |
| :--- | :--- |
| Native species |  |
| Australian smelt | 40 mm (Pusey et al., 2004) |
| Bony bream | 67 mm (Cadwallader, 1977) |
| Carp-gudgeon | 35 mm (Pusey et al., 2004) |
| Flatheaded gudgeon | 58 mm (Pusey et al., 2004; Llewellyn, 2007) |
| Eel-tailed catfish | 83 mm (Davis, 1975) |
| Golden perch | 75 mm (Mallen-Cooper, 2003) |
| Murray cod | 222 mm (Gavin Butler, unpublished data) |
| Murray-Darling rainbowfish | 45 mm (Pusey et al. 2004: for M. duboulayi) |
| Silver perch | 75 mm (Mallen-Cooper, 2003) |
| Spangled perch | 68 mm (Leggett and Merrick, 1987) |
| Trout cod | 150 mm |
| Un-specked hardyhead | 38 mm (Pusey et al., 2004) |
| Exotic species |  |
| Common carp | 155 mm (Vilizzi and Walker, 1999) |
| Eastern gambusia | 20 mm (McDowall, 1996) |
| Goldfish | 127 mm (Lorenzoni et al., 2007) |
| Redfin perch | 60 mm (maximum reported by Heibo and Magnhagen, 2005) |

Note: Values represent the length at 1 year of age for longer-lived species or the age at sexual maturity for species that reach maturity within 1 year.

## Appendix 6 - Historic fish community monitoring data for the Macquarie River

NSW Department of Primary Industries (DPI) and partnerships have pre-existing data from approximately 65 riverine sites within the Macquarie River and Macquarie Marshes channel system collected from 13 programs operating between 1976 and 2012. Of these, 26 sites have been sampled $\geq 1$ time/s during the years of 2006, 2009 and 2012 as part of the Sustainable Rivers Audit/Monitoring Evaluating and Reporting (SRA/MER). In 2000/2001, eight sites were sampled as part of the 'Integrated monitoring of environmental flow' (Growns and Gehrke, 2005). In 1998, four sites were sampled by Astles et al. (2003) during the non-irrigation (June) and irrigation (October) seasons, examining the effects of cold water pollution below Burrendong Dam. Finally, between 2007 and 2012, five sites were sampled annually on the Macquarie River as control sites for the Lachlan Carp Demo Project. The remaining sites have been sampled inconsistently, with little replication or non-standardised techniques between 1976 and 2012.

## Appendix 7 - Water diversion schematic for the Macquarie System

Figure A7.1: Water diversion schematic for the Macquarie River system.


## Appendix 8 - Native fish stocking within the Macquarie River

Table A8.1: Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam, January 2001 - February 2005.

| Date | Species | Number | Hatchery | River | Site | Nearest town | Latitude | Longitude |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 20/01/2011 | Golden perch | 5,000 | Murray Darling Fisheries | Macquarie River | 'Raby Irrigation' | Warren | -31.613051 | 147.778969 |
| 20/01/2011 | Golden perch | 5,000 | Murray Darling Fisheries | Macquarie River | Bryan Egan Weir | Warren | -31.684722 | 147.835785 |
| 20/01/2011 | Golden perch | 5,000 | Murray Darling Fisheries | Macquarie River | Oxley Park fronting Coonamble Road | Warren | -31.696928 | 147.839032 |
| 20/01/2011 | Golden perch | 5,000 | Murray Darling Fisheries | Macquarie River | Warren 'Top <br> Weir' | Warren | -31.734697 | 147.866298 |
| 20/01/2011 | Murray cod | 4,225 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 20/01/2011 | Murray cod | 4,225 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 1/03/2011 | Murray cod | 1,574 | Uarah Fish Hatchery | Macquarie River | Terramungamine Reserve | Terramungamine | -32.169494 | 148.585074 |
| 1/03/2011 | Murray cod | 1,574 | Uarah Fish Hatchery | Macquarie River | Whylandra Crossing Reserve | Whylandra | -32.190047 | 148.494510 |
| 1/03/2011 | Murray cod | 1,574 | Uarah Fish Hatchery | Macquarie River | Minore Falls Reserve | Dubbo | -32.192231 | 148.396239 |
| 1/03/2011 | Murray cod | 1,574 | Uarah Fish Hatchery | Macquarie River | Brumagen Creek Reserve | Narromine | -32.233601 | 148.364787 |
| 1/03/2011 | Murray cod | 1,574 | Uarah Fish Hatchery | Macquarie River | Scabbing Flat Reserve | Geurie | -32.430641 | 148.810245 |

Table A8.1 (cont'd): Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam, January 2001 - February 2005.

| Date | Species | Number | Hatchery | River | Site | Nearest town | Latitude | Longitude |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1/03/2011 | Murray cod | 1,574 | Uarah Fish Hatchery | Macquarie River | Peach Tree Reserve | Ponto | -32.446781 | 148.777073 |
| 1/03/2011 | Murray cod | 1,575 | Uarah Fish Hatchery | Macquarie River | Ponto Falls Reserve | Ponto | -32.465147 | 148.818204 |
| 16/01/2012 | Golden perch | 4,546 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 16/01/2012 | Golden perch | 4,546 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 16/01/2012 | Murray cod | 2,273 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 16/01/2012 | Murray cod | 2,273 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 16/01/2012 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | 'Raby Irrigation' | Warren | -31.613051 | 147.778969 |
| 16/01/2012 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | Bryan Egan Weir | Warren | -31.684722 | 147.835785 |
| 16/01/2012 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | Oxley Park fronting Coonamble Road | Warren | -31.696928 | 147.839032 |
| 16/01/2012 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | Warren 'Top Weir' | Warren | -31.734697 | 147.866298 |
| 2/04/2012 | Golden perch | 1,137 | Murray Darling Fisheries | Macquarie River | Minore Falls Reserve | Dubbo | -32.192230 | 148.396240 |
| 2/04/2012 | Golden perch | 1,138 | Murray Darling Fisheries | Macquarie River | Butlers Falls Reserve | Dubbo | -32.315866 | 148.621385 |
| 2/04/2012 | Murray cod | 757 | Murray Darling Fisheries | Macquarie River | Minore Falls Reserve | Dubbo | -32.192230 | 148.396240 |

Table A8.1 (cont'd): Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam,

| Danuary 2001 - February 2005. |  | River | Site | Nearest town | Latitude |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $2 / 04 / 2012$ | Species | Number | Hatchery | Longitude |  |  |  |
| $13 / 01 / 2013$ | Golden <br> perch | 1,298 | Murray Darling <br> Fisheries | Murray Darling <br> Fisheries | Macquarie <br> River | Terramungamine <br> Reserve | Dubbo |

(continued)

Table A8.1 (cont'd): Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam, January 2001 - February 2005.

| Date | Species | Number | Hatchery | River | Site | Nearest town | Latitude | Longitude |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 13/01/2013 | Murray cod | 865 | Murray Darling Fisheries | Macquarie River | Peach Tree Reserve | Geurie | -32.446781 | 148.777073 |
| 13/01/2013 | Murray cod | 871 | Murray Darling Fisheries | Macquarie River | Ponto Falls Reserve | Geurie | -32.465147 | 148.818204 |
| 24/01/2013 | Golden perch | 3,409 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 24/01/2013 | Golden perch | 3,409 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 24/01/2013 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | 'Raby Irrigation' | Warren | -31.613051 | 147.778969 |
| 24/01/2013 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | Bryan Egan Weir | Warren | -31.684722 | 147.835785 |
| 24/01/2013 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | Oxley Park fronting Coonamble Road | Warren | -31.696928 | 147.839032 |
| 24/01/2013 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | Warren 'Top Weir' | Warren | -31.734697 | 147.866298 |
| 24/01/2013 | Murray cod | 2,280 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 24/01/2013 | Murray cod | 2,280 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 18/01/2014 | Golden perch | 3,785 | Murray Darling Fisheries | Macquarie River | Gin Gin Bridge | Gin Gin | -31.915770 | 148.083448 |
| 18/01/2014 | Golden perch | 757 | Murray Darling Fisheries | Macquarie River | Turkey Farm Reserve | Narromine | -32.142028 | 148.234459 |
| 18/01/2014 | Golden perch | 757 | Murray Darling Fisheries | Macquarie River | Rotary Park, Narromine | Narromine | -32.225730 | 148.245399 |

Table A8.1 (cont'd): Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam, January 2001 - February 2005.

| Date | Species | Number | Hatchery | River | Site | Nearest town | Latitude | Longitude |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 18/01/2014 | Golden perch | 1,514 | Murray Darling Fisheries | Macquarie River | Lions Park West | Dubbo | -32.245484 | 148.598802 |
| 18/01/2014 | Golden perch | 757 | Murray Darling Fisheries | Macquarie River | Sand Beach Dubbo | Dubbo | -32.254978 | 148.592877 |
| 18/01/2014 | Golden perch | 1,514 | Murray Darling Fisheries | Macquarie River | Bril Bral Reserve | Geurie | -32.414511 | 148.724246 |
| 18/01/2014 | Golden perch | 1,523 | Murray Darling Fisheries | Macquarie River | Scabbing Flat Reserve | Geurie | -32.430641 | 148.810245 |
| 18/01/2014 | Golden perch | 1,514 | Murray Darling Fisheries | Macquarie River | Peach Tree Reserve | Geurie | -32.446781 | 148.777073 |
| 18/01/2014 | Murray cod | 2,021 | Murray Darling Fisheries | Macquarie River | Gin Gin Bridge | Gin Gin | -31.915770 | 148.083448 |
| 18/01/2014 | Murray cod | 2,020 | Murray Darling Fisheries | Macquarie River | Terramungamine Reserve | Dubbo | -32.169495 | 148.585074 |
| 18/01/2014 | Murray cod | 2,020 | Murray Darling Fisheries | Macquarie River | Dundullimal Reserve | Dubbo | -32.282718 | 148.602318 |
| 18/01/2014 | Murray cod | 2,020 | Murray Darling Fisheries | Macquarie River | Pilchers Reserve | Dubbo | -32.298760 | 148.622601 |
| 18/01/2014 | Murray cod | 2,020 | Murray Darling Fisheries | Macquarie River | Butlers Falls Reserve | Dubbo | -32.315866 | 148.621385 |
| 18/01/2014 | Murray cod | 2,020 | Murray Darling Fisheries | Macquarie River | Bril Bral Reserve | Geurie | -32.414511 | 148.724246 |
| 23/01/2014 | Golden perch | 3,409 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 23/01/2014 | Golden perch | 3,409 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 23/01/2014 | Murray cod | 2,273 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |

(continued)

Table A8.1 (cont'd): Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam, January 2001 - February 2005.

| Date | Species | Number | Hatchery | River | Site | Nearest town | Latitude | Longitude |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 23/01/2014 | Murray cod | 2,272 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 23/01/2014 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | 'Raby Irrigation' | Warren | -31.613051 | 147.778969 |
| 23/01/2014 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | Bryan Egan Weir | Warren | -31.684722 | 147.835785 |
| 23/01/2014 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | Oxley Park fronting Coonamble Road | Warren | -31.696928 | 147.839032 |
| 23/01/2014 | Murray cod | 2,500 | Murray Darling Fisheries | Macquarie River | Warren ‘Top Weir' | Warren | -31.734697 | 147.866298 |
| 31/01/2014 | Murray cod | 2,525 | Uarah Fish Hatchery | Macquarie River | Brummagen Reserve | Narromine | -32.227029 | 148.353422 |
| 1/02/2014 | Murray cod | 2,525 | Uarah Fish Hatchery | Macquarie River | Timbrebongie Falls Reserve | Timbrebong | -32.130191 | 148.247750 |
| 1/02/2014 | Murray cod | 2,525 | Uarah Fish Hatchery | Macquarie River | Rotary Park, Narromine | Narromine | -32.225730 | 148.245399 |
| 22/01/2015 | Golden perch | 3,409 | Murray Darling Fisheries | Macquarie River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| 22/01/2015 | Golden perch | 3,409 | Murray Darling Fisheries | Macquarie River | Narromine boat ramp | Narromine | -32.225730 | 148.245399 |
| 22/01/2015 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | 'Raby Irrigation' | Warren | -31.613051 | 147.778969 |
| 22/01/2015 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | Bryan Egan Weir | Warren | -31.684722 | 147.835785 |
| 22/01/2015 | Golden perch | 3,750 | Murray Darling Fisheries | Macquarie River | Oxley Park fronting Coonamble Road | Warren | -31.696928 | 147.839032 |

Table A8.1 (cont'd): Murray cod and golden perch stocking in the Macquarie River downstream of Burrendong Dam, January 2001 - February 2005.

| Date | Species | Number | Hatchery | River | Site | Nearest town | Latitude | Longitude |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $22 / 01 / 2015$ | Golden <br> perch | 3,750 | Murray Darling <br> Fisheries | Macquarie <br> River | Warren 'Top <br> Weir' | Warren | -31.734697 | 147.866298 |
| $22 / 01 / 2015$ | Murray cod | 2,272 | Murray Darling <br> Fisheries | Macquarie <br> River | Trangie Ski Hole | Trangie | -31.950525 | 148.152076 |
| $22 / 01 / 2015$ | Murray cod | 2,272 | Murray Darling <br> Fisheries | Macquarie <br> River | Narromine boat <br> ramp | Narromine | -32.225730 | 148.245399 |

