

Scoping study: Impacts of managed flows on fish spawning and recruitment

Impacts of managed flows on fish spawning and recruitment

Scoping Study for Project R5002

**Prepared for:
Murray-Darling Basin Commission**

by

Karina C. Hall, Paul Humphries, Adam Richardson and Matthew Vogel



Murray-Darling Freshwater Research Centre

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EXECUTIVE SUMMARY

This scoping study was undertaken to provide essential background for a Murray–Darling Freshwater Research Centre (MDFRC) project entitled, ‘Impact of managed flows on fish spawning and recruitment’. The aim of this project is to investigate the links between altered flows, the timing of fish spawning, and the levels of subsequent juvenile recruitment into riverine fish populations, across the Murray–Darling Basin. To achieve this, several hypotheses are to be tested, regarding the ways in which flow alteration may influence the recruitment dynamics of native and alien fish species, which have different durations of spawning.

The main objectives of the scoping study were to:

- 1) Review briefly the available scientific literature on recruitment and spawning in freshwater fishes.
- 2) Further develop conceptual and predictive models of fish recruitment timing and ‘success’ in lowland rivers.
- 3) Scope the future of flow management in selected rivers for the main study, in conjunction with appropriate water agencies within each study region.
- 4) Further develop appropriate hypotheses, indicators and suggested sampling designs for the project's second phase.

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1 INTRODUCTION

The status of native freshwater fish stocks can indicate the overall health of a river system (Harris & Gehrke 1997). Hence, for many years, the declines in abundance and distribution of many native fish populations across the Murray–Darling Basin have been major concerns for government, recreational fishers, conservation groups, scientists and the wider community.

Attempts to maintain fish populations through expensive re-stocking programs have met with variable success. A more effective, and probably ethical, strategy for population recovery would be through natural recruitment. Unfortunately, the conditions required for the successful recruitment of many freshwater fish species remain unknown, especially with respect to the natural timing and environmental conditions required for spawning, and the subsequent survival of larvae and juvenile fish.

The flow regimes of most major Australian rivers have been fundamentally altered through water resource development and regulation (Arthington & Pusey 2003). The construction of barriers such as dams and weirs, and the extensive diversion and/or extraction of water have left many rivers with reduced total flows, altered flood cycles — flood frequency, duration and extent — or seasonal reversal of flows. Under regulated conditions, water is typically stored during natural flood periods and released during subsequent dry periods (Lloyd et al. 2003). Indeed, within the Murray–Darling Basin there are few rivers remaining with near-natural flow regimes (Sheldon et al. 2000).

Previous research in the southern Murray–Darling Basin suggests that many fish species still breed under changed flow conditions, but that high abundances of juveniles do not necessarily result (Humphries et al., 2002). This suggests that recruitment failure, rather than lack of spawning, may be the controlling factor underpinning the continued low population size of a number of species. Establishing the appropriate flow regimes that would provide suitable conditions for recruitment remains a challenging but, ultimately, likely the only sustainable means of maintaining viable fish populations.

This scoping study was undertaken to complete the first phase of a project to be undertaken by the Murray–Darling Freshwater Research Centre (MDFRC) and Charles Sturt University entitled, ‘Impacts of managed flows on fish spawning and recruitment’. The aim of the project is to investigate the links between altered flows, the timing of fish spawning, and the levels of subsequent juvenile recruitment into riverine fish populations, across the Murray–Darling Basin. To achieve this, several hypotheses are to be tested, regarding the ways in which flow alteration may influence the recruitment dynamics of native and alien fish species, which have different durations of spawning.

The main objectives of this scoping study were to:

- 1) Review briefly the available scientific literature on recruitment and spawning in freshwater fishes.
- 2) Further develop conceptual and predictive models of fish recruitment timing and success in lowland rivers.
- 3) Scope the future of flow management in selected rivers for the main study, in conjunction with appropriate water agencies within each study region.
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2 RECRUITMENT VARIABILITY

Fish populations persist through time by the production of new individuals, or ‘recruits’ to replace members of the population lost through mortality or emigration. The ‘level of recruitment’ each year is determined by the number of new individuals that survive through all early life stages to mature and join the reproductively active adult population. In very basic terms, the number of new recruits (N_t) will be determined by the initial number of fertilised eggs (N_0), mortality rates (M) and the time taken to reach recruitment (t), according to the exponential equation $N_t = N_0 e^{-Mt}$ (Jobling 1995). Even small changes in M or t result in large variations in the number of new recruits at time t . Therefore, any factors that influence mortality rates or the time taken to reach recruitment will indirectly affect the level of recruitment and year class strength in any given year.

The total number of individuals that can survive a given life stage is generally controlled by the amount and availability of the key resource required for that stage, and the ability of individuals to access it (White 2001). Density-dependent factors such as competition and predation are more likely to determine which individuals survive rather than how many survive. These latter factors, however, do influence natural selection and the evolution of life history traits, which in turn affect the interaction between species and their environment.

Environmental conditions are not stable, but highly variable both spatially and temporally (Jobling 1995). The amount and availability of a key resource is likely to vary over time and some years, or even days, will be more favourable for survival than others. Thus large year-to-year fluctuations in recruitment are likely to arise in response to short-term changes in the environment. For most species, the key resource for survival is likely to be the availability of food, which is likely to exhibit considerable variation in response to climatic variation.

The initial number of fertilised eggs (N_0) is set by the size and condition of the adult spawning population, which is influenced by the amount of resources available to the

adult population. Maternal condition is known to affect fecundity, egg size and the yolk content of eggs. These factors in turn influence the subsequent size and yolk reserves of hatchlings, and their potential survival and growth rates.

Mortality rates (M) are generally high and variable during the earliest life stages of fish — the fertilised egg, larval and juvenile stages — due to the many rapid changes in morphology, ecology and behaviour that occur at these stages (Bradford & Cabana 1997). Year class strength and recruitment variability are, consequently, thought to be largely determined during these very early stages (Houde 1997).

The time taken to reach recruitment (t) is largely determined by the growth rates achieved during each life history stage. Growth rates are influenced by species-specific genetic characteristics and environmental factors such as food availability and temperature (Jobling 1995). For any given level of development, well fed larvae generally grow faster and reach recruitment earlier than poorly fed ones. Similarly, temperature has a positive influence on growth rates up to an upper threshold tolerance level which is generally species specific.

Other environmental factors — natural or human induced — may directly or indirectly affect early life history mortality and growth rates by limiting or enhancing larval access to resources or sources of mortality. For example, pollution may directly increase mortality rates through poor health and decreased tolerance to disease. Alternatively, altered flow regimes may physically move larvae away into or away from areas of high food concentration or predation, or restrict access to food through poor connectivity between spawning and nursery areas — for example, the main river channel and floodplain wetlands.

In temperate areas, where climate varies considerably, it is generally accepted that the timing of adult spawning has evolved such that it coincides with the timing of optimal conditions for juvenile survival (Trippel *et al.* 1997). Therefore, any changes to conditions through human activities, such as changes to flow regimes, may adversely affect recruitment levels. There has been a marked decline in the abundance of many freshwater fish species in Australia and the world (Matthews, 1998). River regulation and flow alteration undoubtedly have played a role in this process (Lloyd *et al.*, 2004;

Lytle and Poff, 2004). The persistence of some species, however, goes against this trend. At least in the south-eastern portion of the Murray-Darling Basin, the native species which are currently thriving, despite dramatic changes to the flow environment are those with relatively protracted breeding seasons — for example, Australian smelt (*Retropinna semoni*) and flathead gudgeon (*Philypnodon grandiceps*) (Humphries et al., 1999; 2002). These species tend to be opportunists and, perhaps, are able to overcome alterations to the flow regime through the buffering capacity of the life history styles. Why some species persist and others decline is a fundamental question that fish researchers and river managers must address if restoration has any chance of succeeding. The various hypotheses described in the scientific literature to explain recruitment variability are explored below in relation to the possible recruitment failure of certain fish species.

2.1 Match/mismatch hypothesis

One hypothesis that attempts to explain large annual variations in the level of recruitment of temperate fish populations is the ‘match/mismatch’ hypothesis (Cushing 1990). It is based on the contrast between the relatively fixed time of spawning for many temperate fish species — controlled by predictable biotic factors such circadian rhythms in response to day length — and the highly variable time for peak production of food for larval stages — controlled by more variable abiotic factors such as climate. This can result in a natural match or mismatch between the timing of larval and food production, which in turn varies the level of successful recruitment from year to year. Indeed, the timing of food production each year may be more influential on recruitment success than the total amount of food produced.

Laboratory experiments designed to test this hypothesis with Atlantic cod larvae (Gotceitas *et al.* 1996) indicated that the amount of food and the timing of food availability both influenced larval growth rates. Larvae have only a short window of opportunity — usually in the order of a few days — within which to locate appropriate food before starvation occurs. Then, and at all subsequent stages of development, larvae need to encounter large numbers of prey of the appropriate size to survive.

Factors that influence either the timing of spawning or the production of larval prey —

water temperature, flow regime and connectivity, for example — could have a direct influence on the level of recruitment to the adult population. The operation of some water storages has resulted in a change in the timing of peak riverine primary and secondary production, and these peaks may no longer coincide with the cues for the onset of spawning for some fish species. Hence, altered flow regimes may have resulted in a ‘mismatch’ between cues for spawning and the optimal conditions for larval survival. Such a mismatch might result in failed or minimal recruitment for those species in that year. Recruitment undoubtedly varied significantly between years under natural flow conditions, however, altered flow regimes may have increased the frequency of mismatch’ events, leading to an overall reduction in recruitment success and declines in population numbers.

To test the hypothesis of whether there is a correlation between the timing of spawning, larvae variability and prey abundance; time series data of fish eggs, larvae and zooplankton abundances may be analysed (Johnson 2000).

2.2 *Window of opportunity hypothesis*

One prediction from the match/mismatch hypothesis is that as spawning duration decreases, interannual recruitment variability should increase (Mertz & Myers 1994). In other words, the probability of larvae encountering sufficient quantities of prey for survival, once they have used up their yolk, declines with decreasing length of the spawning period.

Taking the match/mismatch hypothesis one step further, it has been suggested that the success of species such as flathead gudgeon and Australian smelt may be due to their extended spawning period (Humphries et al. 2002). A longer spawning period would potentially increase the likelihood of larvae surviving during what may be a relatively brief period of optimal recruitment conditions — the prey abundance peak (Humphries et al. 2002). Conversely, the lack of success of species with relatively short spawning periods may be due to the limited opportunity these species have to produce larvae in coincidence with peaks in optimal conditions.

There are some differences between the match/mismatch hypothesis and the ‘window of

opportunity' hypothesis presented for the first time here. The window-of-opportunity hypothesis states that: fish which spawn over a 'protracted' period have a recruitment advantage, *in any one year*, over fish which spawn over a 'brief' period. This is because the probability that a proportion of the larvae of protracted species will encounter a period when conditions are optimal for recruitment is greater than for larvae of 'brief' species.

2.3 *Alternative hypotheses*

Generalist v specialist

Rather than the timing of optimal conditions being crucial, it may in fact be the range of conditions that a species can take advantage of which determines the extent of successful recruitment. Species with flexible life history strategies that can survive under a wide range of environmental conditions may be able to maintain high levels of recruitment amidst altered flow patterns. For example, generalist feeders with non-specific larval food requirements that can exploit a range of food species may have greater recruitment success in an altered environment, particularly if those food species have differing times of peak production. The larvae of Australian smelt are non-specific feeders and, rather, eat a variety of small sized food items (King, 2005). Species with highly specialised life history traits that exploit only a narrow range of optimal conditions may be less likely to encounter those optimal conditions under a regime of altered flows. In other words, it may not be the timing of the window of opportunity *per se*, but rather the 'breadth' of that window that determines patterns of recruitment variability for each species.

Maternal condition hypothesis

This hypothesis also relies on the 'bigger is better' hypothesis and other growth rate-related hypotheses such as the 'stage duration hypothesis'. These hypotheses maintain that the fastest growing and, hence, largest larvae have the highest survival rates (Miller et al. 1988), so the period during the spawning season when the largest individuals spawn is likely to be the time of the highest survival rates (Trippel *et al.* 1997). Larger and older individuals tend to spawn earlier in the spawning season (Trippel *et al.* 1997) and they produce larger eggs with more yolk reserves. This is particularly relevant for short-lived fish that may have alternative reproductive strategies: some individuals

delay maturity to return to spawn at a much larger size than others.

Flood pulse concept and flood recruitment hypothesis

The flood pulse concept postulates that the inundated floodplain provides more food productivity to the main river channel than downstream transport (Junk *et al.* 1989; Bayley 1991). This is particularly relevant if flooding coincides with warm temperatures to increase rates of invertebrate production. Fish are thought to spawn in response to increases in flow and flooding so that recruitment coincides with the high densities of food flushed from the floodplain into the main river channel. Thus, it follows, that the lack of flooding in many regulated systems may have decreased the levels of larval food supply from floodplain areas or removed spawning cues for some species.

Based on the results of aquaculture studies, many Australian native fish were thought to spawn in response to increases in flow or water level. More recent studies on wild populations, however, suggest that some of these species may actually spawn independent of high flows and flooding (Humphries *et al.* 1999), and that some species apparently spawn every year at approximately the same time, irrespective of variations in environmental conditions (Humphries *et al.* 2002). Murray cod (*Maccullochella peelii peelii*) larvae were found in the Broken River at approximately the same time every year for 5 years, even though environmental conditions varied significantly during that time, including some years with no high flows during a severe drought (Humphries 2005).

The flood recruitment hypothesis was further explored for native freshwater fishes of the Murray–Darling Basin by King (2002; 2004a). This study demonstrated that a number of species can successfully spawn and recruit in the main channel without flooding or access to the floodplain environment, and that there was an abundant source of prey in the epibenthic zone of the main channel for larval fish food (King, 2004b). King *et al.* (2003) concluded that only a small number of Murray–Darling fishes have life history characteristics that would enable them to make use of the inundated floodplain for recruitment. In particular, many species spawn in spring–summer, but floods in the region where that study took place tend to occur in the late winter period as snows melt. Recent results from the flooding of Barmah Forest on the Murray River,

seems to confirm that some species, notably golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*) will indeed spawn in response to increases in flow (King, personal communication), if this occurs at an appropriate time.

Low flow recruitment hypothesis

In contrast to the flood recruitment hypothesis, the low flow recruitment hypothesis suggests that some native fish species spawn at a time to coincide larval hatching with the period of predictable low flows during summer (Humphries et al. 1999). Smaller volumes of water warmed by summer temperatures can create favourable conditions for invertebrate prey production which, in turn, provide high prey densities which can accelerate larval growth. Periods of low flow may be more predictable and of longer duration than floodplain inundation periods in some temperate areas. Furthermore, low flows may provide an abundance of slackwater habitats in the main channel for egg substrate and larval refuge (King 2004a; Humphries et al. in press). River regulation that has resulted in seasonal flow inversions — with increased flows during summer for irrigation or domestic use — may, therefore, have reduced the extent of slackwater habitat for egg substrate and larval refuge or interfered with the production of in-channel larval food. Those species most dependent on low flows for recruitment would be the most adversely affected in systems with seasonal flow inversions (Humphries et al. in press).

Member/vagrant hypothesis

Variations in fluid transport processes may aid or hinder larval retention in areas of high food availability or successful migration of larvae from spawning to nursery feeding grounds (Cushing 1990). For example, the inundated floodplain may have provided the primary source of habitat for spawning and/or larval refuge/feeding for some species. A reduction in floodplain inundation as a result of river regulation may have reduced the quantity and/or quality of suitable spawning and or nursery habitat for some species. Alternatively, the spawning areas of some Murray–Darling fishes may be limited to certain parts of the Murray–Darling Basin, and recolonisation and movement into other areas may be restricted by barriers, such as weirs (Koehn et al. 2004).

2.4 *Proposed project*

The ‘Impact of managed flows on fish spawning and recruitment’ project originally proposed to address three main questions:

1. Is there differential recruitment for ‘protracted’ spawners?
2. Is the temporal distribution of spawning dates synchronous within and between years for selected species?
3. What environmental conditions are most closely linked to spawning activity and recruitment success in a suite of brief and protracted spawning species?

To address the first question, the proposed methodology will involve quantitatively sampling Australian smelt to assess temporal variability in spawning and recruitment in regulated and unregulated rivers in three geographic regions of the Murray–Darling Basin — Australian smelt will be targeted because they are distributed widely throughout the Murray–Darling Basin and have a protracted spawning period. Smelt larvae will be collected monthly over two breeding seasons to determine the temporal distribution of spawning dates and variation in abundance of larvae produced. At the end of the spawning season, new recruits (juvenile smelt) will be collected and their spawning-date distributions back-calculated from otolith microstructure analysis to determine the timing of peaks in recruitment success during the spawning period. Environmental variables and food concentrations will also be monitored at each sampling time to determine any correlations in the timing of peak spawning, peak prey abundance and peak recruitment success. To assess the suitability of Australian smelt as a focus species for this methodology, a brief review of the known biology of Australian smelt has been included (Section 3).

The methodology to answer the second question will utilise the otoliths of juveniles of other species collected during the two years of sampling. Selected species will be grouped according to the length of their spawning season — brief, protracted or otherwise — and their spawning-date distributions back-calculated from otolith microstructure analysis. The spawning-date distributions will be compared with those of juvenile smelt to determine if there is any synchrony between species and/or between

years for the timing of peak survival. It was hypothesised that species with spawning-date distributions that overlap with the time of peak recruitment of smelt, or peak food abundance, should show good recruitment in that year, and that those with brief spawning periods that mismatch with the time of peak recruitment of smelt, or peak food abundance, should show poor levels of recruitment.

To answer the third question, the spawning-date distributions of survivors will also be compared with temporal distributions of environmental variables and food concentrations to determine which variables correlate with the time of peak recruitment success for each species. To explore the feasibility of this methodology further, Section 4 provides a brief summary of the main spawning traits of Murray–Darling fish species.

3. Biology of Australian smelt

The Retropinnidae is a small family of salmoniformes from the Southern Hemisphere, consisting of only four species (McDowall 1996b). Two species are endemic to Australia: Australian smelt, which is widespread and abundant throughout the south-eastern mainland; and Tasmanian smelt (*Retropinna tasmanica*), which occurs only in Tasmania. The remaining two species are endemic to New Zealand, with common smelt (*Retropinna retropinna*) the most abundant and widespread of the two.

New Zealand common smelt have two forms: diadromous individuals that inhabit coastal rivers and spend part of their life-cycle in the marine environment, and lacustrine individuals that inhabit inland lakes and reservoirs (Ward *et al.* 2005). The two forms differ morphometrically and can be readily distinguished from each other. Diadromous fish tend to be larger at a given age than the lacustrine fish and obtain overall larger maximum sizes (Ward & Boubée 1996). Lacustrine populations can be further divided into truly landlocked populations and those that inhabit lakes with access to lowland rivers. In some lowland lake and river systems both migratory and sedentary forms occur sympatrically (Ward & Boubée 1996).

Tasmanian smelt populations are limited to coastal streams of the isle and are thought to be diadromous, with an obligate marine period. However, little is known of their life history.

Australian smelt can also be divided into coastal and inland populations geographically separated by the high altitude regions of the Great Dividing Range. Individuals of coastal populations tend to be larger than those from inland areas, but unlike other smelts, they apparently do not make regular migrations into the marine environment (Milton & Arthington 1985). At the present time, we do not know if Australian smelt in either coastal or inland river systems migrate prior to spawning or are spatially distributed as stationary populations? This has particular relevance to the design of a sampling strategy for rivers that include spawning areas. Juvenile and adult Australian smelt have been recorded moving upstream at Torrumbarry fishway on the River

Murray (McDowall 1996a) and in large numbers elsewhere in the River Murray (Gilligan & Schiller 2003).

Milton and Arthington (1985) studied a coastal population of Australian smelt near Brisbane. Fish started to mature when water temperatures rose following a period of decreasing temperature when water temperature fell to below 15°C. Mature fish were found over 9 months of the year from June to February, but there was an apparent peak in spawning between July and November. This period coincides with the period of lowest and relatively predictable stream discharges, and suggested that spawning primarily occurred in low-flow pre-flood periods (Milton & Arthington 1985). Juveniles were collected between December and June, the months of normally high stream discharge and intermittent flooding, which suggested that juveniles rather than larvae were the main dispersal stage (Milton & Arthington 1985). It may be assumed that if juveniles and larvae disperse downstream, the adults must eventually return back upstream.

For an inland population in the lower River Murray, the spawning season was relatively short: mature individuals were found from August to December only (Leigh 2002). Juveniles started appearing in November and grew rapidly in size. Juveniles collected later in the season grew at slower initial growth rates than those collected early in the season, despite higher ambient water temperatures.

Size distributions of oocytes in the gonads of mature females indicate that Australian smelt is a multiple batch spawner, with synchronous oocyte development producing discrete batches of eggs every 3–4 days (Leigh 2002). However, it is unknown how many batches of eggs one individual female may lay over her individual spawning period. The protracted spawning season may result from the same females spawning many batches over a long period, or from successive females coming in to spawn for shorter periods.

Australian smelt eggs are tiny — diameter 0.8-1.0 mm; Milward (1966) and Leigh (2002) — negatively buoyant and strongly adhesive. Spawning occurs in shallow littoral areas of slow flowing stream pools over sand substrate; eggs attach to the base of vegetation, debris or sediment (Milton & Arthington 1985). In aquaria, embryos took

approximately nine days to develop and hatch at water temperatures of 13–19°C (Milward 1966). During development, eggs adhered to the bottom of aquaria and newly hatched larvae congregated at the surface or sides. The hatchling larvae were very small — less than 5 mm long; McDowall (1996a) — and residual yolk was absorbed within 2–3 days in captivity. Emaciation ensued if appropriate food was not available.

Ageing results for New Zealand common smelt, determined from otolith analysis, suggested that a small proportion of both diadromous and lacustrine fish may delay maturity and overwinter to spawn the following spring at a larger size (Ward & Boubée 1996). However, all are thought to be semelparous, such that they do not survive to spawn during a second season.

Adult otoliths of Australian smelt from the lower River Murray were very fragile and prone to shattering, especially when dried, and contained ambiguous regions of growth increments towards the outer edges (Leigh 2002). Therefore, it was difficult to age individuals older than 4 months old and the age composition of adult spawning populations could not be determined. Most mature individuals were 44–50 mm in length, but a small number were larger (50–57 mm in length). In addition, a small number of larger but immature smelt were captured in March following the spawning season when all other mature adults had disappeared (Leigh 2002). This suggests that in this lacustrine population a small number of individuals may delay spawning until the following year. Histological examination of gonads suggested that Australian smelt were also semelparous, therefore, those individuals that delayed maturity until the following year to spawn at a larger size would not have spawned previously.

This population structure and life history information should be considered when interpreting the temporal dynamics of recruitment. As discussed in Section 2, larger females generally produce larger eggs, which in turn produce larger hatchling larvae. These larvae may have a survival advantage over larvae from smaller individuals. Thus, if larger individuals spawn at a particular time, it may influence the peak period of larval survival. In the lower River Murray, the proportion of larger individuals was greater early in the spawning season compared to later — an interpretation of length frequency data from Leigh (2002)). Thus, there may be a higher survival rate of larvae spawned early in the spawning season that is independent of food availability or water

temperature.

4 SPAWNING AND RECRUITMENT OF FRESHWATER FISHES

Through the process of natural selection, life history strategies evolve that optimise the survival of new individuals through all life stages to reproduce in natural environments (Bunn & Arthington 2002). Hence, the cues for and timing of spawning for each species would have evolved over time in response to natural conditions that ensured the highest numbers of individuals survived to reproduce.

The natural flow regimes of Australian temperate rivers show extreme interannual variation in response to variation in climate and precipitation (Arthington & Pusey 2003). The Murray–Darling Basin covers a large area that is not homogenous climatically, with high flows occurring during summer in some areas and during early spring in others. Therefore, it is expected that a variety of life history and recruitment styles will have evolved across the Murray–Darling Basin in response to local conditions.

Approximately 30 species of native freshwater fish from 12 families are found in the Murray–Darling Basin incorporating a diverse range of families, sizes and life cycle modes. The timing and location of adult spawning sets the stage for subsequent early life dynamics. Unfortunately, information regarding the spawning cues and preferences of Murray–Darling fishes is very limited and largely derived from aquaculture studies, rather than controlled experiments that incorporate the full range of conditions likely to be encountered in the wild. In particular, factors that might influence circadian rhythms — day length, light intensities and moon cycles, for example — have been largely ignored. Evidence from recent larval fish studies suggest many species consistently spawn at the same time every year despite large interannual variation in patterns of flow and temperature (Humphries *et al.* 2002).

Humphries *et al.* (1999) proposed four major categories of Murray–Darling fishes based

on life history traits, with an emphasis on spawning style and larval first-feeding characteristics (Table 1). Growns (2004) developed this further and attempted to quantitatively categorise a large number of freshwater fishes using a suite of 13 reproductive and larval traits, and multivariate statistics. Growns (2004) described five major reproductive guilds, with two that could be further sub-divided into sub-guilds (Table 1). Species with a protracted spawning period do not fall into a single category, but are spread across a number of guilds. They also encompass a range of maximum sizes and spawning habitats. Most species spawn in spring–summer, with the exception of mountain galaxias, which spawns in winter–spring. A protracted spawning season during spring-summer would increase the chances of coinciding larval occurrence with peaks in prey production, and cover a range of environmental conditions that could influence larval survival and growth rate.

Since maximum prey size is related to the gape size of larvae (King 2005), the size of larvae at first hatching will influence the size and range of potential prey species available during the critical transition from endogenous feeding on yolk reserves to exogenous feeding on prey species. This could be important if prey taxa show differential production with respect to size over the period of peak production. For example, a progression in size from small to larger sized prey species as the season progresses.

Characteristics of eggs and larvae of native and introduced freshwater fish of the Murray–Darling Basin are provided in Table 2 for contextual purposes.

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Table 1 Characteristics of adults and spawning of native and introduced freshwater fish of the Murray–Darling Basin.

Species name	Common name	Family	Mode #	Guild *	Max. size (cm)	Life span (years)	Age-at-maturity (years)	Spawn season	Spawn duration (months)	Migrate to spawn	Trigger to spawn Temp	Trigger to spawn Flows	Spawn habitat
Native													
<i>Ambassis agassizii</i>	Olive perchlet	Chandidae		A	8	4	1	Nov-Dec	2	Move rec.	Min. Inc.	Inc.	Near vege
<i>Anguilla australis</i>	Short-finned eel	Anguillidae		Id	109	25	7-25						>300m deep
<i>Bidyanus bidyanus</i>	Silver perch	Terapontidae	2	D1	60	15	3-5	Oct-Dec	3	Upstream	Min.	Inc.	Open water
<i>Craterocephalus amniculus</i>	Darling River hardyhead	Atherinidae		A	6		1		3				
<i>Craterocephalus fluviatilis</i>	Murray hardyhead	Atherinidae		A	7		1	Oct-Feb	4		Min.		Near vege
<i>C. stercusmuscarum</i>	Fly-specked hardyhead	Atherinidae		A	10	3	1	Oct-Nov	2		Min.		
<i>Gadopsis bispinosus</i>	Two-spined blackfish	Gadopsidae		C2	26		1-2	Oct-Dec	2.5				
<i>Gadopsis marmoratus</i>	River blackfish	Gadopsidae	1	C2	60	6	1-2	Nov-Jan	2.5		Min.		Hollow logs
<i>Galaxias brevipinnis</i>	Climbing galaxias	Galaxiidae		A	28		1	May-Jun	2				
<i>Galaxias olidus</i>	Mountain galaxias	Galaxiidae	3b	A	14	4	1	Jul-Nov	5				Riffles
<i>Galaxias rostratus</i>	Murray jollytail	Galaxiidae	3b	B	15		1	Aug-Sep	2		Min.		
<i>Geotria australis</i>	Pouched lamprey	Geotriidae		D1	67		5	Oct-Dec	3	Upstream			Nests gravel
<i>Hyposelatoris spp.</i>	Carp gudgeon	Gobiidae	3b	C2	7	3	1	Nov-Mar	5	Move rec.	Min.		Rocks, vege
<i>Leiopotherapon unicolor</i>	Spangled perch	Terapontidae		D2	25	5	1	Sep-Dec	4	Move rec.	Min.	Inc.	Open water
<i>Maccullochella macquariensis</i>	Trout cod	Percichthyidae	1	C2	80		3	Sep-Nov	3		Min.		
<i>Maccullochella peelii peelii</i>	Murray cod	Percichthyidae	1	C2	180	30	4-6	Nov-Dec	2	Upstream	Min. Inc.	Inc.	Snags, rock
<i>Macquaria ambigua</i>	Golden perch	Percichthyidae	2	D1	76	15-20	3-4	Nov-Mar	5	Upstream	Min.	Inc.	Open water
<i>Macquaria australasica</i>	Macquarie perch	Percichthyidae		A	46		3	Oct-Dec	3	Yes	Min.		
<i>Melanotaenia fluviatilis</i>	Crimson-spotted rainbowfish	Melanotaeniidae	3b	A	9	3	1	Nov-Feb	4		Min. Inc.		Near vege
<i>Mogurnda aspersa</i>	Purple-spotted gudgeon	Gobiidae		C2	14	4	1	Dec-Feb	3		Min.		Snags, rock
<i>Mordacia mordax</i>	Short-headed lamprey	Mordaciidae		D1	48			Aug-Oct	3	Upstream			Sand, gravel
<i>Nannoperca australis</i>	Southern pygmy perch	Nannopercidae	3b	B	8		1	Aug-Nov	4		Min. Inc.		
<i>Nematalosa erebi</i>	Bony herring	Clupeidae		D2	47	8	1-2	Oct-Dec	3	Move rec.	Min.		Open water
<i>Philypnodon grandiceps</i>	Flathead gudgeon	Gobiidae	3a	C1	12		1	Oct-Apr	7	Yes	Min.		Rock, snags
<i>Retropinna semoni</i>	Australian smelt	Retropinnidae	3a	A	10	4	1	Sep-Feb	6	Move rec.	Min. Inc.		Near vege
<i>Tandanus tandanus</i>	Freshwater catfish	Plotosidae	1	C2	90	15	3-5	Oct-Mar	6	Move rec.	Min.		Nests grave;
Introduced													
<i>Carrassius auratus</i>	Goldfish	Cyprinidae		A	40	5	1		3				Near vege
<i>Cyprinus carpio</i>	Common carp	Cyprinidae		A	120	15	2	Nov-May	7	Upstream	Min.		Near vege
<i>Gambusia holbrooki</i>	Gambusia	Poeciliidae		E	6	3	2 months	Oct-May	8	Move rec.	Min.		Internal
<i>Migurnus anguillicaudatus</i>	Oriental waterloach	Cobitidae		B	25		1		6				
<i>Oncorhynchus mykiss</i>	Rainbow trout	Salmonidae		C1	78	15	2-3	Aug-Oct	3	Upstream			Gravel beds
<i>Perca fluviatilis</i>	Redfin perch	Percidae		D2	45		1	Sep-Nov	3				Near vege
<i>Salmo trutta</i>	Brown trout	Salmonidae		C1	140		3	Apr-Aug	5				
<i>Salvelinus fontinalis</i>	Brook char	Salmonidae		C1	85		3		5				
<i>Tinca tinca</i>	Tench	Cyprinidae		A	70		3		3	Yes			

Modes from Humphries et al. (1999); *Guilds from Grown (2004); Other data from various sources, all of which are included in the reference list.; Move rec. indicates movement has been recorded for that species but not necessarily indicative of a spawning migration.

Scoping study: Impacts of managed flows on fish spawning and recruitment

Table 2 Characteristics of eggs and larvae of native and introduced freshwater fish of the Murray–Darling Basin.

Species name	Common name	Fecundity (no. eggs)	Egg size (mm)	Egg habitat	Egg adhesive	Av. dev. time (days)	Parental care	Av. hatch size (mm)	Larval duration (days)#	Larval habitat	Larval food	Decline in abundance/distribution
Native												
<i>Ambassis agassizii</i>	Olive perchlet	2,350	0.7	Demersal	Yes	6	No	3				*
<i>Anguilla australis</i>	Short-finned eel											*
<i>Bidyanus bidyanus</i>	Silver perch	500,000	2.8	Pelagic, drift	No	1.5	No	3.6	18	Pelagic, drift	Zoop	*
<i>Craterocephalus amniculus</i>	Darling River hardyhead	2,000	1.3	Demersal	Yes	7	No	3.5				*
<i>Craterocephalus fluviatilis</i>	Murray hardyhead	2,000	1.3	Demersal	Yes	6	No	3.4				*
<i>C. stercusmuscarum</i>	Fly-specked hardyhead	2,000	1.5	Demersal	Yes	7	No	4	7	Drift		*
<i>Gadopsis bispinosus</i>	Two-spined blackfish	450	3.5	Demersal	Yes	15	Yes	5				*
<i>Gadopsis marmoratus</i>	River blackfish	2,500	4	In hollow logs	Yes	16	Yes	5	14			*
<i>Galaxias brevipinnis</i>	Climbing galaxias	23,000	1.2	Demersal	Yes	12	No	8.6				*
<i>Galaxias olidus</i>	Mountain galaxias	400	1.5	Boulders in riffles	Yes	21	No	9.5				*
<i>Galaxias rostratus</i>	Murray jollytail	7,000	1.4	Demersal	No	9	No	6.5	5			*
<i>Geotria australis</i>	Pouched lamprey	58,000	1.1	Demersal	No		No	1				
<i>Hyposeletoris spp.</i>	Carp gudgeon	2,000	0.4	Hard bottom	Yes	2	Yes	1.9	12	Backwaters	Zoop	
<i>Leiopotherapon unicolor</i>	Spangled perch	100,000	0.8	Demersal	No	2	No	2				*
<i>Maccullochella macquariensis</i>	Trout cod	10,000	3.3	Demersal	Yes	8	Yes	8.3	28	Drift		*
<i>Maccullochella peelii peelii</i>	Murray cod	200,000	3	Snags, rock	Yes	9	Yes	7.5	27	Drift	Zoop	*
<i>Macquaria ambigua</i>	Golden perch	500,000	3.5	Pelagic, drift	No	1.5	No	4	23	Pelagic, drift	Zoop	*
<i>Macquaria australasica</i>	Macquarie perch	110,000	2	Demersal	Yes	10	No	7	10	Drift		*
<i>Melanotaenia fluviatilis</i>	Crimson-spotted rainbowfish	150	0.9	Vege, snags	Yes	5	No	4	9	Still	Algae	*
<i>Mogurnda aspersa</i>	Purple-spotted gudgeon	1,300	2	Hard bottom, rocks	Yes	6	Yes	3.5				*
<i>Mordacia mordax</i>	Short-headed lamprey	14,000	1	Demersal	No		No	1				
<i>Nannoperca australis</i>	Southern pygmy perch	4,000	1.3	Demersal	No	3	No	3.5	11			*
<i>Nematalosa erebi</i>	Bony herring	230,000	0.8	Pelagic, drift	No		No	3				
<i>Philypnodon grandiceps</i>	Flat-headed gudgeon	900	1.8	Rock, snags	Yes	5	Yes	3.8	9	Drift		
<i>Retropinna semoni</i>	Australian smelt	1,000	0.9	Vege, sand	Yes	9	No	5	5	Backwaters	Zoop	
<i>Tandanus tandanus</i>	Freshwater catfish	20,000	3	Nests, sand, gravel	No	7	Yes	7.2			Zoop	*
Introduced												
<i>Carrassius auratus</i>	Goldfish	400,000	1	Vege	Yes	7	No	4	13	Drift	Zoop	
<i>Cyprinus carpio</i>	Common carp	2,000,000	1.5	Vege	Yes	3	No	5	17	Drift	Algae	
<i>Gambusia holbrooki</i>	Gambusia	315	Live young	Internal	-	24	No	2	-	Still		
<i>Migurnus anguillicaudatus</i>	Oriental waterloach	20,000	1.3	Demersal	No	3	No	3.4				
<i>Oncorhynchus mykiss</i>	Rainbow trout	3,000	4	Gravel beds	No	35	Yes	14.5				
<i>Perca fluviatilis</i>	Redfin perch	200,000	2.5	Demersal	Ribbons	8	Yes	5	53	Drift	Zoop	
<i>Salmo trutta</i>	Brown trout	3,000	4	Demersal	No	42	Yes	25				
<i>Salvelinus fontinalis</i>	Brook char	5,000	4.2	Demersal	No	50	Yes	38				
<i>Tinca tinca</i>	Tench	900,000	1	Demersal	Yes	5	No	4.5				

Estimated in most cases by subtracting the rapid development time from King et al. (2003) by the egg development time. Data from various sources, all of which are included in the reference list.

5 PROPOSED METHODOLOGY

As stated in Section 2.4, the proposed methodology will involve quantitatively sampling larval and juvenile Australian smelt in regulated and unregulated rivers of three distinct geographic regions of the Murray–Darling Basin. It will be no mean logistical feat to collect quantitative scientific data over such a large area and adequately achieve the project objectives — posed as questions, also in Section 2.4. The project relies, therefore, on a well-designed and robust methodology. Following is a detailed description of the proposed methodology including a discussion of the selected rivers, sampling design, field sampling methods and laboratory procedures.

5.1 *Selection of rivers*

The ecological effects of altered flow regimes would be investigated, ideally, by the comparison of before-and-after data from an impacted system with that of a non-impacted control system. However, changes to flow regimes have often been instituted gradually over decades and, as such, long-term data is rarely available. The alternative approach is to compare spatial ecological data between rivers with historically similar flow regimes that are now similar apart from a change in the flow regime of one (Closs *et al.* 2004).

For the comparison of rivers with different hydrological regimes, it is imperative that they are matched as closely as possible in all other respects — this should reduce the likelihood that the two rivers differed naturally, irrespective of current flow regime. This applies to fundamental natural features of the rivers, such as a common species pool, geology, vegetation, water chemistry and gradient; and other anthropogenic pressures, such as urbanisation, vegetation clearance, agricultural development, snag removal and riparian grazing. Other anthropogenic pressures may have produced simultaneous ecological impacts that are difficult to separate from those arising from altered flow regimes (Bunn & Arthington 2002).

It is highly unlikely that any two rivers possess exactly the same characteristics, therefore matching rivers perfectly is probably impossible. However, as hydrology is a major determinant of aquatic community structure (Allan 1995; Statzner and Higler 1986), selecting rivers of similar annual discharge, with geographic proximity and similar influences, should ensure that differences in the fish communities, if they occur, are likely to be as a result of differences in hydrological regimes.

The Murray–Darling Basin is climatically heterogenous and natural flow regimes vary considerably among regions. The relationship between flow regime and fish recruitment dynamics are also likely to vary among regions. For this reason, it is proposed that the study be conducted in three different climatic regions of the Murray–Darling Basin: northern, southern and western regions.

5.1.1 Regions

The Border Rivers Catchment is located in the northern region of the Murray–Darling Basin and straddles the Queensland–New South Wales border. It covers an area of almost 50 000 km² and is comprised of three main rivers — the Dumaresq, Macintyre and Barwon — associated smaller effluent streams and a complex floodplain (Kingsford 1999). The major rivers originate in the high altitude areas of the Great Dividing Range and flow, generally, in a westward direction. Stream gradient gradually decreases from the headwaters and the rivers flow into lowland floodplain areas. The entire northern region of the Murray–Darling Basin is characterised by high rainfall and floods during the spring–summer period, the period of highest annual temperature. In recent years, irrigated agriculture in the region has expanded considerably and annual water diversions have increased from 63 GL in 1988 to more than 242 GL in 1998 (Abawi *et al.* 2001).

The major rivers of north-eastern Victoria, in the southern region of the Murray–Darling Basin, have considerably greater annual flow than rivers in the northern region. For example, the combined catchment area of the major rivers in north-eastern Victoria — the Upper Murray and Mitta Mitta, Kiewa, Ovens, Broken and Goulburn — is almost 50 000 km², but the mean annual runoff is almost ten times greater than the Border River

Catchment (9890 GL compared with 1100 GL; Crabb 1997). Major river systems of north-east Victoria originate in the high altitude areas of the Great Dividing Range and flow in a northerly or easterly direction across lowland floodplain areas to the River Murray. River gradients decrease with river length. In the southern region, flooding generally occurs during winter–spring due to a combination of high rainfall and snowmelt; therefore, flooding is normally accompanied by low water temperatures. The degree of regulation varies considerably among rivers, from the virtually unregulated Ovens River to the heavily regulated Mitta Mitta and Goulburn rivers.

The western region of the Murray–Darling Basin consists primarily of dryland river ecosystems and lowland river floodplains. Most rivers are typical of arid region rivers: extremely ephemeral and flow for only a few months of the year during the monsoonal summer season in high rainfall areas (Puckridge *et al.* 2000). The River Murray and the Darling River are the only two permanent rivers in the region are heavily regulated. Periods of high flow and flooding are naturally intermittent and unpredictable. Selection of rivers for study in this region is restricted by these naturally irregular flow patterns, and by remoteness and river regulation. It is recommended that two regulated rivers with significantly different flow regimes be used for this study, due to the reasons given in Section 5.1.1. The Lindsay Island system covers approximately 15 000 hectares between the Murray and Lindsay rivers and is an important site for current and future environmental flow research. Anabranches within the area have different flow regimes depending on the location of their outlets from the River Murray.

5.1.2 Rivers

Northern region —Macintyre River (unregulated)

Only the upper reaches of the Dumaresq and Macintyre Rivers remain unaffected by river regulation (Figure 1). The Severn River, which flows into the lower reaches of the Macintyre River, has been regulated since Pindari Dam was constructed in 1969. The original storage capacity of Pindari Dam was 37,500 ML, but this was increased eightfold in capacity to 312 000 ML in 1994 (Kingsford 1999). Upstream of the junction with the Severn River, the Macintyre River remains unregulated and has a natural flow regime of

spring–summer flooding (Figure 2). The Macintyre River upstream of the Severn River has a mean annual discharge (1990–2004) of over 125 GL.

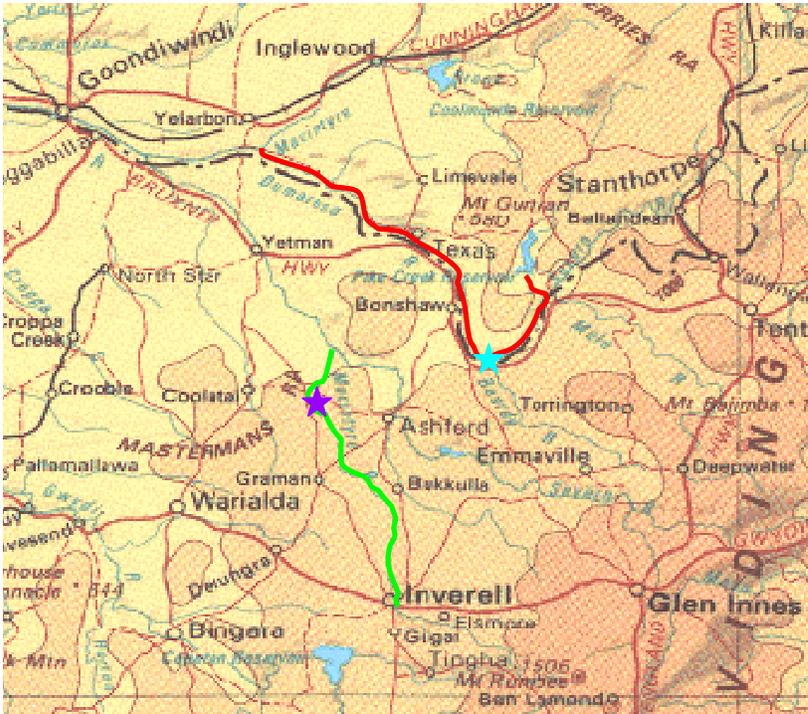


Figure 1 Map of the northern region study area with selected river sections highlighted: red line indicates regulated section of Dumaresq River downstream of Glenlyon Dam; and green line indicates unregulated section of Macintyre River downstream of Inverell; aqua star indicates the location of the Roseneath gauging station; and the purple star indicates the location of the Wallangra gauging station.

Northern region — Dumaresq River (regulated)

The Dumaresq River has been regulated since 1976, when Glenlyon Dam was built in its headwaters with a storage capacity of 253 000 ML. The Dumaresq joins the Macintyre River east of Goondiwindi (Figure 1). Water extraction from Glenlyon Dam — and a number of dams on both the Macintyre and Dumaresq rivers — and other private off-river storages have decreased the amount of water reaching Goondiwindi from about 95% in 1969 to 37% in 1995 (Abawi *et al.* 2001). Downstream of Glenlyon Dam, the Dumaresq River has a mean annual discharge of almost 260 GL, and the flow regime has been altered such that baseline flows throughout autumn–winter are higher than natural and the magnitude and frequency of flooding events is reduced (Figure 2).

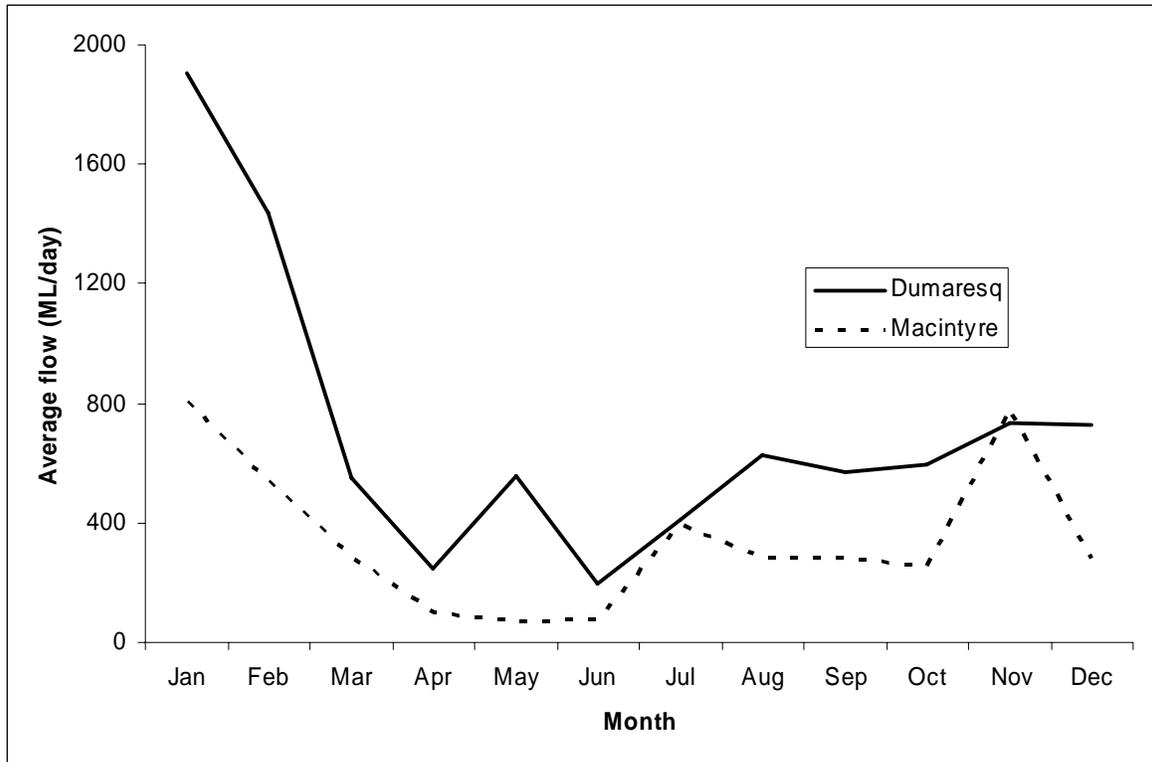


Figure 2 Average monthly flow rate (MLday^{-1}) of the Macintyre River at the Wallangra gauging station (1990–2004), upstream of the junction with Severn Creek, and the Dumaresq River at Roseneath gauging station (1990–2004), downstream of Glenlyon Dam.

Southern region — Ovens River system (unregulated)

The Ovens River system is one of the least regulated in the whole Murray–Darling Basin (Cottingham *et al.* 2001). The Ovens River catchment covers approximately 778 000 hectares and includes the Ovens, Buffalo and King rivers and associated tributaries.

Average annual discharge ranges from 200 GL for the King River to 1750 GL for the Ovens River (Cottingham *et al.* 2001). Climate and rainfall vary considerably throughout the catchment from the mountainous alpine region to the vast lowland river floodplains.

There are only two small impoundments in the system — Lake Buffalo on the Buffalo River and Lake William Hovell in the headwaters of the King River. The flow regime in the lower reaches is relatively natural: the effects on summer–autumn flows are minimal and there is virtually no effect on winter–spring flood events. In fact, the lower section of the Ovens River, downstream of Wangaratta, has been designated a ‘Heritage River’ because of its intact floodplain and riverine habitat (Cottingham *et al.* 2001).

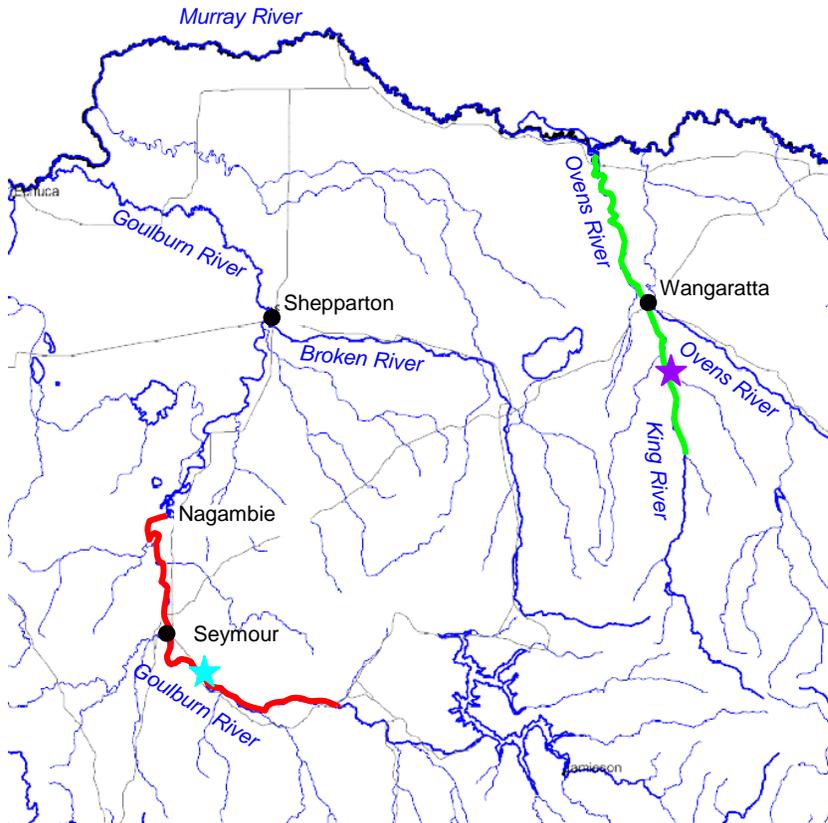


Figure 3 Map of the southern region study area with selected river sections highlighted: red line indicates regulated section of Goulburn River upstream of Lake Nagambie; green line indicates unregulated sections of Ovens and Kings rivers downstream of Edi; aqua star indicates the location of the Trawool gauging station; and the purple star indicates the location of the Docker Rd Bridge gauging station.

Southern region — Goulburn River (regulated)

The Goulburn River basin is the largest in Victoria, covering over 1.6 million hectares and with a mean annual discharge of 3040 GL (GBCMA 2004). Catchment terrain varies from alpine mountains along the Great Dividing Range to extensive lowland river floodplains in the north. Flow regimes in the mid Goulburn River region — between lakes Eildon and Nagambie — are significantly altered by regulation of Lake Eildon, a large reservoir with a capacity of 3390 GL. Operation of this reservoir has reduced flows during the winter–spring flood period and increased summer–autumn flows dramatically.

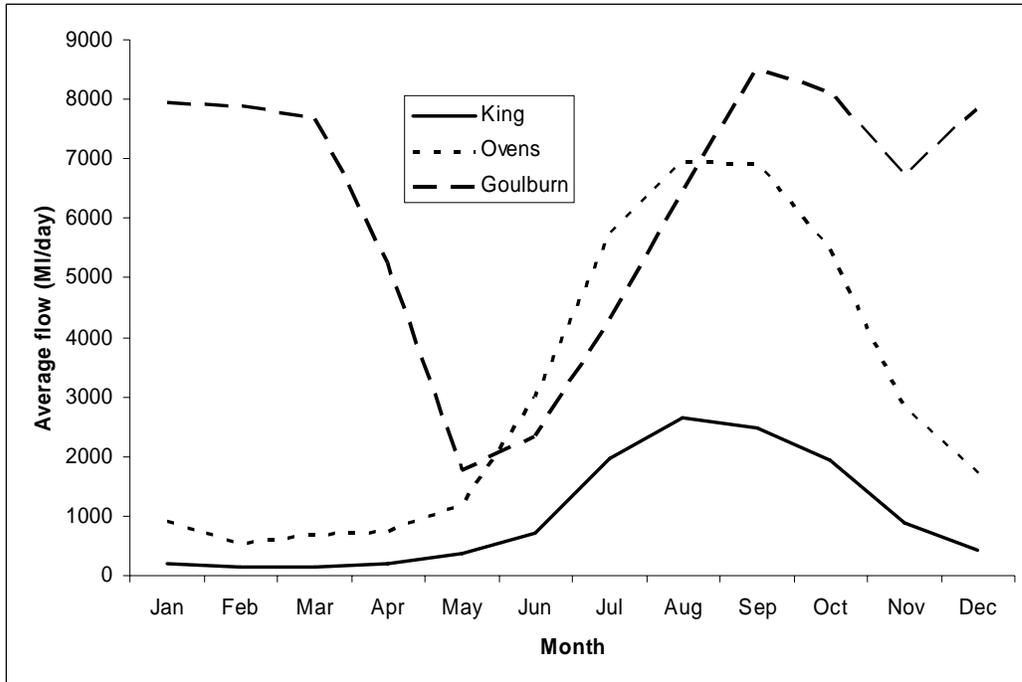


Figure 4 Average monthly flow rate (MLday⁻¹) of the King River at Docker Road Bridge gauging station (1974–2004), the Ovens River at Wangaratta gauging station (1987–2000), and the Goulburn River at Trawool gauging station (1990–2004).

Western region — Lindsay River (low flow)

The Lindsay River is an anabranch of the River Murray that leaves the river upstream of Lock 7. The upper reaches of the Lindsay River generally floods during spring, when flows reach 4000 MLday⁻¹ (Meredith *et al.* 2002), but has lower flows during the remainder of the year. For this reason, the flow regime may be comparable to the natural ephemeral regimes of the region. The average annual discharge of the Lindsay River was 465 GL between 1992 and 2005.

Western Region — River Murray (regulated)

The River Murray is a highly regulated river with a mean annual discharge of 11 000 GL (MDBMC 1995). Due to the immense size of the River Murray in comparison to the Lindsay River — over twenty-three times the annual discharge — it is probably an inappropriate choice as the regulated river in this study for the western section. It would be difficult to separate the effects due to altered flow regime from those of natural differences as a result of vast differences in river magnitude.

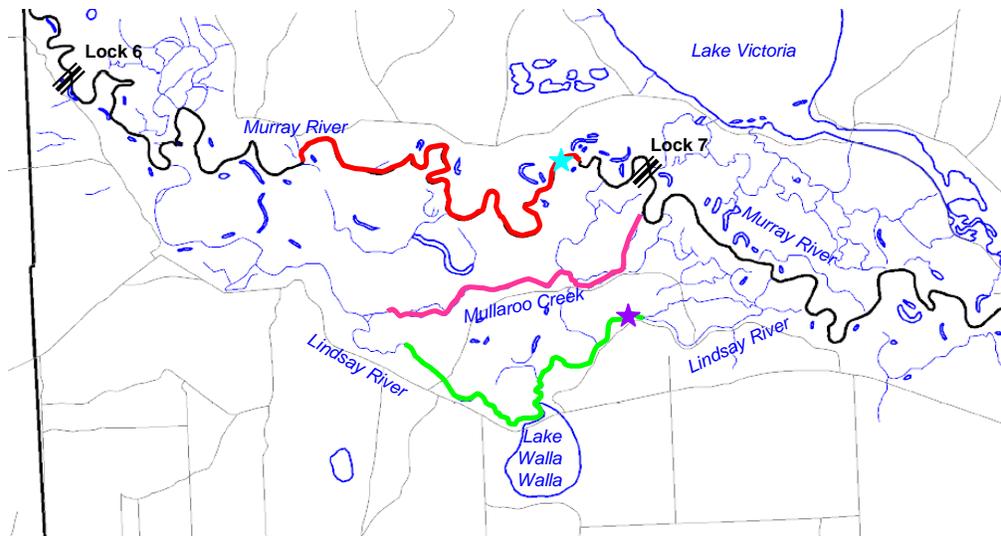


Figure 5 Map of the southern region study area with selected river sections highlighted: red line indicates regulated section of River Murray downstream of Lock 7; pink line indicates high flow section of Mullaroo Creek; green line indicates low flow section of Lindsay River; aqua star indicates the location of the River Murray gauging station; and the purple star indicates the location of the Lindsay River gauging station.

Western region — Mullaroo Creek (high flows)

Mullaroo Creek is a fast-flowing anabranch of the River Murray and maintains high flows for much of the year due to the influence of the weir pool upstream of Lock 7 (Meredith *et al.* 2002). It effectively floods every year — compared to one year in three naturally — and the seasonality of floods and high flows has changed from June–August to January–August, coinciding with irrigation demand (Sharpe *et al.* 2004). Average flows in Mullaroo Creek (785 MLday^{-1}) are greater than that of the upper Lindsay River (4 MLday^{-1} ; Sharpe *et al.* 2004).



Figure 6 Photographs of Lindsay River (left) showing lagoon with no flow, and Mullaroo Creek (right), showing consistent high flows.

5.1.3 Reaches

It is recommended that one section of lowland river be selected from each of the rivers to be investigated. For regulated rivers, the section with the most altered flow regimes would ideally be sampled — for example, the section of Goulburn River above Lake Nagambie shows considerably more change in flow regime than that below Lake Nagambie. It is important that the ‘unregulated’ river section selected simulates a natural flow regime, even though dams and abstractions will effect the hydrograph — for example, the King River approximates a natural flow pattern despite the effect of Lake William Hovell and abstractions for irrigation.

It is recommended that at least 2–3 replicate reaches be sampled within the chosen section of each river. This will provide some indication of the spatial variation in patterns that might confound temporal variation comparisons.

The spatial variation in Australian smelt abundance and distribution is unknown. If dispersal is limited, smelt may be spatially distributed as stationary local populations. Alternatively, if there is large-scale longitudinal dispersal of adults, larvae and juveniles, there may be spatial segregation of different aged individuals along the length of a river. For example, an upstream spawning migration of adults may produce a high density of eggs and early larval stages in upstream reaches followed by downstream dispersal of progressively older eggs/larvae/juveniles. For example, American shad (*Alosa sapidissima*) show a size/age-biased juvenile spatial distribution along the length of the Hudson River in America (Limburg 1996). If such a scenario existed (e.g. hypothetical scenario in Figure 7) and only one reach was sampled, it may incorrectly be assumed that the remainder of the river was the same. In addition, sampling replicate reaches along a single river, would indicate any spatial synchrony or cyclic patterns in recruitment dynamics that can be directly related to climatic variation (Grenouillet *et al.* 2001).

Spatial replication of sampling units within reaches

It is recommended that the sampling units be randomly allocated within each reach at each sampling time when possible, to avoid pseudo-replication. It is acknowledged that the

specific habitat requirement of some sampling methods (e.g. drift nets need to be attached to accessible snag structures within concentrated flow habitat of the reach) will limit the number of habitats available for selection.

Temporal replication of sampling units within reaches

Sampling should commence in September 2005 and should be repeated each month until at least March 2006.

Sampling of larvae by boat at night has proved most effective (Humphries et al. 2002), however, sampling by hand trawl will have to be carried out by day, because a pilot study showed that it was not possible, both for sampling efficiency and for safety reasons to do this at night. Sampling of epibenthic and planktonic prey should occur at the same time that larvae are sampled.

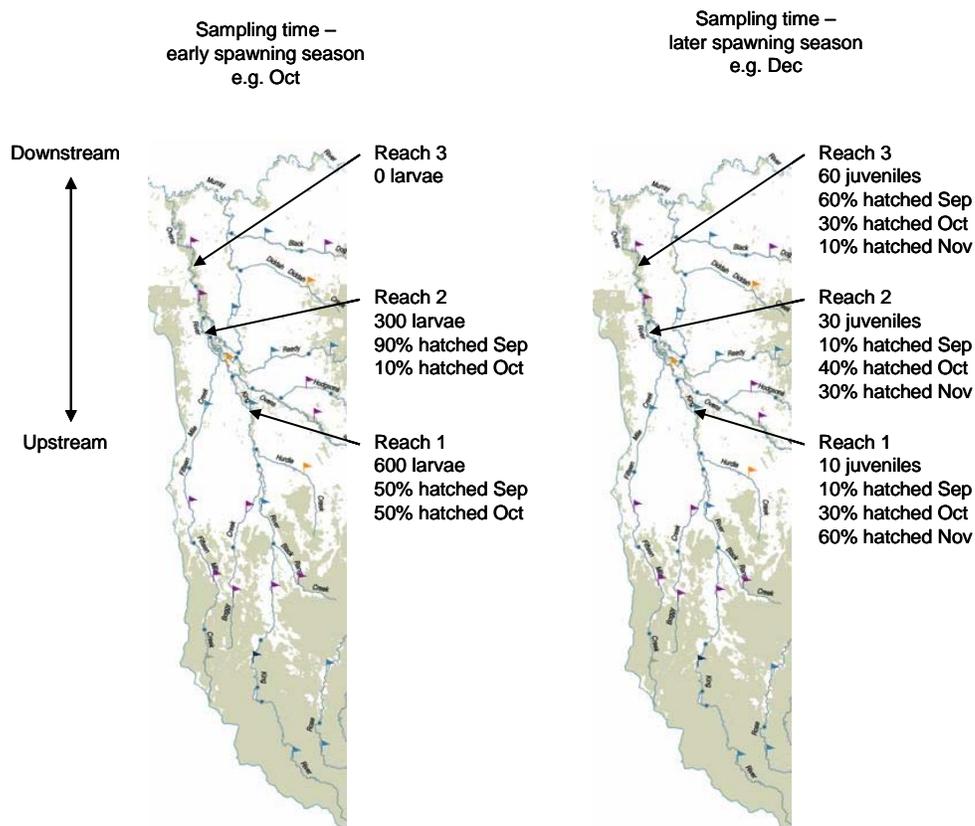


Figure 7 Hypothetical spatial distributions of smelt larvae and juveniles in the Ovens River in the instance of

spatial segregation of different aged individuals as a result of large-scale longitudinal dispersal, at two sampling times: (a) early in the spawning season, e.g. around October; and (b) later in the spawning season, e.g. around December.

5.2 *Field sampling*

5.2.1 Fertilised eggs, larvae and juveniles

Unless all early life stages are sampled effectively, accurate measures of abundance, growth and mortality needed to understand the recruitment dynamics processes will be difficult to establish.

The extent of smelt spawning would be ideally determined through temporal sampling of adults and examination of their maturity state; however, that method was considered unnecessarily destructive in respect to the potential gains. The ripe stage of live adults can be determined from live adults (Milton & Arthington 1985), but few would survive the procedure due to the fragile skin of smelt. Recently hatched larvae are to be sampled — as part of the regular sampling program — to provide a measure of the abundance of potential recruits, so their presence can be used to indicate recent spawning events and the duration of the spawning season. It should be noted that egg development takes 8–10 days and newly hatched larvae are very small (Milward 1966), so some differential egg or early larval mortality may have already occurred prior to the time of capture.

The hydrological conditions of the rivers are likely to vary extensively over the duration of the spawning and larval seasons, from extremely high flows and bulk discharges during and following flood events to very low constricted flows during the dry periods. The quantity of water to be sampled, therefore, will vary with each sampling trip. It is recommended that a variety of quantitative methods to sample larvae and juvenile fish be trialled in the early stages of the project and ineffective methods dropped as the project proceeds.

Methods could include:

- Seine nets — eight by two metres with a mesh size of 1 mm in the main panel and 500 µm in the bunt. Net dragged through known area of water during the day.
- Boat trawls — trawl net of mesh size 500 µm fitted with flow meter and towed through

the surface water behind a vessel (boat or kayak) for a set time period. Trawling should be conducted at night targeting the still and slower flowing parts of river reaches.

- Hand trawls — small trawl net with a mesh size of 500 μm , deployed from the edge of the channel and pulled through surface waters by hand for a set distance. Hand trawls should be collected during the day and target slackwater areas.
- Drift nets — mesh size of 500 μm and fitted with a flow meter in the opening, passively soaked for a set time period (from dusk until dawn) at the top and bottom of each reach for directional sampling.

Other non-quantitative methods may also be trialled as a back-up, such as:

- Sweep net electrofishing for a set time period.
- Light traps passively soaked for a set time period.

These methods have effectively captured larval and juvenile smelt in previous projects (Humphries & Lake 2000, Humphries et al. 2002, King & Crook 2002, Meredith et al. 2002, Humphries 1995).

5.2.2 Environmental variables

Battery-powered continuous data loggers should be deployed at each site at the start of the study to record temporal variation in water temperature. In addition, in each reach and on each sampling occasion, an instantaneous measure of temperature, conductivity, turbidity and dissolved oxygen should be collected with a Horiba, or similar instrument, to calibrate the data loggers and provide extra environmental information.

5.2.3 Food availability

To sample zooplankton abundances, plankton nets with a mesh size of 50 μm are to be deployed from the boat for a set time period during boat trawls. To sample epibenthic meiofauna, a small battery-powered pump attached to a long pole will be tapped over the surface of the littoral substrate until 20 l of water has been collected. The pumped material will pass through a net of mesh size 50 μm .

Certainly in times of low productivity will need to sample a large quantity of water to collect sufficient quantity of plankton for laboratory processing. Also need to ensure method of collection is quantitative with respect to quantity of water processed.

5.3 *Laboratory processing*

Fertilised eggs, larvae and juveniles

Samples will be sorted and eggs and fish identified under a low power dissecting microscope. We will primarily refer to Serafini and Humphries (2004) and McDowall (1996b) for identification of larvae, juveniles and adults.

The developmental stage of fish will be determined using Serafini and Humphries (2004). Lengths of fish will be measured to 0.1 mm.

The “characteristics of survivors” approach is made possible through otolith microstructure analysis, to retrospectively describe the distinctive growth characteristics of juveniles that did survive and infer the characteristics of those that did not from previously collected larval otolith samples. The primary objective will be to determine from which time of the breeding season the juveniles were originally spawned. This will be done by back-calculation of otoliths, assuming daily growth increments can be read. The following list of procedures will be investigated if we have time and may prove very informative.

For the analysis of otoliths of larvae, the following characteristics may be useful to determine the range of early growth patterns:

- back-calculated hatch date, usually indicated by a hatch mark near the nucleus of a sectioned otolith;
- otolith diameter at the hatching mark, which may be related to the size of larvae at hatching;

For the analysis of otoliths of juveniles, the above characteristics plus the following additional characteristics may be useful:

- widths of increments in the larval region of juvenile otoliths, to retrospectively

estimate prior larval growth rates of juvenile survivors;

- widths of increments in the recently deposited juvenile region of the otolith, to infer current growth rates of juvenile survivors from different hatch dates;
- or total diameter of otoliths divided by number of increments (age), to estimate average growth rates over the life of juvenile survivors from different hatch dates.

Food availability

Particulate organic matter analysis method of meiofauna biomass and size structure (Masson et al. 2004).

For a pilot study, the samples should be divided in half, with half kept for sorting by hand to compare with the destructive quantitative measure.

For one half of the samples: Filter the preserved sample through a series of four sieves with decreasing mesh size (500 μm , 200 μm , 100 μm and 50 μm) to obtain four size fractions (500–1000 μm , 200–500 μm , 100–200 μm and 50–100 μm) (Seda & Dostalkova 1996).

Re-suspend each fraction in 600 ml distilled water.

Filter sample through pre-ashed and pre-weighed (= filter weight) (GF/C (Whatman) glass fibre filters. Rinse with distilled water to remove any residual fixative.

Dry at 60°C for 24 h, desiccate and re-weigh using a micro-balance (= dry weight).

Ash at 500°C for 3 h and cool overnight.

Dry at 60°C for 3 h, desiccate and re-weigh using a micro-balance (= ash weight).

Zooplankton organic biomass in each size fraction = (organic carbon biomass: $\mu\text{g C}$)
= ash weight – dry weight
= ash free dry weight (AFDW) per litre

For the second half of the samples: Filter through a series of four sieves with decreasing mesh size (500 μm , 200 μm , 100 μm and 50 μm) to obtain four size fractions (500–1000 μm , 200–500 μm , 100–200 μm and 50–100 μm). Re-suspend each fraction in 600 ml distilled water. Then sort by hand and remove all zooplankton individuals from remaining phytoplankton and detrital matter. Retain the remainder as a separate “residue” sample. Sort zooplankton individuals to lowest taxonomic level possible to indicate potential species dominating each size fraction and count individuals of each.

Re-suspend residue samples in distilled water and treat as above.

This will provide an indication of the proportion of AFDW of non-zooplankton material retained in each of the size fractions.

Then for sampling procedure, just divide sample into half and do biomass calculations on half and fix the other half for taxonomic compositions later if necessary.

6 References

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