Impacts of Managed Flows on Fish Spawning and Recruitment

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Paul Humphries (CSU)
Glenn Wilson (UNE)
Adam Richardson (MDFRC)
Tanya Ellison (UNE)

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Impacts of Managed Flows on Fish Spawning and Recruitment


Murray–Darling Basin Commission
GPO Box 409
Canberra ACT 2601
Ph: (02) 62790100  Fax: (02) 62488053

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For further information contact:

<table>
<thead>
<tr>
<th>Dr. Paul Humphries</th>
<th>Adam Richardson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charles Sturt University</td>
<td>Murray–Darling Freshwater Research Centre</td>
</tr>
<tr>
<td>PO Box 789, Albury, NSW, 2640</td>
<td>PO Box 991, Wodonga, VIC, 3689</td>
</tr>
<tr>
<td>Ph (02) 60519920</td>
<td>Ph (02) 60582300</td>
</tr>
<tr>
<td>Email: <a href="mailto:phumphries@csu.edu.au">phumphries@csu.edu.au</a></td>
<td>E-mail: <a href="mailto:Adam.Richardson@csiro.au">Adam.Richardson@csiro.au</a></td>
</tr>
</tbody>
</table>


Cover image: (clockwise from top left) Dumaresq River; cross-section of Australian smelt otolith; Ovens River; Murray cod larvae; recruitment bottleneck (centre).

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EXECUTIVE SUMMARY

This study tested aspects of the ‘window-of-opportunity’ hypothesis (a variation on the ‘match/mismatch’ hypothesis), which attempts to relate the timing of production of prey to the timing of production of fish larvae and subsequent recruitment of fish. 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 spawning species will encounter a period when conditions are optimal for recruitment is greater than for larvae of brief spawning species. This study tested the ‘window-of-opportunity’ hypothesis in the context of fishes in lowland rivers of the Murray–Darling Basin, with the primary aim of comparing the estimated hatch date distribution of early stage larvae with that of juveniles at the end of the spawning period (recruits) for a protracted spawning species: Australian smelt. This species was chosen because previous work has indicated that it can spawn for many months each year, and, given that it is widespread in the Murray–Darling Basin, it is likely to occur in a range of river types. We also sought to compare and contrast the relative timing of spawning and recruitment of other native and alien fishes in rivers which have substantially regulated flows (flow-managed rivers) and in rivers which have mostly unregulated flows (non flow-managed rivers), with the premise being that river regulation alters the rearing environment for young fishes and thus may result in different patterns of spawning and recruitment between these two types of rivers. We chose three regions of the Murray–Darling Basin — the ‘Southern Region’ in the south-eastern part of the Basin (north-eastern Victoria); the ‘Lower Region’ in the lower, south-western part of the Basin (western Victoria); and the ‘Northern Region’ in the north-eastern part of the Basin (south-eastern Queensland) — to look for broad-scale patterns throughout this large area, and established paired rivers of similar geomorphologies, sizes, discharge volumes, and ones which would have likely had similar fish faunas prior to regulation. We also recorded physico-chemical variables — especially flow and temperature — and sampled food availability, to determine if there were any relationships between these variables and peak recruitment in the rivers sampled.

The specific aims of this study were:

- To determine whether recruitment is proportional or disproportional to the abundance of newly hatched larvae for a protracted spawner: Australian smelt.
- To determine whether spawning and recruitment are synchronous between flow-managed and non flow-managed rivers for fish in general.
- To determine whether spawning and recruitment are synchronous between years for fish in general.
- To determine whether timing of peak recruitment is synchronous for protracted and short spawners.
- To determine if environmental variables or food availability are correlated with peak recruitment of Australian smelt in a subset of rivers sampled.

Sampling of larval and juvenile fish was carried out at three reaches of the Ovens (non flow-managed) and Goulburn (flow-managed) rivers in the Southern region, at three reaches in the Lindsay River (non flow-managed) and Mullaroo Creek (flow-
managed) in the Lower region, and also in the Mole (non flow-managed) and Dumaresq (flow-managed) rivers in the Northern region of the Murray–Darling Basin, monthly during the breeding seasons of 2005/06 (between September/October and February) and 2006/07 (between August and February). Fish sampling was conducted using seines and hand trawls, mostly, although other (largely ineffective) methods were used in the first season. Seining was by far the most effective method and was used for the majority of analyses, except for descriptions of presence/absence and the occurrence of larvae and juveniles of species in rivers. Zooplankton samples were collected monthly from each reach. Temperature was logged hourly at all reaches, and duplicate physico-chemical variables were recorded each month. Hatching dates of early stage larvae (proto- and flexion larvae) were compared with those of juvenile recruits that survived until the end of the breeding season in 2006/07 using back-calculated ages estimated from otolith increments.

A total of 19 species — 14 native and 5 alien — was collected from the six rivers, and most showed evidence of spawning and recruitment in both years of the study. Because of small and patchy abundances, however, only Australian smelt could effectively be compared among all rivers, whilst a small suite of species could be compared for a subset of rivers.

Our results showed that overall faunal differences were regionally based. However, substantial differences in species composition were also found between pairs of rivers (flow-managed versus non flow-managed) within regions. These differences were consistent with previous work comparing unregulated and regulated systems. In addition to this, however, we found that in the Southern and Northern regions, common species tended to begin spawning earlier, and spawn for longer, in flow-managed rivers than in their non flow-managed counterparts. In the Lower Region the opposite was the case. The results, nevertheless, point to the fact that timing and duration of breeding of fish in rivers are likely influenced more by local differences in discharge regimes than broader climatic and geographic influences. The implication of these results is that fish are flexible and will probably respond to alterations to flow regimes if managers impose them.

We concluded that classifying fish as either brief or protracted spawners does not include the element of flexibility that was apparent in many of the species studied. Instead we suggest that fish be grouped into ‘brief’ spawners (ones that only spawn for one or two months), ‘flexible’ spawners (ones whose spawning period varies by river and by year), and ‘protracted’ spawners (ones that spawn consistently for more than two months). Only Gambusia holbrooki fitted the last criteria. Our results do not support the ‘window-of-opportunity’ hypothesis, because the premise upon which this is built was not met — we now classify Australian smelt as a ‘flexible’ spawner, and its success cannot be purely because of its supposed protracted spawning behaviour. However, it is important to recognize that our results were limited to the sampling methods we used, and not entirely consistent with other studies in similar areas, and so we encourage others to modify this classification as more data are collected.

Despite the lack of utility of the ‘window-of-opportunity’ hypothesis in explaining recruitment success, most species spawned over a long enough period to indicate that recruitment was not proportional to larval production. In other words, the hatching date distributions of early-stage larvae did not match that of juveniles at the end of the
spawning season. This was shown best for Australian smelt — in most cases the early hatching events resulted in relatively poor recruitment, and later hatchings resulted in relatively good recruitment. The only exception to this pattern was for the Ovens River, where early larvae and juvenile hatching distributions coincided.

Whereas timing and duration of spawning seemed to be influenced by local factors, timing of recruitment tended to be similar for rivers within regions, except for the Ovens/King and the Goulburn systems. Our preliminary comparisons of environmental variables with food availability suggested that temperature (positively) and discharge (negatively) influence production of the smallest size classes of zooplankton and that the first significant rise in zooplankton coincided with peak Australian smelt recruitment in the Ovens River.

Management recommendations from this project are:

- To classify native fish species in the Murray–Darling Basin based on their life history strategies, and to use these to select representative species for targeted management.
- To recognize that the small species in the Murray–Darling Basin are critical to this system’s function, and a greater emphasis on their study and management be made.
- To recognize that river regulation-effects of fish are overlayed — and sometimes subservient to — regional factors and to, therefore, manage Murray–Darling Basin rivers and fish faunas not as a single unit, but as a number of climatic and zoogeographic zones.
- To give high priority to amelioration of cold-water pollution in flow-managed rivers, as temperature depressions are likely to delay or prevent the increase in zooplankton biomass that may be critical to the recruitment of many species of fish.

Scientific recommendations from this project are:

- To develop and test other fish recruitment hypotheses, besides the ‘window-of-opportunity’ hypothesis in lowland rivers.
- To further investigate the timing of spawning and recruitment of Australian smelt in more flow-managed and more intact rivers in each region to increase our capacity to draw meaningful conclusions from patterns observed in the present study.
- To initiate a series of adaptive management experiments to investigate the feasibility to influence spawning time and duration of native fish species in flow-managed rivers through manipulation of temperature and flow in flow-managed rivers.
- To process the rest of the zooplankton samples for non flow-managed rivers that were investigated as part of this study.
- To initiate adaptive management experiments in which discharge and temperature are manipulated in flow-managed rivers, to determine if it is possible to influence zooplankton production to levels that are critical for fish recruitment.
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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 (MDBC Native Fish Strategy, 2003). Attempts to sustain fish populations through re-stocking programs have met with variable success. A more effective long-term strategy for population sustainability 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 larval and juvenile fish (Fuiman & Werner, 2002).

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 limiting factor driving the continued low population size and more restricted distributions of a number of species. Establishing the appropriate environment that would provide suitable conditions for recruitment remains a challenge but, ultimately, is likely to be the only long-term means of sustaining native fish populations.

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. 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 one or more key resources 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. Large year-to-year fluctuations in recruitment are a likely response to short term changes in the environment. For most species, a key resource for survival is likely to be the availability of food, which is likely to exhibit considerable variation in response to climatic variation.

Mortality rates 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 is largely determined by the growth rates achieved during each life history stage, and 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 anthropogenic, 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 into or away from areas of high food concentration or predation, or restrict access to food through poor connectivity between spawning and nursery areas — the main river channel and floodplain wetlands, for example.

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. River regulation and flow alteration have undoubtedly played a role in the process of decline in abundance and distribution of Australia’s, and the worlds’, freshwater fishes (Lloyd et al., 2003; Lytle & 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 — Australian smelt (Retropinna semoni) and flathead gudgeon (Philypnodon grandiceps), for example (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 their life history strategies. They also match well the description of opportunist species in a three-way classification of life history strategies of South American freshwater fishes (Winemiller, 1989). 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.

There are several hypotheses that relate recruitment of fish to maternal and environmental conditions in freshwater. Generalist species, such as the opportunistic Australian smelt and flathead gudgeon, may be able to take advantage of a range of conditions, feed and grow well when young, and, consequently, fare better than more specialist species. The ‘bigger-is-better’ 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 with alternative reproductive strategies — some individuals delay maturity to return to spawn at a much larger size than others. The flood pulse concept contends that periodic floodplain inundation brings a burst of productivity to the main
river channel (Junk *et al.*, 1989; Bayley, 1991) and that this productivity is important for the recruitment of fish (the flood recruitment hypothesis of Harris & Gehrke, 1995). The relevance of this hypothesis has been questioned in recent years (Humphries *et al.*, 1999; King *et al.*, 2003) and it has been demonstrated that there is an abundant source of prey in the epibenthic zone of the main channel for larval fish food (King, 2004a). However, it may still be pertinent to long-lived species which only need to recruit sporadically. Furthermore, recent results from the flooding of Barmah Forest on the Murray River seem to confirm that some species, notably golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*) spawn in response to increases in flow and recruit if this occurs at an appropriate time (King, unpublished data).

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). This hypothesis is based on the contrast between the relatively fixed time of spawning for many temperate fish species — controlled by predictable biotic factors, such as 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 a limited period or window-of-opportunity — usually in the order of a few days — within which to locate appropriate food before starvation occurs. Especially at this time, and probably 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 (Munn & Brusven, 2004), 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 low recruitment for those species in that year. Recruitment undoubtedly varies significantly between years under natural flow conditions, although altered flow regimes may have increased the frequency of mismatch events, leading to an overall reduction in recruitment success and declines in population abundances.

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 Australian smelt and flathead gudgeon 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. This development of the ‘match/mismatch’ hypothesis we call the ‘window-of-opportunity’ hypothesis’ (Fig. 1.1).

![Diagram showing relative numbers of hatching/recruits over time within the year.](figure1.png)

**Figure 1.1:** Schematic representation of the ‘window of opportunity hypothesis’.

There are some essential differences between the ‘match/mismatch’ hypothesis and the ‘window-of-opportunity’ hypothesis. 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.*

The ‘window-of-opportunity’ hypothesis could be considered as a variation of the ‘match/mismatch’ hypothesis, since the latter does not specifically consider the duration of spawning, but, more generally, the timing of spawning relative to the timing of the available food.

We decided to investigate aspects of the ‘window-of-opportunity’ hypothesis in the context of fishes in lowland rivers of the Murray–Darling Basin, with the primary aim
of comparing the estimated hatch date distribution of early stage larvae with that of recruits — juveniles at the end of the spawning period — for one *protracted* spawning species: Australian smelt. This species was chosen because previous work has indicated that it can spawn for many months each year, and, given that it is widespread in the Murray–Darling Basin, it is likely to occur in a range of river types.

In addition, as stated above, it is thriving under regulated conditions. We also sought to compare and contrast the relative timing of spawning and recruitment of native and alien fishes in rivers which have substantially regulated flows and in rivers which have mostly unregulated flows, with the premise being that river regulation alters the rearing environment for young fishes and, as such, may result in different patterns of spawning and recruitment between these two types of rivers. We chose three regions of the Murray–Darling Basin to look for broad-scale patterns and established paired rivers of similar geomorphologies, sizes, discharge volumes, and ones which would have likely had similar fish faunas prior to regulation. We also recorded physicochemical variables — especially flow and temperature — and sampled food availability to determine any relationships between these variables and peak recruitment in the rivers sampled.

The specific aims of this study were:

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- To determine whether spawning and recruitment are synchronous between years for fish in general.
- To determine whether timing of peak recruitment is synchronous for protracted and brief spawners.
- To determine if environmental variables or food availability are correlated with peak recruitment of Australian smelt in a subset of rivers sampled.
2 MATERIALS AND METHODS

2.1 Study area and study reaches

This study was undertaken within three broad regions of the Murray–Darling Basin — the Ovens/King and Goulburn catchments in the upper Murray sub-basin (Southern Region), the Lindsay and Mullaroo anabranch system in the lower Murray sub-basin (Lower Region) and the Mole and Dumaresq rivers in the upper Darling sub-basin (Northern Region) (Fig. 2.1). Two river systems were selected within each region — one that historically receives regulated flows and a second whose flow is largely, or entirely, unregulated. Three reaches were selected in each river system in September 2005, and the reach length and distance from the upstream-most reach in each river calculated using Google Earth Pro (Table 2.1). Three reaches were selected to take into account variation within each river — but there was no intention to compare results among reaches — and to integrate samples for all reaches within rivers. The multiple reaches also meant that if larval or juvenile fish moved out of one reach they had a good chance of ending up in a similar environment to one of the other reaches being sampled. This, however, was a dubious concern, given the general lack of movement of Australian smelt (Hughes & Crook, 2006) — only three juveniles were found in all of the drift samples collected in the Northern Region.

Figure 2.1: Map of the Murray–Darling Basin, showing the three study regions and rivers. Map courtesy of the Murray–Darling Basin Commission.
In the Southern Region (Fig. 2.2), the Ovens/King river system was selected as the non flow-managed system and the Goulburn River was selected as the flow-managed system. The Goulburn River receives regulated flows from several major weirs and dams upstream, including Lake Eildon. Three reaches on the largely unregulated Ovens/King river system were chosen (Peechelba, Warby Range Road and Oxley) — these reaches were chosen because of the similarities in geomorphology, substrate and pattern of flow regime. Three reaches on the Goulburn River (Northwood Road, Telegraph Road and Hubert Miller’s property) were chosen because of the similar altitude to the reaches in the Ovens/King river system, and their shared similarities in geomorphology, substrate and flow regime. The major difference in flow regime between the Ovens/King and the Goulburn systems is that the former typically experiences a lengthy period of low flow over the summer/early autumn period, whereas the latter experiences high flows over much of the same period.

Figure 2.2: Map showing location of the three reaches in the Ovens/King and Goulburn systems in the Southern Region.

In the lower region (Fig. 2.3), the Lindsay River was selected as the non flow-managed river and the nearby Mullaroo Creek was used as the flow-managed system. Three reaches were chosen along the upper Lindsay River. The three reaches on Mullaroo Creek were chosen because of their similar altitude, geomorphology and substrate to the Lindsay River, and because this system differed considerably in flow regime to the upper Lindsay River. Preliminary investigations suggested that the target species, Australian smelt, was present. The major difference in flow regime between the Lindsay and Mullaroo systems is that the former typically experiences a
protracted period of low flow over the summer/early autumn period, whereas the latter experiences a protracted period of high velocity current over much of the same time. The actual discharge is similar for the two systems, but the steepness of the gradient of the Mullaroo means that velocities are always greater than for the Lindsay.

![Map showing location of the three reaches in the Lindsay and Mullaroo systems in the Lower Region.](image)

Figure 2.3: Map showing location of the three reaches in the Lindsay and Mullaroo systems in the Lower Region.

In the Northern Region (Fig. 2.4), the Dumaresq River was selected as the flow-managed river, and receives regulated flows from Glenlyon Dam on the upstream tributary, Pike Creek. The nearby Mole River was selected as the non flow-managed river. The choice of the Mole, rather than the Macintyre (as in the Scoping Study) was due to preliminary investigations having revealed that the Macintyre did not contain the species needed (especially Australian smelt) to make the study viable as a multi-regional comparison. The Mole River experiences peak discharge over summer but flow remains relatively stable throughout this period, with rainfall events causing only relatively short-lived fluctuations. Throughout winter and spring, flows in this river tend to be low. The Dumaresq, on the other hand, experiences a more erratic discharge pattern due to releases from Glenlyon Dam — releases which produce large velocity and depth changes within the channel. Flows from Glenlyon Dam are often artificially high throughout late winter and spring (in particular), due to irrigation releases, although these will typically extend into February. In summary, the differences in discharge between these rivers are more in terms of spring flows and the daily variability within spring-summer peak intervals.
Figure 2.4: Map showing location of the three reaches in the Mole and Dumaresq Rivers in the Northern Region.
Table 2.1: Summary of study reach characteristics for the six study river systems.

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<td>Goulburn (flow-managed)</td>
<td>Hubert’s</td>
<td>HUB</td>
<td>S 37°06.883', E 145°13.259'</td>
<td>960</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Telegraph Road</td>
<td>TEL</td>
<td>S 37°04.868', E 145°09.164'</td>
<td>1220</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Northwood Road</td>
<td>NOR</td>
<td>S 36°52.989', E 145°06.671</td>
<td>930</td>
<td>38</td>
</tr>
<tr>
<td><strong>Lower</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lindsay River (non flow-managed)</td>
<td>Upper Lindsay 1</td>
<td>UL1</td>
<td>516416, 6219236</td>
<td>550</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Upper Lindsay 2</td>
<td>UL2</td>
<td>514968, 6220742</td>
<td>700</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Upper Lindsay 3</td>
<td>UL3</td>
<td>513504, 6221350</td>
<td>500</td>
<td>3.2</td>
</tr>
<tr>
<td>Mullaroo Creek (flow-managed)</td>
<td>Mullaroo 1/2</td>
<td>MC1/2</td>
<td>520918, 6225851</td>
<td>750</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mullaroo 3</td>
<td>MC3</td>
<td>520140, 6225233</td>
<td>620</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Mullaroo 4</td>
<td>MC4</td>
<td>518795, 6226030</td>
<td>650</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>Northern</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mole (non flow-managed)</td>
<td>Six-Mile Crossing</td>
<td>SMC</td>
<td>S 29°03’05.00”, E 151°42’01.54”</td>
<td>1326</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Bellanboe</td>
<td>BEL</td>
<td>S 29°02’40.72”, E 151°31’20.04”</td>
<td>447</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Mingoola</td>
<td>MIN</td>
<td>S 28°59’45.28”, E 151°31’20.04”</td>
<td>498</td>
<td>25</td>
</tr>
<tr>
<td>Dumaresq (flow-managed)</td>
<td>Haystack</td>
<td>HAY</td>
<td>S 29°10’33.84”, E 151°22’32.73”</td>
<td>1004</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Bonshaw</td>
<td>BON</td>
<td>S 29°03’46.26”, E 151°17’09.51”</td>
<td>641</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Texas</td>
<td>TEX</td>
<td>S 28°52’49.79”, E 151°10’34.32”</td>
<td>1091</td>
<td>52</td>
</tr>
</tbody>
</table>
2.2 Field methods

Field sampling was undertaken monthly throughout the spring–autumn period of peak fish spawning activity, from September or October 2005 to February 2007. For the Northern and Lower regions, sampling was undertaken from October 2005 to February or March 2006 — February was missed in the Northern Region — and for the Southern Region, from September 2005 to February 2006. In all regions, sampling was also undertaken between August 2006 and February 2007.

Sampling was carried out using a range of gears designed to catch larval and juvenile stages of a variety of fishes. Two techniques were also used to sample zooplankton assemblages likely to be key prey items for larval fishes. For northern reaches, unavoidable delays with gear acquisition meant that only a limited number of gears could be used in October 2005, though sampling on each subsequent field trip matched that used in the other two regions in both years. The various fish-sampling methods were:

*Seine net.* An 8 × 2 m net, constructed of 2 mm mesh with a 500 µm cod-end, was used monthly at each reach, with five replicate hauls taken per reach in 2005/06 and a minimum of five and a maximum of eight hauls taken per reach in 2006/07. The variable number of hauls taken in the 2006/07 season was because it was decided from the previous season’s results, that seine netting was the most effective for collecting Australian smelt larvae and juveniles, and so as many seine hauls were taken per reach as was practicable — in regards to both time and accessibility to appropriate sampling habitats within a reach. This was deployed by two operators, one who walked out from the bank and then, when the net was at full stretch, walked in an arc back to the bank. Locations where seines could be carried out were limited, but randomized wherever possible. Maximum depth and area fished were estimated. This technique collected both larvae and juveniles/adults of a range of species.

*Drift net.* Two 1.5 m drift nets of 500 µm mesh with a circular 0.4 m diameter opening were deployed at each reach in the late afternoon and left overnight to sample drifting larval fishes. These were collected as soon as practical the next morning. These were set facing perpendicular to the current, and tethered either between two ‘star pickets’ or behind a snag or submerged tree branch. A General Oceanics analog flow meter was used to calculate the volume of water filtered by each net. One net was set at the upstream and downstream end of each reach.

*Throw net.* A 500 µm net of 1 m length and 300 mm diameter conical opening was used to target pelagic larval stages by day. This net was attached to a 5 m rope and was thrown and retrieved from the bank 5 times per replicate haul. Five random, replicate samples were taken from each reach.

The sampling methods for pelagic zooplankton and epibenthos were:


**Boat trawl.** A 53 µm meshed conical net with a 250 mm diameter opening was used to collect pelagic zooplankton prey items. Five replicates of 30 seconds duration were taken randomly at each reach, whilst driving the boat at a constant speed, zig-zagging across the river.

**Zooplankton pump.** A small bilge pump connected to a 12 volt battery and a 2.5 m length of clear 15 mm diameter hose was used to sample zooplankton taxa along the bank. Five random, replicates were obtained from each reach, with each sample comprised 20 litres of pumped water, filtered through a 53 µm trawl net.

StowAway Onset® temperature loggers were deployed at each reach, and set to record the water temperature at hourly intervals, in parts of the river which we hoped to remain wetted throughout the study period.

All samples from each gear-type were preserved in 95% ethanol upon capture, with all further processing being undertaken in the laboratory.

### 2.3 Laboratory methods

In the laboratory, fish specimens were sorted from other debris and invertebrate fauna and preserved in 95% ethanol. Fishes were identified to species wherever possible (Allen et al., 2002), and larvae identified and staged (protolarvae, flexion, post-flexion, meta larvae) using Serafini & Humphries (2004). Other fish were classed as either juveniles or adults.

### 2.4 Otolith processing and analysis

All larval and a subset of juvenile fish were aged from daily increments present on sagittal otoliths (Fig. 2.5) using standard methods (Tonkin et al., 2008a). Both sagittae were removed, placed on a glass slide in Crystalbond® adhesive and one otolith was polished using fine lapping film, until a flat surface was achieved and increments were clear from the margin through to the hatching check near the primordium. Otoliths were examined under a compound microscope, photographs taken of every otolith and images captured on image analysis software. Maximum length (the longitudinal axis) and width (at right angles to maximum length) of each otolith were measured using the software. Increments were counted directly from the otolith and from the image and an average taken. All otoliths were read by two readers — the second reader ignorant of the previous estimate of age. If the two estimates differed by more than 5%, the otolith was discarded.

Validation of daily increment formation and hatch checks for Australian smelt has been shown by Tonkin et al. (2008a). McCasker (unpublished data) has demonstrated likewise for carp gudgeons and unspecked hardyheads, that of flathead gudgeon by Serafini & Humphries (unpublished data), but validation of daily increments and hatch checks for gambusia have not been established to date.
Length/age relationships were explored for each species. Linear, von Bertalanffy and Gompertz equations were fitted to all fish from each species analysed and from each river in the 2006/07 spawning season (as per Tonkin et al., 2008b). Gompertz and von Bertalanffy both gave excellent fits to the data, but, because all fish from all months were used, this effectively gives an approximation of growth over the larval and juvenile periods, thereby averaging the effect of temperature differences on growth rate. This is a problem, when the aim is to backcalculate ages from length for fish from individual months, where water temperature differences occur. For this reason, fish from individual months were used instead. In most cases, Gompertz and von Bertalanffy models were not able to be resolved, usually because of the relatively small range of lengths of the sample of fish; however, linear relationships generally fitted well and provided reliable estimates of age from length. Log-transforming the data did not improve the fit. In the cases where there were not enough fish to enable a good linear model, or the model for a particular month was non-significant, fish from the adjacent month with the closest mean water temperature were included in the data set and the linear relationship calculated. For example, there were only three juvenile Australian smelt collected from the Lindsay River in February 2007, and so they were combined with those from January, and a length-age relationship constructed. All length-age equations are shown in Appendices.

Back-calculated hatch dates were estimated for the remaining juveniles of each species analysed using the linear length-age relationships described above. Estimated ages were rounded to the nearest whole number and then hatch dates determined as the date of
capture minus estimated age. Frequency histograms of hatch dates were constructed in a similar manner to that of length.

Finally, hatch date distributions for early stage larvae (protolarvae and flexion larvae) were compared with hatch date distributions of recruits (juveniles from February or, if there were not enough individuals, from January and February combined) to determine if proportional or disproportional recruitment had occurred. In other words, for proportional recruitment, both distributions should be similar; whereas for disproportional recruitment, the distributions will be offset. Hatch date distributions are presented as relative frequencies.

### 2.5 Food availability

Plankton and epibenthic samples were processed only for the Ovens River at Warby Range Road and for the Goulburn River at Northwood Road for the 2006/07 spawning season. However, this allows for a comparison of zooplankton biomass in the heavily regulated Goulburn with the largely unregulated Ovens Rivers.

The particulate organic matter analysis method of meiofauna biomass and size structure of Masson et al. (2004) was largely followed. In brief, each sample was divided in half, and both halves were filtered through a series of four sieves with decreasing mesh size (500 µm, 212 µm, 106 µm and 53 µm) to obtain four size fractions (+500 µm, 212–500 µm, 106–212 µm and 53–106 µm) (Seda & Dostalkova, 1996). Each fraction was then re-suspended in 600 ml distilled water and zooplankton removed from one of the pairs of fractions under a dissecting microscope. Zooplankton of each size-class were then filtered through pre-ashed and pre-weighed GF/C Whatman glass fibre filters (= filter weight) and rinsed with distilled water to remove any residual fixative. Samples (filters and zooplankton) were dried at 60°C for 24 h, desiccated and re-weighed using a microbalance (= dry weight). Samples (filters and zooplankton) were then placed in a muffle furnace at 500°C for 3 h, desiccated and re-weighed using a microbalance (= ash free dry weight). The difference between the final two weights (dry weight – ash free dry weight) gave the organic carbon biomass of the zooplankton. The other fraction was preserved for later taxonomic resolution.

To investigate relationships between discharge and temperature and biomass of zooplankton and epibenthos, simple regressions were carried out using the mean of the previous week’s temperature and discharge and the mean of the previous month’s temperature and discharge versus dry weight of zooplankton and epibenthos. This was investigated for each size fraction. Once it was established that ‘mean of previous month’ had the best fit for temperature and discharge, a stepwise forwards multiple regression analysis was conducted for each size fraction. Discharge and temperature were both log_{10} transformed, but data for dry weight of zooplankton or epibenthos did not need to be transformed. Parameters were included in the model that best fitted the data. Analyses were performed in SPSS v14.
2.6  Environmental data

Daily flow data were obtained for each study river from either Thiess Environmental (Victoria), the Victorian Department of Sustainability (http://dse.vic.gov.au/waterdata), or the New South Wales Department of Water and Energy (http://waterinfo.nsw.gov.au) (Table 2.2).

Table 2.2: Summary of flow gauges used to examine hydrological variability in each study river system throughout the study period.

<table>
<thead>
<tr>
<th>Region</th>
<th>Stream</th>
<th>Flow gauge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern</td>
<td>Ovens and King rivers</td>
<td>King River @ Docker Road Bridge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovens River @ Peechelba</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovens River @ Wangaratta</td>
</tr>
<tr>
<td></td>
<td>Goulburn River</td>
<td>Goulburn River @ Seymour</td>
</tr>
<tr>
<td>Lower</td>
<td>Lindsay River</td>
<td>Lindsay River upstream of Walla</td>
</tr>
<tr>
<td></td>
<td>Mullaroo Creek</td>
<td>Murray River, below Rufus River</td>
</tr>
<tr>
<td>Northern</td>
<td>Mole River</td>
<td>Mole River @ Donaldson</td>
</tr>
<tr>
<td></td>
<td>Dumaresq River</td>
<td>Dumaresq River @ Roseneath</td>
</tr>
</tbody>
</table>

Duplicate spot temperature, conductivity, pH and turbidity measurements were made at each reach in each month of sampling, using either an Horiba Water Quality Checker 10® or a Hydrolab Quanta®.

2.7  Data analysis

Three reaches were chosen in each river to account for variation within each river. There was no intention to compare among reaches within rivers. Thus, for all analyses, fish are pooled by river. No statistical assumptions were violated by this approach, because numbers of larvae were not calculated per unit sampling effort — per seine, for example (see below).

Although sampling included drift nets, hand trawls and seine nets, the results from this last method included most of the fish collected during the study. Thus, virtually all analyses and results will be solely based on seine net data. The only exceptions are the presence of species and life stages (Table 3.1), and the duration of occurrence of larvae and juveniles (Fig. 3.7), when all methods were included to be comprehensive. In the duration of occurrence figure, juveniles that were clearly from the previous year’s spawning season were not included.

A non-metric multidimensional scaling analysis was performed on presence/absence data for larvae and juveniles of species present in reaches within rivers and regions for the
2005/06 and 2006/07. The Bray-Curtis dissimilarity measure was used. The PRIMER® package was used to carry out the procedures.

This study primarily focused on Australian smelt — our target species — because of its presumed protracted spawning behaviour, and because its distribution included the three regions of the Murray–Darling Basin that formed the basis of this study. We had also intended to include a subset of species to analyse further for patterns of spawning and recruitment. The subset was to include selected protracted spawners and brief spawners, but, in the end, we were forced to choose only those species that were sufficiently abundant for further analyses. This limited comparisons between rivers within regions, and between regions. We were able to compare the timing of spawning for carp gudgeons, flathead gudgeons, gambusia, hardyheads, Murray cod and common carp for several rivers and regions. We were able to analyse the timing of recruitment for carp gudgeons in the Ovens and Lindsay rivers, for flathead gudgeons in Lindsay River and Mullaroo Creek, for gambusia in the Ovens and Goulburn rivers, and for hardyheads in Lindsay River and Mullaroo Creek.

The presence of protolarvae (first stage larvae) was used as a surrogate for the duration of spawning, although it is recognized that protolarvae are not zero day’s old — the mean age of protolarval smelt was 7.4 days. Since protolarvae indicated spawning, and since all species included in this study produce many hundreds of eggs — and, hence, many larvae — each spawning, it was considered that to calculate mean number of protolarvae per seine net would potentially give an inaccurate estimate of abundance. It may be that the seine collected a shoal of the larvae from one pair of adults, or many pairs. Instead, the number of seines which contained a species was expressed as a percentage of the total number of seines carried out. The results from this approach were compared with calculating mean abundances, and comparable results, indicating peak spawning, were achieved.

Length-frequency histograms (standard length) were constructed from all larvae and juveniles of Australian smelt from each river and region. Length-frequency histograms for other species were constructed only for those rivers for which it was considered worthwhile to analyse further (as stated above). Analyses were performed in MS Excel and SPSS v 14.
Figure 2.6: Ovens River at Warby Range Road.

Figure 2.7: Goulburn River at Hubert Miller’s.
Figure 2.8: Upper Lindsay River at site #2.

Figure 2.9: Mullaroo Creek originates at its divergence from the Murray River.
Figure 2.10: Mole River at Mingoola.

Figure 2.11: Dumaresq River at Haystack.
3 RESULTS

For the rest of this report, the names of the rivers/creeks are abbreviated such that OV = Ovens, GO = Goulburn, LI = Lindsay, MU = Mullaroo, MO = Mole and DU = Dumaresq.

3.1 Environmental variables

Historical discharge regimes, daily discharge during the study period, as well as temperature patterns, all indicated that the pairs of rivers in each region are not uniform (Figs. 3.1–3.3). However, in each case, during spring and summer, discharge was much greater in the three flow-managed rivers than in the non flow-managed (unregulated) rivers. For the Southern Region, the difference in discharge historically is not so great in spring, but is very different from November onwards (Fig. 3.1). For the Lower Region, the difference is most marked in early spring, and then from January onwards (Fig. 3.2). For the Northern Region, the Mole River always has lower discharge than the Dumaresq, but this difference increases from December onwards (Fig. 3.3).

The Southern Region discharge in the 2005/06 season was high for both rivers in the spring. Indeed, OV flooded during this period and then receded to low levels for the rest of the study period (Fig. 3.1), whereas irrigation releases in GO kept the discharge of this river high throughout the summer. During the 2006/07 season, discharge was low in OV, but moderate throughout for GO. Discharge patterns were similar in the Lower Region in each spawning season, with MU particularly high during the spring and summer of 2005/06 and moderately high in 2006/07 (Fig. 3.2). The LI discharge was high in spring of 2005, but declined to low levels for the rest of the study period. By contrast, discharge patterns were very similar for the two Northern Rivers in the first season (Fig. 3.3), and in the spring of the second season. However, discharge was very low throughout the summer of 2006/07 in MO, but releases in DU kept the discharge in this river high during this time.
Figure 3.1: Seasonal daily discharge patterns in the Goulburn, King and Oven rivers. (a) Long-term variability from the Goulburn River at Seymour (1975-2007, solid line), King River at Docker (1974-2007, dashed line) and Ovens River at Pechelba (1990-2007, dotted line). (b) Daily variability in the Goulburn River at Seymour (solid line) and Ovens River at Wangaratta (dotted line), July 2005 to March 2007.
Figure 3.2: Seasonal daily discharge patterns in the Mullaroo Creek and Lindsay River. (a) Long-term variability from the Mullaroo Creek (2001-2007, solid line) and Lindsay River (1992-1994, 1998-2001, 2004-2005, dotted line). (b) Daily variability in the Murray River (solid line, left axis) and Lindsay River (dotted line, right axis), July 2005 to March 2007. Note: there is no gauge in the Mullaroo Creek, so discharge follows the same pattern as the Murray River, just upstream, but is, of course, considerably lower.
Temperature patterns were generally very similar for the pairs of rivers over the study period (Fig. 3.4), except for the summer of the 2005/06 season, when temperatures in GO were much lower than those in the Ovens/King system. The temperatures in this first season reached almost 30 °C in OV, whereas they generally fluctuated between 16 and 20 °C in GO. This was not, however, the case in the 2006/07 season, when temperatures for this pair of rivers were very similar. It must be noted, though, that temperatures for the Ovens/King system for this season are from the King River because the loggers at each of the OV reaches were either stolen or lost — however, comparison of spot temperatures in OV and the King River showed that they were indeed similar.
Spot measurements of dissolved oxygen and pH showed little consistent differences between pairs of rivers, although conductivity was typically higher in the Ovens than GO, and in LI compared with MU, and in DU compared with MO (Fig. 3.5). Turbidity was also consistently higher in OV than in GO.
3.2 Fish species and life stages collected

A total of 19 species — 14 native and 5 alien — were collected from the three regions during the study (Table 3.1). Of these, only Australian smelt, common carp and gambusia were common to all rivers in all regions. Five species — Australian smelt, carp gudgeons, unspecked hardyhead, common carp and gambusia — were common to all three regions, but not rivers. Species unique to the Southern Region were Galaxias spp. and brown trout. There were no species unique to the Lower Region. Species unique to the Northern Region were purple-spotted gudgeon, olive perchlet, freshwater catfish, spangled perch and goldfish.
The Northern Region consistently contained the most species — 13 and 12 species collected from DU in 2005/06 and 2006/07, respectively (Table 3.1). LI was the most species-poor river in 2005/06 (seven species collected) whereas GO was the most species-poor in 2006/07 (five species collected). GO had the lowest proportion of native species (50% and 60% in the two seasons, respectively), whereas MU and MO were similar (≈ 80%). The total number of species or number of native species were not consistent when comparing flow-managed rivers with non flow-managed ones, and only the Southern Region showed the predicted situation — fewer total species and fewer native species in the heavily flow-managed GO than in the non flow-managed OV.

An MDS on combined larvae and juveniles presence/absence data, grouped reaches primarily by Region, and then by river type (flow-managed vs non flow-managed), although OV reaches were sometimes closer to Lower reaches, than they were to the GO reaches (Fig. 3.6).

Figure 3.6: Non-metric multidimensional scaling analysis of presence/absence data for fish larvae and juveniles combined, for each of the reaches in the Southern, Lower and Northern Regions and for the 2005/06 (closed circles) and 2006/07 (open triangles) sampling seasons. Key to numbers = reaches: Dumaresq 1-3, Mole 4-6, Goulburn 7-9, Ovens/King 10-12, Mullaroo 13-15 and Lindsay 16-18. Ellipses group reaches by Region.

In many cases, larvae and juveniles were present in samples for all species collected (Table 3.1). Notable exceptions to this pattern were that Australian smelt were not collected as larvae in MO in 2006/07, carp gudgeons were not collected as larvae in GO and MU, *Galaxias* sp. were not collected as juveniles in OV in 2006/07, juveniles of Murray cod were rarely caught in any system, larval purple-spotted gudgeons were rarely caught, and larvae and juvenile common carp only occurred sporadically. Adults of most small-bodied fishes were present, but were almost always absent for larger-bodied fishes. Sample sizes for the larvae and juveniles of all species collected are given in Appendix I.
Table 3.1: Presence or absence of larvae (L), juvenile (J) or adult (A) fish from each species in each river and the total number of species and number of native species collected during the 2005/06 and 2006/07 spawning seasons.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species name</th>
<th>Season 05/06</th>
<th>Season 06/07</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ovens</td>
<td>Goulburn</td>
</tr>
<tr>
<td>Silver perch</td>
<td><em>Bidyanus bidyanus</em></td>
<td>J</td>
<td>J</td>
</tr>
<tr>
<td>Oliver perchlet</td>
<td><em>Ambassis agassizi</em></td>
<td>J</td>
<td>J</td>
</tr>
<tr>
<td>Freshwater catfish</td>
<td><em>Tandanus tandanus</em></td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Spangled perch</td>
<td><em>Leithiopon unicolor</em></td>
<td>A</td>
<td>L</td>
</tr>
<tr>
<td>Common carp</td>
<td><em>Cyprinus carpio</em></td>
<td>L, J</td>
<td>L, J</td>
</tr>
<tr>
<td>European perch</td>
<td><em>Perca fluviatilis</em></td>
<td>L, J</td>
<td>J</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>Carassius auratus</em></td>
<td>J</td>
<td>J</td>
</tr>
<tr>
<td>Gambusia</td>
<td><em>Gambusia holbrooki</em></td>
<td>J</td>
<td>J</td>
</tr>
<tr>
<td>Trout</td>
<td><em>Salmo trutta</em></td>
<td>J</td>
<td>J</td>
</tr>
<tr>
<td>Total no. of species</td>
<td></td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>No. of native species</td>
<td></td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>
3.3 **Timing of spawning and recruitment: overall fauna**

Since sampling started late in 2005/06, most of the emphasis will be placed on the second season’s results. Nevertheless, we will invoke the results from the first season when they are relevant. Comparisons will mostly be between pairs of rivers, but general patterns will be described first.

It is apparent that protolarvae (indicating recent spawning) were present in LI for longer than any other system during this study (Fig. 3.7). For this river, Australian smelt, carp gudgeon, flathead gudgeon and unspecked hardyhead spawned for, usually, between three and six months. The duration of spawning was next greatest for species in GO, where Australian smelt, *Galaxias* sp. and common carp generally spawned for between three and four months. In most other systems, spawning was evident only for one or two months at most, and in many cases in the Northern Region, spawning could only be inferred by the presence of later stage larvae or juveniles. Murray cod and gambusia were never collected as protolarvae, and so that is why only later stage larvae are shown in this figure. In virtually all cases for small-bodied species, juveniles were present in the same month or the month after spawning commenced, and continued to be collected throughout the study period.

**Southern Region: Ovens versus Goulburn**
For those species that the two rivers shared, spawning generally started earlier, and went on longer, in GO than in OV (Fig. 3.7). This was most marked for Australian smelt, *Galaxias* sp. and common carp. The only exception to this was probably gambusia, which occurred as later-stage larvae between December and February in both rivers in 2006/07.

**Lower Region: Lindsay versus Mullaroo**
For those species that the two rivers shared — which was the majority — spawning began earlier and extended for longer in LI than in MU (Fig. 3.7). This was true for Australian smelt, carp gudgeons, flathead gudgeon in both seasons and for unspecked hardyhead in 2005/06.

**Northern Region: Mole versus Dumaresq**
For those species that the two rivers shared — which, again, was the majority — there were very few protolarvae collected (Fig. 3.7). However, at least for Australian smelt, carp gudgeons and unspecked hardyhead, spawning likely began in DU a month before MO. It was difficult to make an assessment for the other species because of their patchy occurrences.
Figure 3.7: Duration of occurrence of protolarvae (black), other larvae (grey) and juveniles (dotted line) of fish species collected from each river in the three regions of the Murray–Darling Basin collected during the 2005/06 and 2006/07 spawning seasons.
### 3.4 Timing of spawning and recruitment: Australian smelt

Estimates of timing of spawning come from presence of protolarvae and backcalculations of ages from lengths, as well as interrogation of length-frequency histograms, as these can indicate when fish may have spawned in a previous month, but protolarvae failed to be present in samples.

It is clear that Australian smelt spawned and recruited in each year and in each river, despite the absence of protolarvae from samples in MO (Figs. 3.7 & 3.8).

Based on the presence of protolarvae, spawning occurred for the longest period in GO and shortest in DU (Fig. 3.8).

![Australian smelt frequency of occurrence](image)

**Figure 20:** Frequency of occurrence of Australian smelt protolarvae in seine samples collected from the Ovens, Goulburn, Lindsay, Mullaroo, Mole and Dumeresq systems for the 2005/06 and 2006/07 spawning seasons. * = samples not taken.
Spawning commenced first in LI (in August) and finished latest in GO (in January). Spawning commenced at a similar time in both Southern Region rivers and length-frequencies and back-calculated ages suggest that spawning may have started as early as late August in both rivers (Figs. 3.9 & 3.10).

It seems likely that a similar pattern is evident for both Lower Region rivers. Length-frequency histograms confirmed this, with a large cohort of fish in MU in September, mostly between 12 and 16 mm, which would have been produced in the mid-late August, which is when LI larvae were first detected (Fig. 3.9).

Length-frequencies also suggested that for most rivers there was only one or two cohorts of larvae produced (at the month resolution) or that persisted throughout the study period (Fig. 3.9). The exception to this was GO, where we know, from protolarvae, that fish spawned for four months — this was also shown from length-frequencies. Again, in all cases, except GO, there was usually one length mode and occasionally two (e.g. January in DU) that predominated throughout the season.

The patterns observed and conclusions drawn from the presence of protolarvae and from length-frequency histograms were broadly supported by back-calculated hatching dates of Australian smelt from the rivers (Fig. 3.10). Thus, there were generally only one or two cohorts that persisted throughout the season in most rivers. Back-calculated hatching dates for GO in October, December and January, however, indicated the presence of at least three cohorts, albeit in low numbers. Additionally, more than two cohorts may have been present in LI in January, but two predominated, whilst more than two may have been present in MU in December, but, similarly, two predominated. If we summarise this in another way, and just look at the relative distribution of hatching dates of juveniles collected in January or February and compare these with the distribution of hatching dates of protolarvae and flexion larvae from the rivers, we can see that in each case there is usually one cohort which predominates, sometimes a smaller cohort as well, but with GO, there are definitely three cohorts, albeit with the middle one predominating (Fig. 3.11). This figure also shows that fish in the Lower and Northern regions spawned earlier than their Southern counterparts and with similar frequencies. What is also apparent is that recruitment is disproportionate for all rivers except OV. In each of the former cases, the first spawning period was the strongest, but most recruits had hatch dates coinciding with the smaller, second wave of spawning. In OV, this was not the case, with peak spawning coinciding with peak recruitment.

Peak recruitment approximately coincided in Lower and Northern region rivers, between early and mid-October (Fig. 3.11). Peak recruitment occurred in OV in mid-November and in early December in GO.
Figure 3.9: Length-frequency histograms for Australian smelt larvae and juveniles in seine samples collected from the Ovens, Goulburn, Lindsay, Mullaroo, Mole and Dumersq systems for the 2006/07 spawning season. For sample sizes, see Appendix IIa
Figure 3.10: Hatch date-frequency histograms for Australian smelt larvae and juveniles in seine samples collected from the Ovens, Goulburn, Lindsay, Mullaroo, Mole and Dumersq systems for the 2006/07 spawning season. For sample sizes, see Appendix IIa
Figure 3.11: Hatch date frequency distributions for all Australian smelt protolarvae and flexion larvae (solid line) and juveniles from February (dashed line) for the Ovens, Goulburn, Lindsay, Mullaroo, Mole and Dumersq systems for the 2006/07 spawning season. Sample sizes for protolarvae + flexion larvae and juveniles, respectively, are given in brackets.
3.5 *Timing of spawning and recruitment: carp gudgeon*

Collection of carp gudgeon protolarvae was patchy, which is understandable given that they have a short larval period (Fig. 3.12). Nevertheless, it was apparent that carp gudgeons spawned and recruited in each year and in most rivers. The exceptions were GO, where none of this species group was collected, and in MU in the 2006/07 season, when protolarvae were only collected in February and cohorts could not be detected up to that point (Fig. 3.13). We will concentrate our analyses on OV and the Lower Region rivers for this species, since this was where they were most abundant in samples.

Spawning occurred for the longest in each year in LI (for at least five months in both years) based on length-frequencies, despite the fact that we did not collect protolarvae from reaches in December 2005, December 2006 or January 2007 (Fig. 3.12).

![Figure 3.12: Frequency of occurrence of carp gudgeon protolarvae in seine samples collected from the Ovens, Goulburn, Lindsay, Mullaroo, Mole and Dumaresq systems for the 2005/06 and 2006/07 spawning seasons. * = samples not taken.](image-url)
Spawning began first in LI in at least September — and perhaps as early as August (Fig. 3.12) — and early stage larvae were still being collected when sampling ceased in February in the Lower Region. There were no early stage larvae collected from MU until February (as stated above) and indications from length-frequencies suggest that no spawning and recruitment had occurred in this river until January or, at the earliest, December. Spawning may, however, have taken place after sampling ceased. Although collection of protolarvae was sporadic, spawning almost certainly commenced in OV in November and was still occurring in February when sampling ceased (Figs. 3.12 & 3.13). Few carp gudgeon protolarvae were collected from the Northern Region, although spawning undoubtedly occurred in both rivers in both years, based on the presence of early stage larvae (Fig. 3.7); but low abundances of fish prevented any useful analyses of length-frequencies.

Length-frequencies and back-calculated hatching dates suggest that for the three rivers analysed — OV, LI and MU — there were multiple cohorts of larvae and juveniles present (Fig. 3.13 & 3.14). By February, the juveniles of each river comprised fish which had hatched between early November and late January in OV (no real peak), between early October and early February in LI (again, no real peak) and between early November and early February in MU.

We almost certainly did not cover the entire spawning period for carp gudgeons. However, it can be determined that the peak in hatch dates for early stage OV fish was early December, and the peak in recruit hatching dates was only just offset by approximately two weeks — probably the margin of error of analyses (Fig. 3.15). Hatch dates of early stage larvae in LI, on the other hand, were scattered between mid-November through February, but the peak in recruit hatching dates was mid December, right in the middle period of spawning.

In a similar pattern for Australian smelt, there was little indication of disproportionate recruitment in OV, but good indication of this in LI (Fig. 3.15). For MU, unfortunately, there were larvae collected only in February, and, as such, we were unable to make any conclusions regarding proportionate or disproportionate spawning in this river.
Figure 3.13: Length-frequency histograms for carp gudgeon larvae and juveniles in seine samples collected from the Ovens, Lindsay and Mullaroo systems for the 2006/07 spawning season. For sample sizes, see Appendix IIb.
Figure 3.14: Hatch date-frequency histograms for carp gudgeon larvae and juveniles in seine samples collected from the Ovens, Lindsay and Mularoo systems for the 2006/07 spawning season. For sample sizes, see Appendix IIb.
3.6 **Timing of spawning and recruitment: flathead gudgeon**

The occurrence of flathead gudgeon protolarvae in our samples was not unlike that of carp gudgeon protolarvae — sporadic in the rivers of the Southern and Lower regions, although it was more consistently collected in LI than the other rivers (Fig. 3.16). Flathead gudgeon did not occur in the Northern Region. Despite the patchy collection of protolarvae, it was apparent from the presence of later stage larvae and juveniles that fish spawned and recruited in both years in all rivers. We will concentrate our analyses on Lower Region rivers for this species, since this was where fish abundance was greatest.

Spawning occurred for the longest in each year in LI, for probably at least three months in both years based on length-frequencies, despite the fact that we did not collect protolarvae from this river in December 2005 (although post-flexion larvae were present) (Fig. 3.17).
Figure 3.16: Frequency of occurrence of Flathead gudgeon protolarvae in seine samples collected from the Ovens, Goulburn, Lindsay and Mullaroo systems for the 2005/06 and 2006/07 spawning seasons. * = samples not taken.

In 2006/07, spawning began first in LI in September — juveniles that occurred in LI in August were most likely from the previous year’s cohorts — and continued until November, with peak spawning in September and October (Figs. 3.16 & 3.17). There was no indication that spawning continued after sampling ceased, since no protolarvae and no new cohorts were evident after December.

Abundances of juveniles of flathead gudgeon collected in the latter stages of the spawning season were small and, therefore, it is difficult to draw solid conclusions from our results. However, there were probably several cohorts represented as juveniles in January and February in LI and MU (Figs. 3.17 & 3.18). The final cohort suggests that surviving juveniles hatched between mid October and the end of November, and peaked in abundance in mid November in LI, but there were no clear peaks evident for MU.

The peak in hatch dates for early stage LI flathead gudgeon was late September to early October, whereas the peak in recruit hatching dates was approximately one month later, in mid-late October (Fig. 3.19). Thus, for LI, there was disproportionate recruitment, with more recruits coming from the latter part of the spawning season.
Figure 3.17: Length-frequency histograms for flathead gudgeone larvae and juveniles in seine samples collected from the Lindsay and Mullaroo systems for the 2006/07 spawning season. For sample sizes, see Appendix IIc.
Figure 3.18: Hatch date-frequency histograms for Lathead gudgeon larvae and juveniles in seine samples collected from the Lindsay and Mullaroo systems for the 2006/07 spawning season. For sample sizes, see Appendix IIc.
Figure 3.19: Hatch date frequency distributions for all flathead gudgeon protolarvae and flexion larvae (solid line) and juveniles from February (dashed line) for the Lindsay River for the 2006/07 spawning season. Sample sizes for protolarvae + flexion larvae and juveniles, respectively, are given in brackets.

3.7 Timing of spawning and recruitment: gambusia

We collected late stage larvae of gambusia in all regions, rivers and for both spawning seasons (Fig. 3.20). Thus, there was a good indication that this species spawned and recruited consistently in the range of river types and geographical locations. We will concentrate our analyses on the Southern Region, since this was where abundances were greatest, but, as explained below, solid conclusions regarding the relative timing of recruitment and spawning cannot be made.

Spawning occurred for the longest in each year in Northern Region rivers — for at least five months — and it is likely that spawning continued when sampling ceased in each year in all rivers (Fig. 3.20).
In 2006/07, spawning began first the Northern Region rivers, in October, then in Lower Region rivers, in November, and finally in December in the Southern Region (Fig. 3.20). The frequency of samples from Southern and Lower regions containing larval gambusia fluctuated throughout the sampling period without discernible peaks. However, frequencies peaked in February in both MO and DU, although the latter also showed a high frequency of occurrence in October.
Length-frequencies for OV and GO indicated, as was expected from the presence of larvae in samples, multiple and mixed cohorts progressing through the months (Fig. 3.21). By February, there were at least three, but possibly four, cohorts present in both rivers — one with a mode of about 7 mm, a second with a mode at 10-11 mm, a third with a mode at about 14 mm, and a fourth with a mode at 17-18 mm.

It is risky to draw further conclusions from these results in regard to the relative timing of recruitment of gambusia (Fig. 3.22). Juvenile gambusia clearly grew very rapidly between December and February, such that they became adults within a few months of spawning — this rapid growth to reproductive age is consistent with current knowledge of their life history. We limited length measurements to juveniles for all species including gambusia. For other species this was fine, but for gambusia their there were repercussions for data collected and subsequent analyses. This included 2006/07 cohorts becoming ‘lost’ to our analyses and so estimates of hatching date distributions for this seasons will be biased in favour of younger fish, and the ability to distinguish between 2005/06 and 2006/07 recruits becomes problematic because of decreasing growth rates and merging of length-frequency modes. We, therefore, do not think it scientifically prudent to continue with these analyses.
Figure 3.21: Length-frequency histograms for gambusia larvae and juveniles in seine samples collected from the Ovens and Goulburn Rivers for the 2006/07 spawning season. For sample sizes, see Appendix IId.
3.8 Timing of spawning and recruitment: unspecked hardyhead

The occurrence of unspecked hardyhead protolarvae in our samples was sporadic in the Southern and Northern region, and completely absent from samples in GO (Fig. 3.23). Protolarvae were more consistently collected in LI than the other rivers, however. Despite the sporadic collection of protolarvae, it was apparent from the presence of later stage larvae and juveniles, that fish spawned and recruited in both years in all rivers, except GO. We will concentrate our analyses on Lower Region rivers for this species, since this was where fish abundances were greatest.

Spawning occurred for the longest period in LI in each year, likely for at least five months in 2005/06 and for at least two months (but more likely four) in 2006/07 (Fig. 3.23). The occurrence of later stage larvae and length-frequencies showing cohorts of small fish from November to February, add weight to the likelihood of spawning being of similar duration in MU. It is likely that spawning continued after our sampling ceased and, therefore, it is impossible to determine the timing of peak spawning or to infer much about relative timing of recruitment.
In 2006/07, spawning commenced first in DU in October, with all other rivers showing evidence that spawning began a month later, in November (Fig. 3.23).

By February, there were multiple cohorts present in both LI and MU (Fig. 3.24). However, the dominant cohort in LI was of young fish about 9 mm standard length (SL) with another substantial cohort about 15 mm, which hatched between early January and early February. The dominant cohort in MU was of fish at around 20 mm, which hatched in mid-December (Fig. 3.25).
As spawning most likely continued beyond the sampling period, conclusions regarding proportionate or disproportionate recruitment cannot be deduced.

Figure 3.24: Length-frequency histograms for unspecked hardyhead larvae and juveniles in seine samples collected from the Lindsay and Mullaroo systems for the 2006/07 spawning season. For sample sizes, see Appendix IIe.
Figure 3.25: Hatch date-frequency histograms for unspotted hardyhead larvae and juveniles in seine samples collected from the Lindsay and Mullaroo systems for the 2006/07 spawning season. For sample sizes, see Appendix II.

Murray cod spawned in virtually all rivers in both years (Fig. 3.7 & 3.26) — the only exception was that larvae were never collected from GO. There were also indications of Murray cod larvae are rarely caught as protolarvae and so indications of spawning time are based on the presence of older-stage larvae, usually postflexion and metalarvae. Murray cod larvae are usually caught as protolarvae and so indications of spawning time are likely to have occurred between two and three weeks prior to larval hatching. These are likely to be between 10 and 19 days post-hatch (Humphries, 2005) and so spawning time is likely to have occurred between two and three weeks prior to larval hatching.
recruitment in some rivers, with juveniles being present in samples in OV in 2005/06 and in MO and DU in both years.

Larvae were collected for two months in OV in each year of the study, and in three months in MU in 2005/06, but for all other river and year combinations, they were collected in one month only (Fig. 3.26). Larvae were first collected in the Northern Region rivers and in MU in October 2005/06, but was not collected until November in LI and OV. This pattern mostly persisted in the following season, with larvae being present in samples in October in 2006/07 in the Northern Region, but not until November in the Southern and Lower regions. Frequencies of larvae in seine samples were always low, rarely occurring in more than 20% of samples.

Figure 3.26: Frequency of occurrence of Murray cod larvae in seine samples collected from the Ovens, Goulburn, Lindsay, Mullaroo, Mole and Dumaresq systems for the 2005/06 and 2006/07 spawning seasons. * = samples not taken.
3.10 Spawning of common carp

Common carp spawned in both years in all rivers in the Southern and Lower regions of the study area, except in MU in 2006/07 (Figs. 3.7 & 3.27). Carp was never collected from Northern Region rivers. Juveniles were also collected in GO and MU in 2005/06 and in OV and MU in 2006/07, so for these rivers in these years carp clearly recruited.

The longest spawning time for carp was in GO in the second spawning season, when protolarvae were collected in each month between October and January (Fig. 33). In all other cases, protolarvae were only present in samples in one month. There was some indication that spawning of carp commenced earlier in the Lower than in the Southern Region: protolarvae were collected in October in LI, but not until November in OV and December in GO in 2005/06; and in September in LI, but not until October in GO in 2006/07. Frequencies of carp protolarvae in samples were usually low, except in November 2005 in OV, when they were present in 40% of samples.

Figure 3.27: Frequency of occurrence of common carp protolarvae in seine samples collected from the Ovens, Goulburn, Lindsay and Mullaroo systems for the 2005/06 and 2006/07 spawning seasons. * = samples not taken.
3.11 Does peak recruitment coincide among species?

We were able to compare the timing of peak recruitment in several species for individual rivers (Figs. 3.28 & 3.29). In LI, Australian smelt and flathead gudgeon, whose peak spawning times were less than a month apart, also showed similar timing of peaks in recruitment hatching dates (Fig. 3.28). This was not the case for carp gudgeons, as their spawning occurred over a longer period — and likely continued beyond our sampling period — and showed a peak in recruitment in the middle of the spawning period, which was considerably later than for the other two species. Peak recruitment of Australian smelt and carp gudgeons also did not coincide in OV (Fig. 3.29).

Figure 3.28: Hatch date distributions for Australian smelt, carp gudgeon and flathead gudgeon from the Lindsay River during the 2006/07 spawning season. Sample sizes for protolarvae + flexion larvae and juveniles, respectively, are given in brackets.
In a similar result to that shown above for LI, carp gudgeons spawned later than Australian smelt in OV, and showed a peak in recruitment about one and a half months later.

Figure 3.29: Hatch date distributions for Australian smelt and carp gudgeon from the Ovens/King system during the 2006/07 spawning season. Sample sizes for protolarvae + flexion larvae and juveniles, respectively, are given in brackets.

3.12 Exploring recruitment/environmental variable relationships

The biomass of zooplankton collected at Warby Range Road in OV varied significantly by month and size-class, and the interaction between these two; size-class, however, contributed most to this variation (Fig. 3.30a; Table 3.2). Tukey tests indicated that the mean biomass of zooplankton differed significantly among all size-classes, and that whilst there were no significant differences in biomass among August, September and October samples, biomass rose significantly in November from this low base (P<0.001). It was evident that only the two smallest size-classes contributed to this rise, with the two largest size-classes remaining at low levels throughout the study period (Fig. 3.30a). Mean biomass of zooplankton at Northwood Road in GO varied significantly with month, and with the month by size-class interaction, but not by size-class alone (Fig. 3.30b, Table 3.2). Tukey tests indicated that the mean biomass of zooplankton in October was significantly greater than that in September and February. No other significant differences occurred.
Table 3.2: Mean squares (MS) and significance levels of ANOVA of log$_{10}$ transformed biomass of zooplankton at Warby Range Road (Ovens River) and Northwood Road (Goulburn River) comparing months and size classes. Df = degrees of freedom. *P<0.05, **P<0.01, *** P<0.001.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS Ovens</th>
<th>MS Goulburn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>6</td>
<td>0.837***</td>
<td>0.142**</td>
</tr>
<tr>
<td>Size class</td>
<td>3</td>
<td>1.763***</td>
<td>0.068</td>
</tr>
<tr>
<td>Month x size class</td>
<td>18</td>
<td>0.208***</td>
<td>0.076*</td>
</tr>
<tr>
<td>Error</td>
<td>56</td>
<td>0.012</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Figure 3.30: Mean biomass of four size-classes of zooplankton per 30s trawl from (a) Warby Range Road on the Ovens River and (b) Northwood Road on the Goulburn River, collected between August 2006 and February 2007. Size classes are 53-106 µm, 106-212 µm, 212-500 µm and >500 µm.
Comparing the hatch date distributions of Australian smelt recruits with the biomass of zooplankton in the OV and GO, it is evident that the peak in recruitment in the Ovens coincided with the first appreciable rise in biomass of the smallest size fraction (53-106 µm) of zooplankton (Fig. 3.31). This was not, however, the case with GO — a peak in zooplankton biomass occurred in mid-October, whereas the peak in recruitment in early December.

Figure 3.31: Hatch date frequency distributions for all Australian smelt protolarvae and flexion larvae (solid line) and juveniles from February (dashed line) overlayed on the mean biomass of the two smaller size fractions of zooplankton for the Ovens and Goulburn rivers for the 2006/07 spawning season.

Mean biomass of zooplankton in the smallest size fraction was significantly and positively related to ‘mean water temperature for the previous month’ for OV at Warby
This relationship was a negative power relationship. No significant relationships were found for any of the size fractions in OV. This relationship was a negative power relationship. No significant relationships were found for any of the size fractions in OV.

There was also a significant, but negative relationship between ‘mean discharge for previous month’ and mean biomass of both the smaller size fractions of zooplankton in OV. This relationship was a negative power relationship. No significant relationships were found for GO or for the two larger size fractions of zooplankton in OV.

![Graphs showing regression relationships](image)

Figure 3.32: Regression relationships for ‘mean temperature for the previous month’ and ‘mean discharge for the previous month’ with mean weight of zooplankton, for the Ovens River at Warby Range Road and the Goulburn River at Northwood Road during the 2006/07 spawning season.

Multiple regression results, including log10 discharge and log10 temperature as independent variables and the two smallest size fractions of zooplankton as the dependent variables, give highly significant results, and explained 99.0% and 94.4% of the variation, respectively (Table 3.3). However, in both these models, the temperature component was negative. No significant relationships were found for any of the size fractions in GO or for the two larger size fractions of zooplankton in OV.

Table 3.3: Values for stepwise multiple regression for biomass of two size fractions of zooplankton versus log10 mean discharge for the previous month and log10 temperature for the previous month for the Ovens River at Warby Range Road between August 2006 and February 2007.

<table>
<thead>
<tr>
<th>Dep. Var</th>
<th>Constant</th>
<th>Log10 discharge</th>
<th>Log10temp</th>
<th>df</th>
<th>R²</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt 53-106 µm</td>
<td>71.702±8.807</td>
<td>-16.415±1.256</td>
<td>-27.095±4.735</td>
<td>5</td>
<td>0.990</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Wt 107-212 µm</td>
<td>51.617±10.529</td>
<td>-10.466±1.635</td>
<td>-22.795±6.165</td>
<td>5</td>
<td>0.944</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>
4 DISCUSSION

4.1 Consistency of flow management of rivers and limitations of this study

A major strength of the present study — that it included three geographically discrete and distant regions of the Murray–Darling Basin — was also one of its weaknesses. One implicit objective was to investigate whether patterns in spawning and recruitment in fish were influenced more by flow management or regional factors. However, whilst we attempted to find three flow-managed rivers — one in each region — whose flow was managed in a similar way, we were not entirely successful and our experience serves to underline the truism that no two rivers are the same.

The Southern Region included the heavily regulated Goulburn River, which typically has flows greatly enhanced over the summer period usually coinciding with much lower temperatures because of bottom releases from Lake Eildon. Whilst this typical scenario occurred for the Goulburn River in the first season of this study, the storage level in Lake Eildon was so low during the second season that irrigation releases were substantially less than the previous year, and temperature differences between the Goulburn and Ovens rivers were negligible. Indeed, differences in rainfall and runoff between the two years of the study meant that interannual differences in flow and temperature conditions were considerable. The Ovens and King rivers continued to flow throughout the 2005/06 spawning season, but ceased to flow in the second season. Because of their location in the catchment, the Lower Region rivers typically have relatively low flows in winter, leading up to increases in spring and early summer, before returning to low flows over the rest of summer. Mullaroo Creek essentially mirrors the hydrology of the River Murray, but at a lower overall discharge. Thus, it experiences high flows over the summer period because of upstream releases. Such releases occurred in both years, but were considerably higher during the 2005/06 season. The Northern Region rivers typically experience their highest flows over the late spring/early summer period, but the flow-managed Dumaresq River typically experiences enhanced flows over the summer period because of releases from Glenlyon Dam. Because of substantial rainfall over the summer of 2005/06, the discharge in the Mole and Dumaresq rivers were relatively similar, however, in the following year, the Mole remained low over the summer period, whereas the Dumaresq experienced substantially enhanced flows.

In broad terms, the flow-managed rivers in each region typically experience enhanced discharges, because of dam releases upstream, usually during the summer — the spawning and recruitment period of many species of fish. However, the timing of the natural versus enhanced flows varied by river and region. Furthermore, interannual variation in rainfall and runoff meant that flow conditions between the two years were not the same.
4.2 **Australian smelt as a model for investigating fish recruitment in the Murray–Darling Basin**

Using model organisms to explore natural history, evolution, physiology and the like, is as old as biology itself. And choosing the correct species for investigation is important for many reasons: it must be present in the regions of interest; it must be able to be obtained in sufficient and quantifiable numbers for statistical and/or experimental purposes; it must exhibit the characteristics that will provide insights into the hypotheses or questions being posed; and, it should be a vehicle that provides a passage to further questions and future study (Ford, 2000). Australian smelt possess most of these characteristics. It is ubiquitous throughout the Murray–Darling Basin in lowland rivers and was collected readily in all three regions included in this study — although larval stages were, as is our experience generally, not as readily collectable as were juveniles. Australian smelt is a pelagic species and can be collected quantitatively during all life stages; it breeds each year and probably does not live more than one or two years; and it is one of the few species that seems to be thriving under regulated conditions. One unexpected result, however, was that previous observations of spawning patterns — that this species is a consistent protracted spawner (Humphries *et al*., 2002 and unpublished data) — were not observed in this current study. Nevertheless, it did spawn for a duration long enough in most systems in order to compare the timing of spawning peaks with recruitment peaks — the main objective of the study. These and other issues, including those associated with other species investigated, will be discussed below.

4.3 **Brief or protracted spawners?**

This study stemmed primarily from observations over several years (Humphries *et al*., 1999, 2002), and from other work conducted on life histories of other groups of fishes that classified fish as brief or protracted spawners (Winemiller, 1989). Australian smelt and flathead gudgeon have been classified as protracted spawners, mainly based on work in the Campaspe and Broken rivers (Humphries *et al*., 1999, 2002) and this is also evident from studies of rivers in the Lower Region of the Murray–Darling Basin (Meredith, unpublished data). A brief spawner could be described as one that spawns over only one or two months, whereas a protracted spawner could be described as one that spawns over three or more months, and sometimes up to nine months of the year (Humphries, *et al*., 2002). From our results in the present study, however, the only conclusion possible is that spawning duration depends on the environment in which a species is found. This can, presumably, vary geographically and temporally. For example, Australian smelt could be classified as a protracted spawner in the Ovens and Lindsay rivers in 2006/07, but perhaps not in the previous year, and in the Goulburn in both seasons. However, it would be classified as a relatively brief spawner in the Mullaroo, Mole and Dumaresq systems. Similarly, flathead gudgeon would be classified as a protracted spawner only in the Lindsay River, and a brief spawner in all other rivers where it occurred. Carp gudgeon, not previously described as a protracted spawner — partly because of the problems in determining the numbers that exist in any one location — spawned for at least five months in the Lindsay River, but for only one or two months.
in all other rivers — although in some cases, sampling had ceased while spawning continued. It must be recognized, however, that our sampling relied predominantly on collecting fish larvae using seine nets, and these will, like all methods, be selective. Meredith (unpublished data) collected Australian smelt larvae in the same systems as we did in 2005/06 and 2006/07 using light traps, and, while our duration of larval occurrence estimates for the second season broadly agree with his, this is not the case for the first season. Meredith’s results indicate that spawning continued for some time after our sampling failed to collect any more larvae. In addition Meredith has shown that flathead gudgeon were present as larvae for longer periods than our estimates indicated. It is unclear why this was the case.

A summary of the spawning strategies of species featured in this study indicates that fish can be classified into three categories: brief, protracted or flexible spawners (Table 4.1). There were a number of brief spawners — southern pygmy perch, Murray cod, crimson-spotted rainbowfish, purple spotted gudgeon, olive perchlet, freshwater catfish and European perch — and only one truly protracted spawner — gambusia. There were several flexible spawners — Australian smelt, carp gudgeon, flathead gudgeon, unspecked hardyhead, galaxias, and common carp — whose spawning duration varied depending on which river and in which year they were observed. There were also a number of species with an indeterminate spawning strategy because of a lack of data.

Table 4.1: Classification of species into ‘brief’ (1-2 months), ‘flexible’ (varies with river), ‘protracted’ (>= 3 months) or ‘indeterminate” (not enough data) spawners, based on the presence of protolarvae collected from three regions of the Murray–Darling Basin during the 2005/06 and 2006/07 spawning seasons.

<table>
<thead>
<tr>
<th>Species</th>
<th>Brief</th>
<th>Protracted</th>
<th>Flexible</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian smelt</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carp gudgeon</td>
<td>✔</td>
<td></td>
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<td>Flathead gudgeon</td>
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<td>Unspecked hardyhead</td>
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<td>Galaxias sp.</td>
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<td>Bony herring</td>
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<td>Southern pygmy perch</td>
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<td>Murray cod</td>
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<td>Crimson-spotted rainbowfish</td>
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<td>Silver perch</td>
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<td>Purple-spotted gudgeon</td>
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<td>Oliver perchlet</td>
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<td>Trout</td>
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</table>
We suggest that the above table is a work-in-progress and should be amended as more data, from as wide a range of sites as possible and using a range of methods, are collected. As stated above, our study did not utilize all possible sampling methods and nor was it undertaken over the entire year (just the major portion of the breeding season).

This does not preclude, however, investigating the hatch date distributions of early-stage larvae and comparing these with those of recruits at the end of the spawning season. Even a spawning period as short as two months, especially in spring, when conditions change dramatically over a short time, can provide insights into appropriate conditions for recruitment (see discussion below). These findings have major implications, however, for the applicability of the ‘window-of-opportunity’ hypothesis. In the case of both Australian smelt and flathead gudgeon, as stated above, we previously classified them as protracted spawners and hypothesized that this character was responsible for their success under regulated conditions. The fact that Australian smelt thrives in all rivers studied, and that flathead gudgeon thrives in all rivers within their geographic range — despite the fact that they spawn for varying periods — indicates that protracted spawning alone may not explain their success. Thus, our data do not support the ‘window-of-opportunity’ hypothesis. However, it would be worthwhile conducting further investigations in this area, because of the limitations of our sampling. Furthermore, as discussed in the next section, the perception of what a window-of-opportunity is depends, to a large extent, on scale. And so if a species spawns for a relatively short period, this still may mean that there is a time within that period when conditions are more conducive to recruitment than other times.

### 4.4 Is recruitment proportional or disproportional to the abundance of newly hatched larvae for a protracted spawner — Australian smelt?

For the reasons discussed in the previous section, Australian smelt cannot be regarded as a consistent protracted spawner. It should, perhaps, be re-classified as a flexible spawner. However, it did spawn for a sufficient length of time for comparisons to be made between the timing of hatching of early stage larvae with that of recruits. For all rivers, except the Ovens, recruitment was disproportional to spawning. In other words, peak timing of spawning — as indicated by hatching dates of early stage larvae — did not coincide with peak recruitment. Furthermore, in each case, peak spawning occurred at least one month prior to peak recruitment. For the Ovens River, however, fish hatched and presumably spawned for only a relatively short period, and most recruits derived from this peak period. This is discussed further below, in relating recruitment to environmental variables and food availability.
4.5 Are spawning and recruitment synchronous between ‘flow-managed’ and ‘non flow-managed’ rivers for fish in general?

The first major point here is that there were substantial differences in the fish faunas of pairs of flow-managed and non flow-managed rivers. For example, carp gudgeons, Murray cod and southern pygmy perch were absent from the Goulburn River, but were present in the nearby Ovens River; and bony herring and crimson-spotted rainbowfish were present in Mullaroo Creek, but not in the Lindsay River. The Southern Region (Goulburn/Ovens) example typifies what we know of the effects of river regulation on species in that geographical area (Humphries et al., 2002, 2008; Crook et al., 2006; Tonkin et al., 2007). Indeed, this is another in several examples of the role of river regulation in structuring fish faunas (Gehrke et al., 1998; Humphries et al., 2002, 2008). The upshot of these faunal differences is that for comparative purposes, we must find species which are common to our pairs of rivers.

Secondly, spawning time for the fish faunas of the rivers in general varied dramatically. Some species commenced spawning in August and some apparently did not start spawning until as late as December or January. This is not new information, but underlines the need to recognize that different species have their own particular sets of traits related to spawning commencement and vary in regard to spawning duration (as discussed above).

When pairs of flow-managed and non flow-managed rivers were compared, however, there were substantial differences in the commencement and duration of spawning of the species common to both rivers within a region. Spawning usually commenced earlier in the spawning season and was more protracted in the flow-managed Goulburn than in the non flow-managed Ovens. This was true for our target species, Australian smelt, and several others. Only gambusia did not show this pattern. This pattern of earlier spawning in the flow-managed (regulated) river was also observed in the Northern Region, where fish in the regulated Dumaresq began spawning earlier than in the non flow-managed (unregulated) Mole. By contrast, of those species common to both rivers, fish generally began spawning earlier and spawning was more protracted in the unregulated Lindsay than in the regulated Mullaroo. So, there is no generality here in terms of flow-managed versus non flow-managed. Penaz (1996) suggested that river regulation disrupts cues for movement and spawning and results in less coordinated spawning and perhaps more protracted spawning. This could explain the situation in the Southern and Northern regions, but not what we observed in the Lower Region.

Because of the disparity between the fish faunas that are common to our pairs of rivers, and because of the low abundances of larvae collected of many species, our conclusions about timing of recruitment in flow-managed and non flow-managed rivers are limited to Australian smelt. In the Lower and Northern regions, the timing of peak recruitment was broadly the same. In the first case, Lindsay and Mullaroo Australian smelt recruited best from those larvae hatching in early October. In the latter case, Mole and Dumaresq Australian smelt recruited best between early and mid-October. Only in the Southern Region rivers was there a non-coincidence of recruitment between rivers, with fish in the
Ovens recruiting best from those hatched in mid-November, whereas fish in the Goulburn recruited best from those that hatched in early December. It seems that timing of spawning and recruitment for Australian smelt are more driven by environmental conditions associated with particular geographic regions, rather than the type of discharge patterns that they encounter. The exception to this was in the Southern Region, where it may be that the differences between the discharge regimes of the Ovens and Goulburn rivers are so severe that they dominate regional similarities. Certainly, the disparity between discharge levels during the spawning and rearing times of the Ovens and Goulburn rivers is probably the greatest of all the pairs of rivers. Although the Goulburn is typically much cooler than the Ovens, owing to bottom releases, temperature differences were relatively small during the 2006/07 season — the main season of comparison. Nevertheless, the Ovens/King system was consistently warmer than the Goulburn, which may help to explain the disparity between timing of recruitment. The possible influence of temperature and flow in recruitment will be discussed below.

4.6 Are spawning and recruitment synchronous between years for fish in general?

Conclusions regarding the synchronicity of recruitment for fish between years cannot be made from this study because we were not able to encapsulate the entire spawning period for most species. For the same reason, we are limited in what we can conclude about the timing of spawning between years. Furthermore, this study has underlined the need for intensive sampling to effectively determine spawning time. Collection of protolarve was patchy for many species and probably should not solely be relied upon to indicate that spawning has occurred recently.

4.7 Is timing of peak recruitment synchronous for protracted and brief spawners?

It eventuated that the classification of protracted spawners may not be as useful a concept as we thought when this study commenced. It follows, therefore, that this question, as posed, cannot be answered adequately (or logically). Instead, we compared the timing of peak recruitment for a number of species for selected rivers — for which we achieved mixed results. Peak recruitment of Australian smelt and flathead gudgeon coincided in the Lindsay River, but this was not the case with carp gudgeons, which began spawning later than these two species. A similar pattern of non-coincidence was found in the Ovens River for Australian smelt and carp gudgeons. It is not particularly surprising that different species recruit best at different times within a year. Size, morphology, physiology, movement patterns and habitat use all differ for the larvae of different species (King, 2004b; Price, 2007), despite similarities in diet when very young (King, 2004a). If early stage larvae of Australian smelt and flathead gudgeon feed predominantly in the water column on zooplankton, and carp gudgeon early stage larvae feed on the benthos, then relative abundances of preferred prey may differ temporally
and, consequently, influence their respective timing of recruitment. Notwithstanding, if
the timing of hatching does not coincide among species, then comparing the timing of
peak recruitment is irrelevant. It is clear from our work that the timing of spawning and
hatching varies considerably with species and geographic region.

4.8 Are environmental variables or food availability correlated with
peak recruitment of Australian smelt in the Southern Region?

It appears that peak recruitment in the Ovens River in 2006/07 coincided with the first
significant increase in biomass of the two smallest size fractions of zooplankton (53-106
and 107-212 µm). This was not, however, the case for the Goulburn River. There was
also no significant rise in biomass of the two largest size classes of zooplankton.

We suspect that the nature of our plankton sampling of the Goulburn was such that it did
not accurately reflect the biomass of plankton available to fish larvae. Instead, it likely
represented the amount of storage-derived (Lake Eildon) zooplankton being transported
down a regulated river. Large impoundments are significant sources of production of
zooplankton, and increased releases generally result in increases in advection of
zooplankton downstream (Petts, 1984). Although the relationship between discharge and
zooplankton biomass in the Goulburn River was not significant, there was a positive
trend which implied greater volumes of water released from Lake Eildon led to a greater
abundance of zooplankton. Since our method of zooplankton collection involved zig-
zagging across the river for 30 seconds with a plankton net deployed from a boat, this
meant that the zooplankton in the Goulburn River would have been mostly entrained in
the current. Because the Ovens River experienced a much lower discharge level than the
Goulburn River during the spring and summer — and indeed, ceased to have above-
ground flow at times — collection of zooplankton was predominantly from slow-flowing
and still water. Fish larvae tend to be patchy in space and time and are typically
associated with slow-flowing or still habitats which are conducive to production of high
abundances of prey (Fuiman & Werner, 2002; King, 2004a; Price, 2007). Therefore, we
believe that we more effectively sampled the zooplankton likely to be accessible to fish
larvae in the Ovens River than in the Goulburn River.

The biomass of the two smallest size fractions of zooplankton in the Ovens could best be
explained by, and to a high level of determination, mean discharge and mean temperature
for the previous month. Thus, zooplankton biomass was very much driven by
environmental conditions over the previous month. It is possible that the coincidence of
peak recruitment in the Ovens River was a result of: a) reaching a threshold temperature;
b) reaching a threshold flow; c) the dynamics of either of these two; d) reaching a
threshold level of zooplankton; or, e) mere coincidence. It is unlikely that discharge or
temperature thresholds or dynamics alone could drive recruitment, unless there were
physiological tolerances exceeded. Australian smelt is an opportunist — which, as has
been shown, will breed for long periods of time in some rivers and under a range of
conditions, ranging from about 15 to 28 °C — and displays a broad tolerance range.
However, growth rate does differ with temperature, as might be expected. And if
survival is tied to growth rate, as is found with some species of fish (Trippel & Chambers, 1997), then temperature itself, may play a role in recruitment.

It is far more likely, however, that food availability, responding to a rising temperature and falling discharge, is the proximate cause of recruitment success in Australian smelt. A threshold density of appropriate-sized prey has been hypothesized as being one of the key drivers in recruitment of fish for almost a century (Hjort, 1914), with the time of first feeding seen as a ‘make-or-break’ period. This is where the ‘match/mismatch’ hypothesis and our development of the ‘window-of-opportunity’ hypothesis are relevant. Whilst the ‘window-of-opportunity’ hypothesis almost certainly does not explain the success of Australian smelt under regulated conditions, it is likely that conditions during spawning and hatching are not uniform and that larvae present at one time may encounter sufficient prey to get them through the critical first feeding event, whereas larvae occurring at another time may not. Our results imply that the temperature depression usually experienced by rivers like the Goulburn River, because of bottom releases, would have major effects on the recruitment of fish because of the timing of zooplankton production. To date, much of the investigation on the effects of temperature depressions in rivers has been on spawning, whereas our results indicate that there may be important recruitment effects as well.
5 MANAGEMENT AND SCIENTIFIC RECOMMENDATIONS

This study has provided much new information on patterns in spawning and recruitment of Australian smelt and several other species from three regions in the Murray–Darling Basin. This new information is relevant to the field of general fish ecology and, in particular, to the management of flows in regulated river systems.

Firstly, we believe that classifying fish as brief or protracted spawners is not sufficient and needs revising. We have determined that there certainly are a number of brief spawners (species that only spawn for one or two months), there are also several flexible spawners (species whose spawning period varies by river and by year), but we could only identify one species — gambusia — that could be considered a truly consistent protracted spawner. Of course, our results are limited to only six rivers and to a main sampling method which had its limitations, but this alien species is well known for its high production of young, its rapid maturation and its colonizing ability. The negative finding, in terms of this study, is that our results do not support the ‘window-of-opportunity’ hypothesis. The fundamental premise of this hypothesis is that the success of species — such as Australian smelt and flathead gudgeon, for example — under regulated conditions is due to their protracted spawning period, which allows at least a proportion of spawned larvae to encounter high densities of food to survive and recruit. It appears, therefore, that we do not have enough evidence to confirm that protracted spawning is the key to success and other recruitment models need to be found and tested. This should be a priority for future research.

Scientific recommendation: Development and testing of other fish recruitment hypotheses in lowland rivers.

Management recommendation: The intensive focus on Australian smelt as a model organism for investigation of spawning and recruitment patterns in the Murray–Darling Basin has proved useful, but clearly it does not represent the full gamut of species. Since it is impossible to intensively study all species of native fishes, it is recommended that strategies for the management of the fish fauna of the Murray–Darling Basin — especially where it relates to reproduction — be directed at species that represent the various life history strategies. Moreover, it is important to include management of both small and large-bodied species, since smaller species represent a huge biomass in the rivers and themselves support the top predators in the system including fish (Ebner, 2006) and birds (Richardson 1999). Recent work, providing fish passage for the smaller species, and the current study, are good examples of a change in attitude. But this needs to go further. We must recognize that our riverine ecosystems include a complex of food webs, where each node is interdependent on many others, and the small species are a critical component in this system, and should be managed accordingly.

Secondly, despite the lack of utility of the ‘window-of-opportunity’ hypothesis in explaining recruitment success, most species spawned for long enough duration for
(presumably) conditions to vary sufficiently such that recruitment was not proportional to hatching. In other words, the hatching date distributions of early-stage larvae did not match that of juveniles at the end of the spawning season. Our best data came from Australian smelt and showed that, in most cases, the early hatching events resulted in relatively poor recruitment, and later hatchings resulted in relatively good recruitment. The only exception to this pattern was for the Ovens River, where early larvae and juvenile hatching distributions coincided. The Ovens River is mostly unaffected by river regulation and responds naturally to rainfall events and snowmelt. Thus, the coincidence of spawning and recruitment may be related to consistent and appropriate cues for fish in a relatively ‘natural’ system. The reason we did not observe the same pattern in the Lindsay or Mole rivers is uncertain and suggests that either the Ovens River is an aberration, that the former two rivers have other influences that we do not know of, or that regional effects are more important than we have previously considered (see below). This clearly warrants further investigation.

**Scientific recommendation:** We would like to see more targeted investigation of the timing of spawning and recruitment of Australian smelt in more flow-managed rivers and in rivers where natural flow patterns mostly prevail. The hypothesis would be that the timing of recruitment should match that of spawning in relatively intact systems, but be offset temporally in flow-managed systems. Either this, or the investigation of hypotheses relating to the differences in spawning and recruitment timing among non flow-managed rivers included in this study. It would also be worthwhile to attempt to quantify recruitment for several years, with the hypothesis being that if spawning and recruitment coincide, because of response to appropriate environmental cues, then recruitment should be consistently good in natural systems and more variable in flow-managed systems.

Thirdly, there were, not unexpectedly, substantial differences in the fish faunas of the rivers in the three regions. Overall faunal differences were primarily regionally based, although in some cases the Ovens/King system was more similar to Lindsay River and Mullaroo Creek than it was to the nearby Goulburn River. Important differences were also found between pairs of rivers (flow-managed versus non flow-managed) within regions. These differences are consistent with previous work on such systems. In addition to this, however, we found that in the Southern and Northern regions, common species tended to begin spawning earlier and spawn for longer in flow-managed rivers than in their non flow-managed counterparts. This could possibly be explained by the disruption to environmental cues that river regulation causes. Unfortunately, science is rarely neat and simplistic, and in the Lower Region patterns were reversed — fish in the non flow-managed Lindsay River began spawning earlier and spawned for longer than in the flow-managed Mullaroo Creek. The results, nevertheless, point to the fact that timing and duration of breeding of fish in rivers are likely influenced more by local differences in discharge regimes than broader climatic and geographic influences. The implication of these results is that fish are flexible and will probably respond to alterations to flow regimes if managers impose them. This could be a promising avenue of investigation and adaptive management experiments.
**Scientific recommendation:** A series of adaptive management experiments be initiated to manipulate spawning time and duration of selected species in flow-managed rivers. If our results are more widely relevant — which we believe they are — then it should be possible to alter discharge regimes in flow-managed rivers to encourage fish to spawn earlier or later and for shorter or longer time periods. What types of flow manipulations are used, and which outcome is desired and why, would need to be investigated. We would also welcome more research into the relative influence of region and flow-management type on life history traits, recruitment and population parameters.

**Management recommendation:** Managers need to consider that river regulation effects in the Murray–Darling Basin are nested within several contiguous, large zones with different climatic, geomorphic and hydrological influences. Murray–Darling Basin rivers and fish faunas cannot and should not be managed as a single unit, where it is assumed that they have a similar faunal composition and behave in a similar manner. Indeed, we recommend that the Murray–Darling Basin be divided not so much into catchments or altitudinally-based zones, but into climatic and zoogeographic zones, and managed accordingly. The above scientific recommendation would feed into this process, establishing a hierarchy of influences on fish, and pointing to how management would be most effective.

Fourthly, whereas timing and duration of spawning seems to be influenced by local factors, timing of recruitment tended to be similar for rivers within regions — except for the Ovens/King and the Goulburn systems. Our preliminary comparisons of environmental variables with food availability, suggest that temperature (positively) and discharge (negatively) drive production of the smallest size classes of zooplankton and that the first significant rise in zooplankton coincided with peak Australian smelt recruitment in the Ovens River. The strong relationships between zooplankton and temperature and discharge are extremely interesting in their own right and deserve further investigation. These relationships clearly have important implications for river management.

**Scientific recommendation:** Because we did not have the time to process all our zooplankton samples for all rivers, it would be extremely worthwhile to process these now and quantify whether recruitment of Australian smelt was indeed related to a threshold level. To do this for non flow-managed rivers in the first instance would be a priority. The link between temperature and discharge and zooplankton production in the main channel of lowland rivers also needs more investigation. The results from this study also suggest, as outlined in the previous recommendation, that manipulation of discharge and, more importantly, maintaining natural temperature regimes in rivers may be a way to increase production of the food needed to get larval fish through the critical first-feeding stage of life. Adaptive management experiments related to zooplankton production should be initiated, focusing on the manipulation of discharge and temperature with specific objectives and hypotheses in mind.

**Management recommendation:** Following on from this scientific recommendation, management strategies to ameliorate the effects of cold-water pollution should be given
high priority. If our results are broadly applicable, depressed temperatures below major storages may not only in some cases prevent fish from breeding, but may delay or prevent the increase in zooplankton biomass that may be critical to the recruitment of many species of fish. A high density of small zooplankton (or microbenthos) is probably one of the most important factors related to survival of larvae during early feeding. Management actions designed to increase production of zooplankton during the late spring/early summer period should enhance recruitment of native fishes.
REFERENCES


King AJ. 2004b . Onotogenetic patterns of habitat use by fishes within the main channel of an Australian floodplain river. Journal of Fish Biology 65: 1582-1603.


APPENDICES

Appendix I

Sample sizes of larvae and juveniles of all species collected in 2005/06 and 2006/07 from the Southern, Lower and Northern regions of the Murray–Darling Basin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ovens 2005/06</th>
<th>Ovens 2006/07</th>
<th>Goulburn 2005/06</th>
<th>Goulburn 2006/07</th>
<th>Lindsay 2005/06</th>
<th>Lindsay 2006/07</th>
<th>Mullaroo 2005/06</th>
<th>Mullaroo 2006/07</th>
<th>Mole 2005/06</th>
<th>Mole 2006/07</th>
<th>Dumaresq 2005/06</th>
<th>Dumaresq 2006/07</th>
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</table>

Sample sizes of larvae and juveniles of all species collected in 2005/06 and 2006/07 from the Southern, Lower and Northern regions of the Murray–Darling Basin.
Appendix II

Sample sizes of each species presented in length-frequency histograms and hatch date-frequency histograms.

(a) Sample sizes of Australian smelt presented in length-frequency histograms and hatch date-frequency histograms (Figs. 3.9 & 3.10). If sample sizes used in hatch date-frequency histograms differ from those used in length-frequency histograms, the sample size of the former are given in brackets (this was predominantly because fish from the previous year’s cohort were excluded from analysis).

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<tr>
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<td>57</td>
<td>51</td>
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</tr>
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<td>15 (14)</td>
<td>255 (168)</td>
<td>302 (168)</td>
<td>62</td>
<td>784</td>
</tr>
<tr>
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<td>66</td>
<td>157 (98)</td>
<td>443 (303)</td>
<td>7</td>
<td>217</td>
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<td>122</td>
<td>32</td>
<td>17 (0)</td>
<td>234 (232)</td>
<td>54</td>
<td>198</td>
</tr>
<tr>
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<td>93</td>
<td>5</td>
<td>28</td>
<td>100</td>
<td>177</td>
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<td>105 (0)</td>
<td>213 (0)</td>
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(b) Sample sizes of carp gudgeons presented in length-frequency histograms and hatch date-frequency histograms (Figs. 3.13 & 3.14). If sample sizes used in hatch date-frequency histograms differ from those used in length-frequency histograms, the sample size of the former are given in brackets (this was predominantly because fish from the previous year’s cohort were excluded from analysis).

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<td>218 (0)</td>
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<td>Nov</td>
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<td>189 (7)</td>
<td>128 (0)</td>
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<tr>
<td>Dec</td>
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<td>150 (18)</td>
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<td>Jan</td>
<td>381</td>
<td>113</td>
<td>0</td>
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<tr>
<td>Feb</td>
<td>292</td>
<td>71</td>
<td>194</td>
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(c) Sample sizes of flathead gudgeons presented in length-frequency histograms and hatch date-frequency histograms (Figs. 3.17 & 3.18). If sample sizes used in hatch date-frequency histograms differ from those used in length-frequency histograms, the sample size of the former are given in brackets (this was predominantly because fish from the previous year’s cohort were excluded from analysis).

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<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Feb</td>
<td>33</td>
<td>5</td>
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(d) Sample sizes of gambusia presented in length-frequency histograms and hatch date-frequency histograms (Figs. 3.21 & 3.22). If sample sizes used in hatch date-frequency histograms differ from those used in length-frequency histograms, the sample size of the former are given in brackets (this was predominantly because fish from the previous year’s cohort were excluded from analysis).

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<tr>
<td>Feb</td>
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<td>238</td>
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(e) Sample sizes of unspecked hardyhead presented in length-frequency histograms and hatch date-frequency histograms (Figs. 3.25 & 3.26). If sample sizes used in hatch date-frequency histograms differ from those used in length-frequency histograms, the sample size of the former are given in brackets (this was predominantly because fish from the previous year’s cohort were excluded from analysis).

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<td>Oct</td>
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<td>Nov</td>
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<td>Dec</td>
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<td>17</td>
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<td>Jan</td>
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<td>Feb</td>
<td>141</td>
<td>74</td>
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Appendix 3

Linear length/age relationships for fish species.

a) Linear length/age relationships for Australian smelt, parameters, $R^2$ and sample sizes.

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<th>River</th>
<th>Month</th>
<th>n</th>
<th>$R^2$</th>
<th>Sig.</th>
<th>Equation</th>
</tr>
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<td>Sep</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct</td>
<td>actual values</td>
<td></td>
<td></td>
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<td>Nov</td>
<td>actual values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dec</td>
<td>14</td>
<td>0.96 ***</td>
<td>A = 2.361L-6.786</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jan</td>
<td>13</td>
<td>0.79 ***</td>
<td>A=3.635L-19.67</td>
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<tr>
<td></td>
<td>Feb</td>
<td>16</td>
<td>0.61 ***</td>
<td>3.712L+0.921</td>
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</tr>
<tr>
<td>Goulburn</td>
<td>Sep</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dec</td>
<td>12</td>
<td>0.89 ***</td>
<td>A=2.9413L-11.21</td>
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</tr>
<tr>
<td></td>
<td>Jan/Feb</td>
<td>15</td>
<td>0.95 ***</td>
<td>A=3.5527L-17.333</td>
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</tr>
<tr>
<td>Lindsay</td>
<td>Sep</td>
<td>actual values</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Oct</td>
<td>20</td>
<td>0.8 ***</td>
<td>A=3.63L-13.167</td>
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<tr>
<td></td>
<td>Nov</td>
<td>14</td>
<td>0.84 ***</td>
<td>A=4.7419L-31.534</td>
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<tr>
<td></td>
<td>Dec</td>
<td>15</td>
<td>ns</td>
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<tr>
<td></td>
<td>Jan/Feb</td>
<td>13</td>
<td>0.8 ***</td>
<td>A=4.9622L-9.1769</td>
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</tr>
<tr>
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<td>Sep</td>
<td>actual values</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Oct</td>
<td>19</td>
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<td>16</td>
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<td>A=4.287L+22.017</td>
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<td>Oct</td>
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<td></td>
</tr>
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<td></td>
<td>Nov</td>
<td>24</td>
<td>0.67 ***</td>
<td>A=4.301L-25.849</td>
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<td>24</td>
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<td>Dumeresq</td>
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<td>0.74 ***</td>
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<td></td>
<td>Jan</td>
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<td>0.58 ***</td>
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<td></td>
<td>Feb</td>
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b) Linear length/age relationships for carp gudgeons, parameters, $R^2$ and sample sizes.

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<th>Month</th>
<th>n</th>
<th>$R^2$</th>
<th>Sig.</th>
<th>Equation</th>
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<tr>
<td>Ovens/King</td>
<td>Sep</td>
<td>none</td>
<td>none</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct</td>
<td>none</td>
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<td></td>
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<tr>
<td></td>
<td>Nov</td>
<td>none</td>
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<tr>
<td></td>
<td>Dec</td>
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<td>$A = 3.3625L - 8.1219$</td>
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<td>Sep</td>
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<td>Oct/Nov</td>
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<td></td>
<td>Oct</td>
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Lindsay equation

c) Linear length/age relationships for flathead gudgeons, parameters, $R^2$ and sample sizes.

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<td>Nov/Dec</td>
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(d) Linear length/age relationships for gambusia, parameters, $R^2$ and sample sizes.

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<th>Equation</th>
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<td>$A = 2.5795L - 0.5695$</td>
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<td>$A = 2.804L - 2.1255$</td>
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<td>10</td>
<td>0.91 ***</td>
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<td>$A = 3.7435L - 6.5848$</td>
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<td>Feb</td>
<td>10</td>
<td>0.79 **</td>
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<td>$A = 4.5656L - 17.797$</td>
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e) Linear length/age relationships for hardyheads, parameters, $R^2$ and sample sizes.

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<th>Sig.</th>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Dec</td>
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<td>0.89 ***</td>
<td>A=0.4027L-16.592</td>
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<td>A=2.7189L-4.7565</td>
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<td>Nov</td>
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<tr>
<td></td>
<td>Dec</td>
<td>actual values</td>
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<td>A=3.1138L-1.301</td>
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