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Spawning time and early life history of Murray cod, *Maccullochella peelii peelii* (Mitchell) in an Australian river

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Synopsis

I investigated aspects of the early life history of Murray cod in the Broken River, southern Murray-Darling Basin, Australia. I documented patterns in abundance, length, age and the amount of yolk of drifting free embryos and estimated spawning periods for adult Murray cod in two reaches throughout each breeding season between mid-October and mid-December from 1997 to 2001. Free embryos began drifting in late-October and continued until mid- to late-December. Abundances of drifting free embryos showed no obvious peak in most years and were unrelated to discharge. Length, amount of yolk and age typically varied with sampling date, but only age showed strong and negative correlations with temperature. Thus, it appears that temperature affected rates of development, and that developmental stage, not length or age, was likely to be the determinant for when free embryos left the paternal 'nest'. Most free embryos were estimated to have spent between 5 and 7 days drifting. Spawning of Murray cod in the Broken River usually commenced in mid-October and continued until at least early December. Initiation of spawning was associated with temperatures of 15 °C and above, but discharge was highly variable, and no other environmental variables were consistent across years. The mid-point of spawning – usually in the first week of November – is considered a more significant time, because it likely coincides with peak spawning, and conditions during and immediately after this time are expected to be optimal for the survival of eggs and free embryos.

Introduction

The ultimate aim of reproduction is to distribute gametes in the environment such that their survival is maximised. The myriad ways in which gametes are distributed can be placed into broadly definable groups or modes (Balon 1984). For example, some fishes build nests, lay relatively few, large eggs, which are guarded by the male parent, whereas others distribute huge numbers of minute unprotected eggs into the water column. In both cases, the best conditions for eventual recruitment – when the progeny themselves achieve reproductive capability – may not be in the precise location where the adults have spawned.

For piscivorous species that spawn in aggregations, eggs, embryos, larvae and juveniles risk cannibalism (Folkvord 1997; Henderson & Corps 1997), although predation from other species will also be a threat (see Rice et al. 1997). Intracohort cannibalism can also be a significant cause of mortality at times, especially when food is scarce (Folkvord 1997). Thus, dispersing as fertilised eggs or embryos from a spawning ground may be a way of avoiding at least one source of predation. Furthermore, a single spawning will often produce

large numbers of progeny, the survival of which, during endogenous feeding, is probably maximised by tight aggregations, but once exogenous feeding commences, these tight aggregations may become a liability. Indeed, competition has also been suggested as being one of the driving forces behind dispersal (Waser 1985; McCarthy 1999).

Probably at least as important as cannibalism and competition, however, is the fact that optimal rearing habitats for various stages of development may be different. At least in the first few days of life, however, fishes have limited abilities to determine their location in the environment (Bone et al. 1995). Thus, they are dependent on their parents to place them in conditions which optimise survival and, in a highly dynamic environment like a river, the location and timing of spawning must be influenced by environmental cues which are relatively predictable and which augur well for the future. Determining these cues has been the focus for much research over many years, not least by those who study Australian freshwater fishes (see Koehn & O'Connor 1990; Humphries et al. 1999). This interest may have stemmed from the general perception that many species in Australia do not spawn every year, that if the right environmental cues – e.g. temperature thresholds, rises in river level, etc. – do not occur, fishes would resorb their gonads and postpone spawning until the following year (see Humphries et al. 1999). This perception has given rise to predictions that altering flow and temperature regimes through river regulation would remove spawning cues. Although this may very well be the case for some species, there is growing evidence that many species spawn every year, irrespective of flow conditions (Humphries & Lake 2000; Humphries et al. 2002; King et al. 2003). Threshold temperatures below which spawning will not occur, however, may be more critical than flow.[1] Humphries et al. (2002) suggested that degraded populations of some Murray-Darling Basin fishes are more likely the product of poor recruitment, rather than the absence of spawning. Thus, alterations to riverine ecosystems have allowed a number of species to continue to breed, but the survival of their young has been compromised.

The Murray cod, *Maccullochella peelii peelii* (Mitchell), is one of the native species from the Murray-Darling Basin which spawns every year (Humphries et al. 2002), but which has undergone a decline in abundance and distribution for more than a century (Dakin & Kesteven 1937; Rowland 1989; Harris & Rowland 1996). Although Murray cod populations supported a profitable commercial fishery for several decades, its abundance declined to such an extent, that by the 1950s, it was no longer commercially viable in much of the Murray system (Rowland 1989). Despite the iconic status of the species and the huge number of anecdotes told about it (see e.g. Sinclair 2001), until the last 10 years or so, there has been surprisingly little scientifically rigorous information of the biology and ecology of wild populations. Much of our knowledge of the biology of Murray cod has, therefore, relied on investigations carried out under aquaculture-type conditions[2] (Lake 1967a, b; Rowland 1983, 1988, 1998). Recent work on life history (Humphries et al. 1999, 2002), habitat use,[3] movement[4] and ageing (Anderson et al. 1992; Gooley 1992) in the wild, however, is adding significantly to our knowledge base. Although Murray cod spawn each year – unlike, perhaps golden perch *Macquaria ambigua* (Richardson) – an inability to locate spawning individuals in the wild has meant that reliable identification of spawning times has been elusive, as have the environmental variables associated with them (although, see Langtry's mainly anecdotal accounts[5].

[1] Koehn, J.D. 2001. Impacts of weirs on fish. pp. 59–65. *In*: S. Blanch (ed), The way forward on weirs, Inland Rivers Network, Sydney.

[2] Cadwallader, P.L. & G.J. Gooley. 1985. Propagation and rearing of Murray cod *Maccullochella peeli* at the Warmwater Fisheries Station Pilot Project, Lake Charlegrark. Fisheries and Wildlife Service, Department of Conservation, Forests and Lands, Melbourne, Victoria. 189 pp.

[3] Koehn, J.D. 1997. Habitats and movements of freshwater fish in the Murray-Darling Basin. pp. 27–32. *In*: R.J. Banens & R. Lehane (eds), Proceedings of the 1995 Riverine Environment Forum, Murray-Darling Basin Commission, Canberra.

[4] Koehn, J. & S. Nicol. 1998. Habitats and movement requirements of fish. pp. 1–6. *In*: R.J. Banens & R. Lehane (eds), Proceedings of the 1996 Riverine Environment Forum, Murray-Darling Basin Commission, Canberra.

[5] Cadwallader, P.L. 1977. J.O. Langtry's 1949–50 Murray River investigations. Fisheries and Wildlife Paper, Melbourne, Victoria, No. 13. 70 pp.

Coinciding with the limited knowledge about spawning time, is the limited knowledge of the early life history of Murray cod in the wild. We do know, however, that Murray cod free embryos drift downstream once they leave the natal 'nest'[4,6,7] (Humphries et al. 2002). Indeed, Humphries et al. (2002) referred to them as 'obligate' drifters, because they were collected only rarely in gear other than drift nets. Temporal and spatial patterns in the abundance, age and condition of drifting free embryos, especially over several breeding seasons, are yet to be described, yet are important if we are to understand which processes are most important in influencing recruitment of this species.

In this study, I determined at what age and size and for how long Murray cod free embryos drifted and how much yolk they possessed while drifting in the Broken River, south-eastern Australia. I also estimated the spawning period of Murray cod, using wild-caught free embryos, and explored associations with the commencement and mid-point of the spawning period and ambient environmental conditions.

Materials and methods

Study location and species

The study was conducted in the Broken River, north-eastern Victoria, Australia, which arises at about 1000 m above sea level, just north of the Great Dividing Range and which has a total length of approximately 180 km (Figure 1). The river has a mean annual discharge of about 230×10^6 m^3, with highest flows between June and September, when water temperatures are coldest (~7 °C) and lowest flows between December and April, when water temperatures can reach > 30 °C. The Broken River experiences moderate river regulation, with some storage of winter and spring flows in two impoundments (lakes Nillahcootie and Mokoan) and later release of this over summer. Two substantial weirs (Casey and Gowangardie weirs), downstream of the major impoundments, are impediments to movement of fish upstream, but have little effect on the hydrology of the river. Much of the catchment has been cleared for agriculture.

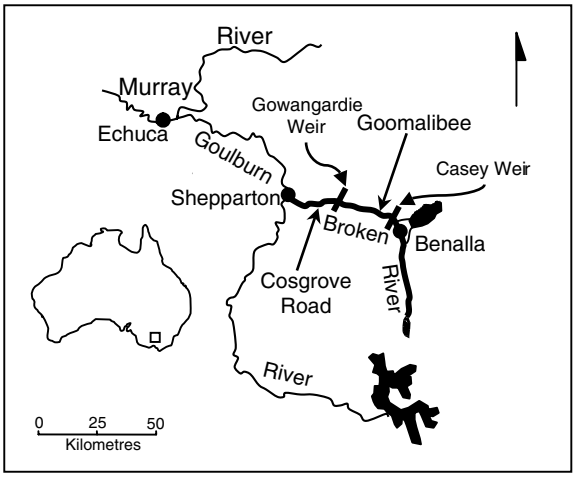

Figure 1. Map of the Broken River catchment, showing location of the two study reaches, Goomalibee and Cosgrove Road.

Historically, there would have been 18–20 native fish species inhabiting the Broken River, although nowadays, this has been reduced to approximately 12 species (Humphries & Lake 2000). One of the species which occurs commonly in the river is Murray cod. This species, whose natural distribution is limited to the Murray-Darling Basin, occurs in a variety of environments, from clear upland streams to turbid lowland rivers and is generally found associated with cover (Harris & Rowland 1996). The species likely reaches maturity at 4 or 5 years and spawns between September and December within the main channel of rivers (Koehn & O'Connor 1990; Humphries et al. 2002), when the temperature rises to about 20 °C (Harris & Rowland 1996). It has been reported to spawn once in a season, either in depressions in the bank or associated with woody debris (Langtry[1], Harris & Rowland 1996), where it deposits from 10000 to 90000 (depending on fish size) large eggs, which are guarded by the male. Incubation time is temperature-dependent, but takes between 3 and 8 days, after which the male continues to guard the young

[6] Meredith, S., B. Gawne, C. Sharpe, N. Whiterod, A. Conallin & S. Zukowski. 2002. Dryland floodplain ecosystems: influence of flow pattern on fish production. Technical Report 1/2002, Murray-Darling Freshwater Research Centre, Mildura, Australia. 41 pp.

[7] Gilligan, D. & C. Schiller. 2003. Downstream transport of larval and juvenile fish in the Murray River. NSW Fisheries Final Report Series No. 50, NSW Fisheries, Narrandera. 136 pp.

for a further 4–10 days, and then the young fish swim up and disperse downstream by drifting. Murray cod undergoes direct development, sensu Balon (1984), and so has no true larval interval; this means that the free embryos begin to feed while still retaining stores of yolk (King 2002) and develop into juveniles not long after. Since it was uncertain if young had begun feeding, but it was certain that they had not reached the juvenile stage, I will refer in this paper to drifting fish as free embryos.

Collection and processing

I collected free embryos from the Broken River weekly between mid-October and the end of December between 1997 and 2001, inclusive, at two locations: Goomalibee in the section of river between Casey and Gowangardie Weirs, and Cosgrove Road, downstream of Gowangardie Weir (Figure 1). I deployed three drift nets at each reach: each was 1.5 m long, had a 0.5 m diameter mouth and tapered to a 90 mm diameter cod end, to which a reducing bottle was fitted. Two of the drift nets were of 500 μm mesh and were used to estimate the abundance of drifting free embryos. The third net was of 1000 μm mesh and its purpose was to collect additional free embryos for the purposes of estimating age and yolk sac mass. I set nets, just prior to dusk and left them for 3 h, where natural obstructions funnelled the majority of flow and so that the top of the mouth of the net was just above the surface of the water. The volume of water filtered was determined from a (General Oceanics®) flow meter placed in the lower third of the mouth of the net.

From 1998 on, I removed observed free embryos from samples immediately upon retrieval of nets and preserved them in 95% ethanol. The remainder of the sample I preserved and sorted the next day, with the two lots of free embryos combined. I used only free embryos preserved immediately (1998 and after) in subsequent length, yolk sac and ageing analyses. In the Results section of this paper, however, I include an estimate of spawning time for Murray cod in 1997, based on well-preserved free embryos in the first and last samples collected for that breeding season.

To investigate the diel patterns in abundance of drifting Murray cod free embryos, I set and retrieved two 500 μm mesh nets every 4 h at each of the Goomalibee and Cosgrove Road reaches between 16:30 on 2 December 1997 and 16:30 the following day. I deployed and retrieved the nets in the same manner as described for the weekly sampling described above.

I separated Murray cod free embryos from the other species from each sample, measured their lengths under a dissecting microscope to 0.1 mm and recorded the presence or absence of a yolk sac. If a yolk sac was present, I dissected and removed it from the alimentary canal. I weighed the body of each free embryo and its yolk sac to 0.0001 g.

To estimate ages of the free embryos of Murray cod, I confirmed daily increment formation on otoliths. I obtained 15 newly hatched free embryos in November 1997, which had been incubated at 20 °C for approximately 7 days, from the Marine and Freshwater Resources Institute, Snobs Creek, Victoria and preserved them in 95% ethanol. I obtained a further two batches of Murray cod, one of fertilised eggs and one of known-age free embryos, in November 2002 from NSW Fisheries Inland Fisheries Research Station, Narrandera, NSW, and these I reared in laboratory aquaria under ambient photoperiod, at 17, 22 or 27 °C for between 8 and 50 days. I fed brine shrimp, *Artemia salina,* to the free embryos every day after they were 10 days old and their yolk sacs virtually gone. Of the November 2002 free embryos, I euthanised approximately 75 fish of a variety of ages with an overdose of benzocaine and preserved in 95% ethanol for a maximum of 4 weeks. For both the 1997 batch and 2002 batches of free embryos, I removed the sagittal otoliths from each fish, after first determining that these otoliths were superior to asteriscae and to lapillae. I used nail polish (ethyl acetate) to mount each pair of sagittae on a glass slide and 5, 3 and 1 μm lapping film to polish them, so that the primordium and outer increments were on a single plane as viewed under 1000× magnification. Distinct hatch checks were apparent in only about half of the 2002 otoliths I examined. Furthermore, the number of pre-hatch increments discernible in otoliths of 1997 and 2002 fish varied considerably, ranging from 2 to 7. Since the number of pre-hatch increments and the presence of a hatch check were variable, I followed a procedure similar to Narimatsu & Munehara (1997). This involved counting the number of increments back from the edge of the otolith cor-

responding to the known age of each free embryo, measuring the diameter of the increment at that point and calculating a mean and median diameter of the sagittae at hatching. In the case of the 1997 otoliths, however, I measured their maximum diameter, because these free embryos had just hatched when they had been preserved. This procedure gave a mean ± 1SE diameter of 55.02 ± 1.00 μm for 11 of the 1997 free embryos (the rest could not be read) and a mean of 55.38 ± 0.96 μm from a total of 61 otoliths of the November 2002 free embryos. Overall, a median diameter at hatching of 55 μm was obtained. Subsequently, I estimated age of the 2002 free embryos by counting the number of increments from immediately outside a diameter of 55 μm to the outer edge of the otoliths and I plotted estimated age and known age. The number of sagittal increments was related linearly to the known age of free embryos. Known age (A_k) could be predicted from the count of the number of increments, or estimated age (A_e), by the equation:

$$A_k = 1.010A_e - 0.761$$

where $R^2 = 0.97$ and the slope of the regression, 1.010 ± 0.048, was not significantly different from 1. Thus, daily increment formation is confirmed and a hatching diameter of 55 μm is considered adequate for subsequent estimates of age.

To estimate the age of wild-caught free embryos, I counted the number of increments, corresponding to days, outside a diameter of 55 μm on the left sagitta, which I had prepared in the same manner as those of the laboratory-reared free embryos. Otoliths which had indistinct increments were discarded. The number of increments for each otolith was verified by a second reader and, if the estimates of the two readers differed by more than 2 days, I discarded the otoliths. Distinct hatch checks were apparent in approximately 1/3 of otoliths and the mean diameter at this point for 104 otoliths was 53.12 ± 0.630 μm. This is sufficiently similar to the value obtained for laboratory-reared free embryos to give added confidence that the designation of a hatch check at 55 μm is a reasonable estimate; indeed the difference between the two is less than half the distance between adjacent increments in this area of the otoliths.

I obtained hydrological data from Goulburn–Murray Water through gauging stations operated by Thiess Environmental Services. Gauging stations are located at Benalla and Casey Weir on the Broken River. I placed temperature data loggers (Onset® Optic Stowaway) in the water at each of the two localities and recorded temperature hourly for the entire period.

Data analysis

I standardised the abundances of free embryos as numbers per 1000 m^3 of water filtered and expressed them as a mean of the two nets deployed on each date in each reach. I examined normal probability plots of standardised abundance, confirmed a normal distribution, before I regressed abundance with daily discharge for each reach, within each breeding season.

I tested variation in abundance of free embryos as a function of the six time periods for the 24 h sampling using one-way ANOVAs on log-transformed $(x+1)$ raw and standardised (per 1000 m^3) data. The results are presented for both types of data, because the estimate of the volume of water filtered for the last time period for one of the nets was unusually low and is, therefore, suspect.

I calculated yolk sac index (YSI) as the weight of the yolk sac expressed as a proportion of the total body weight, including the yolk sac (analogous to the gonadosomatic index) and I present it as a mean ± 1 SE of all individuals for each date and reach.

I present means ± 1 SE of untransformed length, YSI and age, because examination of normal probability plots indicated that data were normally distributed. I performed one-way ANOVAs on these length, YSI and age data, for each breeding season between 1998 and 2001, to test for differences among sampling dates ('Date'). Where mean values for these variables varied with sampling date and where there were sufficient data, I assumed that the most likely environmental influence was temperature and so I performed regressions of length, YSI or age with temperature (mean of 7 days prior to sampling date). I fitted untransformed linear and non-linear regression models to explore the influence of age on YSI for the most complete data sets. All analyses were performed using SYSTAT®.

I estimated the minimum time that free embryos spent drifting by subtracting the number of days spent in the 'nest' from their total age at capture (they may have continued to drift for a considerable number of days beyond this). I assumed time spent in the 'nest' to be a function of water temperature (see Results for rationale) and, because several studies have given the range of time spent in the 'nest' as up to 10 days (Koehn & O'Connor 1990), I have assumed that free embryos were in the 'nest' for 10 days when the temperature of the water was below 18 °C, 8 days when the temperature was between 18 and 20.5 °C, 6 days when the temperature was between 20.6 and 23.5 °C and 4 days when the temperature was greater than 23.6 °C. The assumptions of the relationship between time spent in the 'nest' and temperature are consistent with the relationship between incubation period and temperature presented below. However, I recognise that there will be considerable error around the estimates, and therefore results should be taken as indicative only and are primarily useful in interannual comparisons.

I estimated spawning date (T_s), from collections of free embryos pooled by reach, as:

$$T_s = T_c - A_c - I$$

where T_c = date of collection of free embryo; A_c = age since hatch in days; I = temperature-related incubation time in days.

The number of otolith increments indicated the age since hatching. I estimated time from fertilisation to hatching from relationships of incubation rates with temperature (Tom Ryan, Arthur Rylah Institute for Environmental Research, Department of Sustainability and Environment, Victoria, unpublished data). Ryan found that at 19 °C, Murray cod fertilised eggs took 7 days for 50% to hatch and 8 days for 100% to hatch; at 22 °C, 5 days for 50% to hatch and 6 days for 100% to hatch; and at 25 °C, approximately 4 days for 100% to hatch, with virtually all hatching during the fourth day. I determined the mean daily water temperature during the period when eggs were incubating (10 days prior to hatch) and used it in the estimates of incubation time. If fertilised eggs were incubating in the river at 15–20 °C, I assumed them to have taken 8 days from fertilisation to hatch, at 21–23 °C, 6 days to hatch, and at 24–27 °C, 4 days to hatch. I recognise that there will be some error in these estimations, but it must be realised that identifying the spawning time to ±3 or 4 days is relatively minor overall.

Results

Abundance and timing of drifting free embryos

I first collected free embryos in drift nets between late-October and early November during the 5 years of sampling (Figure 2). I collected free embryos considerably later in 2000 than in previous years, probably because the high flows in the river during that period (see Figure 6) made positioning nets in the main current virtually impossible, and also would have diluted the overall concentration of free embryos in the river considerably. In 1998 and 2001, free embryos were present in the last samples collected in December, and although it is possible that free embryos were still drifting in the Broken River beyond this date, I did not collect them in samples taken in mid-January. Drifting free embryos occurred in samples for between 3 and 9 weeks.

There was no clear peak in the abundance of drifting free embryos in most years, except perhaps on 25 November in 1999 at Goomalibee, when I collected an adjusted abundance of almost 400 free embryos per 1000 m^3 (Figure 2). However, when I determined the mid-point of the occurrence of free embryos for each reach and breeding season, there was only a 10 day difference between the earliest date (20 November 1997, Goomalibee) and the latest date (30 November 2000, Goomalibee). I found no significant relationships between abundance of free embryos and discharge in a reach within a year ($p >> 0.05$).

My diel sampling of Murray cod free embryos in December 1997 indicated that the raw abundance of free embryos differed significantly with time of day (df = 11,5, $F = 4.64$, $p < 0.05$), however, this was not the case for the abundance adjusted to numbers of free embryos per 1000 m^3 (df = 11,5, $F = 1.38$, $p > 0.05$) (Figure 3). Nevertheless, there was a trend, using both measures, for an increase in abundance of free embryos after dusk and a decrease after dawn, followed by an increase around midday.

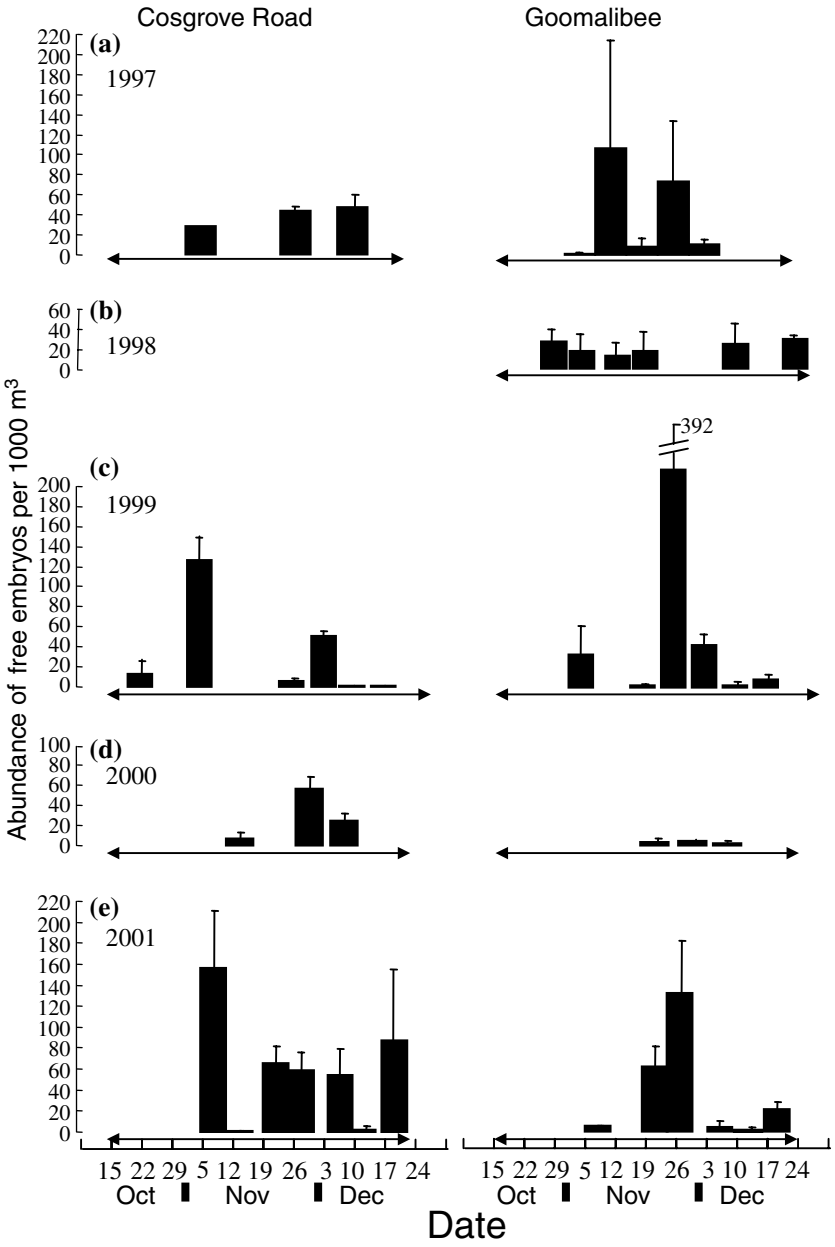

Figure 2. Mean + 1 SE adjusted abundance (per 1000 m^3) of drifting Murray cod free embryos in the Broken River in (a) 1997, (b) 1998, (c) 1999, (d) 2000 and (e) 2001.

Length, amount of yolk and age of drifting free embryos

The mean length of Murray cod free embryos from most samples was usually between 8.5 and 10.0 mm, but ranged between 7.2 and 11.0 mm (Figure 4a). Mean length of free embryos differed significantly among sampling dates at each reach in three of the four years (Table 1). In most years, however, I could detect no obvious patterns in the relationship between length and date of collection, except in 2001 at Cosgrove Road and Goomalibee,

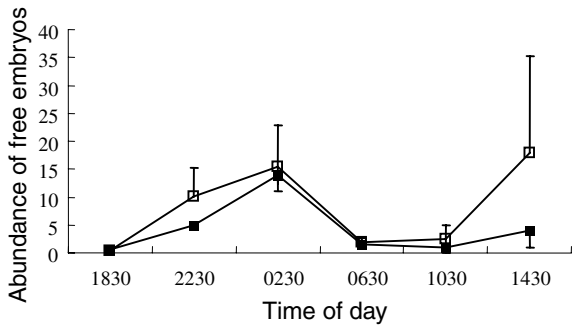

Figure 3. Mean ± 1 SE raw (black boxes) and adjusted abundance (per 1000m^3) (open boxes) of drifting Murray cod free embryos in the Broken River, for 24 h between 2 and 3 December, 1997.

when mean length decreased through time, and in 2000 at Cosgrove Road, when an initial high mean length was followed by a low mean length and then a gradual increase through time (Figure 4a). Regression analyses indicated that mean length of free embryos was significantly negatively correlated with temperature in 2001 only, at both Cosgrove Road ($R^2 = 0.18$, $p < 0.001$, $n = 244$) and Goomalibee ($R^2 = 0.25$, $p < 0.001$, $n = 223$), although only a relatively small amount of variation in length was explained by temperature.

Mean YSI of Murray cod free embryos ranged from 0% to 54% during the 4 years of sampling, although the majority had 20% or less of their body weight comprising yolk (Figure 4b) and the overall median YSI was 3.1%. Mean YSI differed significantly with sampling date in all but one of the possible combinations of year and reach (Table 1). I could observe no obvious pattern in the relationship of YSI with sampling date in most years and at most reaches, except in 1998 at Goomalibee and 2000 at Cosgrove Road, when mean YSI was high in free embryos collected early in the breeding season and declined with time (Figure 4b). Regression analyses for the most complete data sets, indicated that YSI was significantly negatively related to mean temperature for Goomalibee in 1998 ($R^2 = 0.63$, $p < 0.001$, $n = 104$), significantly positively related to mean temperature for Cosgrove Road in 2001 ($R^2 = 0.16$, $p < 0.001$, $n = 62$) and unrelated to temperature at other times.

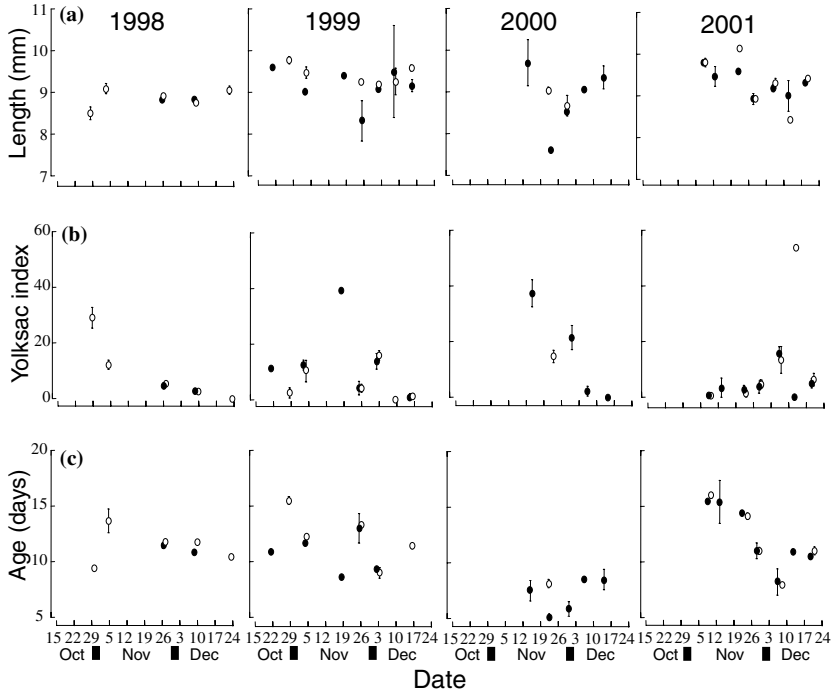

Figure 4. Mean ± 1 SE of (a) standard length, (b) YSI and (c) age of drifting Murray cod free embryos in the Broken River in 1998, 1999, 2000 and 2001. Closed circles = Cosgrove Road, open circles = Goomalibee.

Table 1. Degrees of freedom (df) and mean squares (ms) for the results of ANOVAs of Length, YSI and Age with sampling date, for Murray cod free embryos in the Broken River in 1998, 1999, 2000 and 2001.

	Length				YSI				Age			
	Cosgrove Road		Gomalibee		Cosgrove Road		Gomalibee		Cosgrove Road		Gomalibee	
	df	ms	df	ms	df	ms	df	ms	df	ms	df	ms
1998												
Date	1	0.021	4	1.360**	1	70.306	4	1860.014***	1	1.15	4	20.687***
Error	92	0.120	247	0.172	61	21.497	101	24.580	15	0.308	35	2.261
1999												
Date	6	0.713*	5	1.056***	5	278.655***	5	335.167**	4	10.712**	4	39.708***
Error	121	0.252	235	0.132	26	44.712	43	33.723	13	0.674	37	0.703
2000												
Date	5	1.884***	1	0.433	4	964.356*	–	–	5	14.421*	–	–
Error	85	0.116	20	0.121	27	210.352	–	–	22	1.909	–	–
2001												
Date	6	3.403***	5	11.415***	6	256.486***	5	599.892***	6	96.242***	5	62.817***
Error	237	0.192	217	0.139	55	29.344	37	30.029	54	6.747	34	0.614

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

The mean age of drifting Murray cod free embryos ranged from a minimum of 5 days-old to a maximum of just above 15 days-old (Figure 4c), with the median age being 11 days-old. Mean age differed significantly with sampling date in all but one of the possible combinations of year and reach (Table 1). In only 2001, at both Cosgrove Road and Goomalibee, was a pattern in the relationship of age with sampling date evident: in these cases, mean age of free embryos was more than 15 days-old, early in the breeding season and had declined to approximately 10 days-old by the end of the season. There was a significant positive relationship between age and temperature for free embryos from Cosgrove Road in 2000 ($R^2 = 0.21$, $p < 0.01$, $n = 28$) and significant negative relationships between these variables for both reaches in 1999 (Cosgrove Road, $R^2 = 0.37$, $p < 0.01$, $n = 18$; Goomalibee, $R^2 = 0.61$, $p < 0.001$, $n = 61$) and 2001 (Cosgrove Road, $R^2 = 0.54$, $p < 0.001$, $n = 61$; Goomalibee, $R^2 = 0.58$, $p < 0.001$, $n = 40$).

I found no significant relationships between age and YSI for free embryos at Goomalibee in 1998 and Cosgrove Road in 1999 or 2000. There were, however, significant negative exponential relationships between these two variables for Goomalibee in 1999 ($R^2 = 0.31$, $p < 0.001$, $n = 42$) and for Cosgrove Road ($R^2 = 0.60$, $p < 0.001$, $n = 60$) and Goomalibee ($R^2 = 0.75$, $p < 0.001$, $n = 40$) in 2001.

An estimate of duration of drift of free embryos

In general, I have estimated that Murray cod free embryos had been drifting between 3 and 8 days when collected, although the least number of days (excluding the 2000 results) was 0 and the greatest, 14 (Figure 5). The distributions in 1998 and 2001 were bi-modal, but there was a peak in 1998 at 6 days and in 2001 at between 5 and 7 days. The distribution of days spent in the drift for free embryos in 1999 was unimodal at 4–5 days. I obtained an anomalous result in 2000, when I estimated that in some cases, free embryos had been drifting for negative days and serves to reinforce the unusual results that were obtained for this breeding season as a whole.

Spawning time and duration

My back calculation of ages of drifting free embryos and estimation of incubation duration, indicated that spawning of Murray cod each year in the Broken River generally occurred between mid-October until the beginning of December (Figure 6). The 2000 season was unusual in that spawning commenced considerably later than in other years. This was the only year in which high flows coincided with the spawning time of Murray cod. The earliest that spawning finished was in 1997 (≈25 November), and the latest that spawning

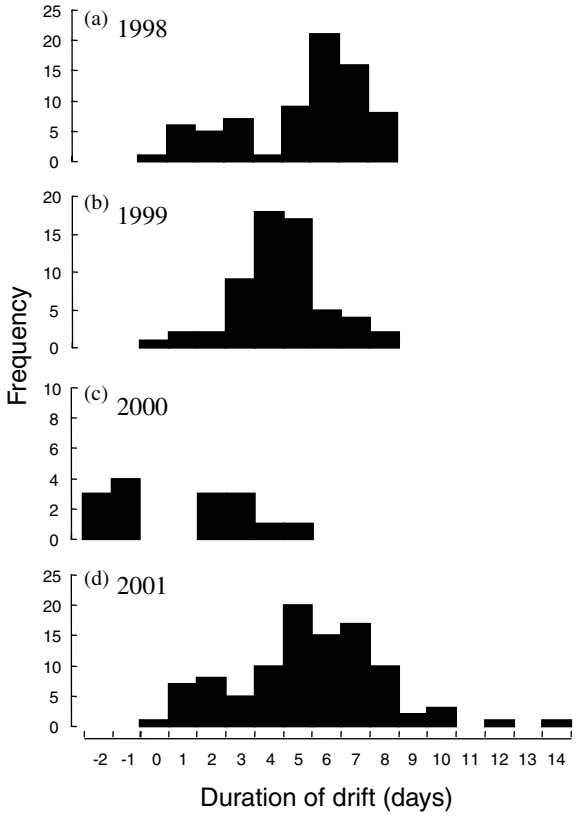

Figure 5. Frequency distributions of estimated duration of drifting of Murray cod free embryos, pooled for reaches, in (a) 1998, (b) 1999, (c) 2000 and (d) 2001.

finished was in 1998 (≈7 December). It must be borne in mind, however, that free embryos in this year and 2001 were probably drifting beyond the last date of collection, and so spawning may have continued on for another week or so.

Fish began spawning coincident with mean temperatures from 14.6 to 23.0 °C, with a range of degree days (cumulative from 1 July) from 1067 to 1860, with flows from a steady base flow of 1.7 m^3 s^{-1} to a rising flow of 23 m^3 s^{-1}, with hours of daylight ranging from 12:47 to 13:37 and with most phases of the moon (Figure 6). If the unusual year of 2000 is omitted, the range of all variables at the time that fish began spawning reduces considerably. Thus, between the earliest date and the latest date that spawning commenced, there was a 13 day difference, a temperature range of 2.5 °C, a difference of 204 degree days and 14 min of daylight.

When I examined the mid-point of spawning I found that, apart from 2000, when this occurred on 17 November, there were only 8 days between the earliest and latest dates (Figure 6). Temperature ranged from 18.4 to 25.6 °C (18.4–21.5 °C without 2000), degree days were between 1526 and 2227 (1526–1692 without 2000), hours of daylight ranged from 13:35 to 14:08 (13:35–13:52 without 2000) and the moon was in a variety of phases. At the mid-point of the spawning period, discharge was generally fluctuating only slightly at base flow, except in 2000, when it was declining from a peak of about 100 m^3 s^{-1}.

Discussion

Drift of free embryos and larvae has only occasionally been documented for Australian freshwater fishes[3,6,7] (see Humphries et al. 2002), although, based on its prevalence in species on other continents (Brown & Armstrong 1985; Pavlov 1994), it is probably considerably more common than its presence in the Australian literature might imply. Because their early life history stages have been caught rarely in other habitats during the first few weeks of life[3] (Humphries et al. 2002; King 2002), Murray cod have been termed 'obligate' drifters (Humphries et al. 2002) and from my results it is likely that they can also be classified as 'active' drifters (sensu Pavlov 1994), although their orientation in the current has not been observed. Their advanced stage of development, relative to some species which drift (Gadomski & Barfoot 1998), diel variation in abundance in the drift, and my observations indicate that Murray cod free embryos are active and strong swimmers and likely to be able to determine their location in the water column of a river.

Prior to major environmental damage and intense fishing pressure, Murray cod were almost certainly far more abundant in lowland rivers than they are today (Rowland 1989). Therefore, the abundance of drifting Murray cod free embryos would have been greater in the past than the maximum that I found in my study (400 free embryos per 1000 m^3). Nevertheless, the densities I recorded are within the range found by other workers for drifting free embryos and larvae of other species elsewhere (Sager 1987; Pavlov 1994;

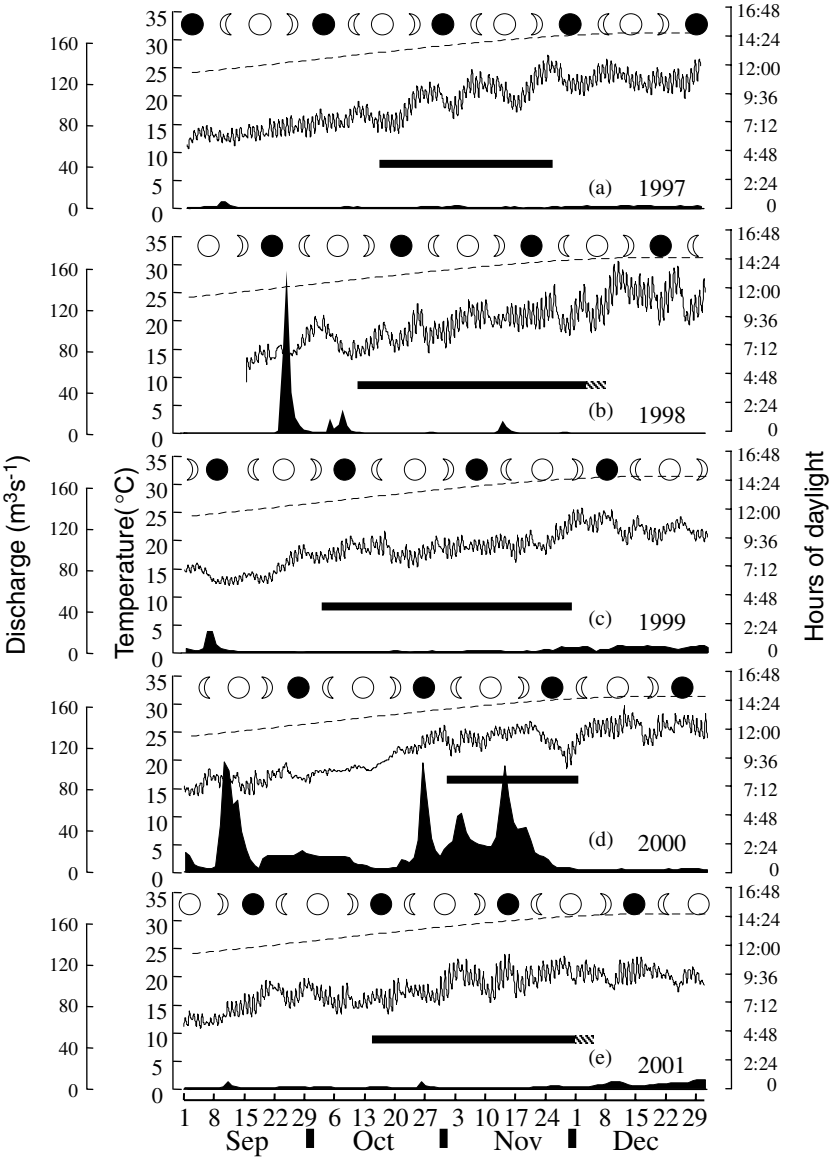

Figure 6. Plots of estimated spawning period (solid horizontal bar), discharge (filled area), temperature (solid squiggly line), hours of daylight (dashed line) and moon phase for Murray cod from the Broken River in (a) 1997, (b) 1998, (c) 1999, (d) 2000 and (e) 2001. Note: hatched bar at end of spawning period in 1998 and 2001 indicate the likelihood of spawning continuing beyond this date due to cessation of sampling (see Methods for details).

Copp et al. 2002), although abundance may be related to the size of the river in which they occur (Copp et al. 2002).

In general, studies have found no relationship of abundance of free embryos and larvae in the drift with discharge (Corbett & Powles 1986; Copp et al. 2002), which is in agreement with my results for Murray cod in the Broken River. However, spring freshets and rises in discharge have been found to coincide with the commencement of drifting behaviour or peak abundance of the free embryos of some species (Carter et al. 1986; Naesje et al. 1986). Daily variation in the abundance of drifting free embryos has been ascribed to daily variation in current speed, moonlight, cloud cover and turbidity, with the strong implication that

light levels are particularly influential (Pavlov 1994).

The range of lengths of Murray cod free embryos that I collected was small relative to that of the amount of yolk (YSI) and age. This can probably be explained because free embryos feed endogenously while in the 'nest' and for the first few days drifting, and are therefore not going to increase in length substantially until they begin to feed exogenously (Chambers & Trippel 1997). Otolith growth and utilisation of yolk of Murray cod free embryos, however, continue during this time (Vogel 2003).

I found relatively little variation in mean length, YSI and age of drifting free embryos within a particular sampling date and reach, which must mean either that the conditions in a particular reach and on a particular date (or in the days preceding collection) had a significant effect on growth and development, and/or that collections comprised individuals from the same brood, which were therefore more likely to exhibit similar values for these three variables. There was no indication that mean age of free embryos collected over successive weeks became progressively greater, as might be expected if free embryos began drifting synchronously at a certain age throughout the river and continued to drift for some time. Indeed, if anything, the evidence indicated just the opposite: that over successive weeks, and generally associated with increasing temperature, the mean age of drifting free embryos decreased. This makes sense, because rate of growth and development are intrinsically related to temperature in the early stages of life (Chambers & Trippel 1997), especially if they have not begun feeding exogenously. In contrast with age, length of Murray cod free embryos showed inconsistent and a generally poorer correlation with temperature. A negative relationship between age and temperature suggests strongly that at higher temperatures, free embryos develop faster, but do not necessarily grow in length substantially, and that once they reach a specific developmental stage, they leave the 'nest' and begin drifting. The small variation in age in any one sample, may also indicate that, once the most developed free embryos begin leaving, the 'nest' becomes less and less safe for the others and so they drift *en masse*. An analogous situation has been shown for smallmouth bass, *Micropterus salmoides* (Sabo & Orth 1994).

Although determining directly for how long free embryos or larvae drift is difficult, some species clearly must drift for several days, especially those that move from rivers to rearing habitats in lakes (Franzin & Harbicht 1992, Mion et al. 1998). My results suggest that Murray cod free embryos may drift for up to 10 days, but more likely for between 5 and 7 days. Species with relatively protracted pre-settlement early life history stages, such as Murray cod, have the potential to drift considerable distances. However, as has been implied above, it is also likely that for many riverine fish species whose early life stages drift, there will be a degree of control over how far they drift. Prior to drifting, whilst in the 'nest', free embryos may have less control over their movements, especially during times of high discharge. My results of the duration of drifting in the year 2000 indicated that some individuals drifted for 'negative' days. In this year, free embryos appeared to be drifting at a younger age, at a lesser developmental stage and with more yolk sac than was usual. The first 2/3 of the spawning period of Murray cod in 2000 coincided with the highest discharges seen in that river for four years, and, although discharge was declining during the period that the free embryos would have been in the 'nest', it is possible that they were displaced prematurely. 'Wash-out' of the young stages of fish by high flows has been recorded elsewhere (Harvey 1987; Pavlov 1994), is consistent with experiments and observations on the abilities of these fish to maintain their position if current velocities increase above critical levels (Corbett & Powles 1986; Franzin & Harbicht 1992) and is unlikely to be advantageous to the survival of young Murray cod: a lesser stage of development may mean greater risk of predation and a reduced ability to orientate. On the other hand, greater dispersal may be facilitated by high flows, thus contributing to colonisation of new areas or downstream lakes (Naesje et al. 1986), as well as aiding gene flow (Bilton et al. 2001).

Despite early work with Murray cod which indicated that a rise in water level was not needed to induce spawning (Lake, 1967a), there has been a perception amongst some scientists, managers, but especially the wider community, that this species, and indeed Murray-Darling Basin species in gen-

eral, spawns in response to increases in discharge (see Humphries et al. 1999). There was large interannual variation in flow conditions associated with the spawning period of Murray cod in my 5-year study, however, it is virtually impossible to rule out that spawning might be initiated by very small increases in discharge (as in e.g. Colorado pikeminnow, Nesler et al. 1998) without knowing what increase is detectable or meaningful to running ripe Murray cod and during which time period preceding the actual event it occurs. It is as reasonable, from my results, to suggest that Murray cod spawns in response to a decrease in discharge. Overall, the range in the time of mid-spawning over the 5 years was only about a week, which suggests that either particular environmental cues are well correlated with date or that circannual rhythms are paramount in influencing when fish spawn.

The timing of spawning is, in many respects, not the issue most relevant to our understanding of the life history of a species of fish: it should be considered in the context of the conditions in which the early life history stages are going to grow, develop, survive or die. Indeed, definitions of life history modes should probably include traits, of both adults and progeny, which relate to recruitment (Winemiller 1989; Humphries et al. 1999). The direct development of Murray cod means that they are well developed and able to actively search for food, while still retaining some of their yolk reserves. Furthermore, they have a large gape and are thus able to take advantage of a range of prey types (King 2002). The species is long-lived and spawns every year at about the time when discharge is relatively predictably declining to base flow levels. It is also the time when temperatures will be maintained above 20 °C for 3 or 4 months, probably promoting invertebrate production and rapid growth in the Broken River, as elsewhere (Mills and Mann 1985). Temperatures which optimise growth during the early life history appear to be critical for survival in many species of riverine fish (e.g. Henderson & Corps 1997; Grenouillet et al. 2001). Nevertheless, once through the free embryo period, juvenile Murray cod should be able to take advantage of a range of conditions at this time and recruit relatively well each year, barring density-dependent effects. Indeed, density-dependence may be a significant influence on year-class strength in many years. Adult Murray cod are known to be piscivorous and establish territories to which they are faithful over prolonged periods[4] and so it is not surprising that their free embryos disperse by drifting. Drifting not only probably reduces the potential for cannibalism (Henderson and Corps 1997), it may also reduce competition with sibs (Waser 1985). Drifting is, however, a double-edged sword: on the one hand potentially reducing one or more sources of mortality and on the other, exposing oneself to others (McCarthy 1999).

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