

# Reproduction in three species of rainbowfish (Melanotaeniidae) from rainforest streams in northern Queensland, Australia

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**Abstract** – The reproductive biology of three species of rainbowfish (Melanotaeniidae) in northeastern Australian rainforest streams was investigated. Two species, *Melanotaenia eachamensis* and *Cairnsichthys rhombosomoides* are endemic to the area, whereas the third, *M. splendida splendida*, is more widespread. The species were all highly fecund, producing many hundreds of eggs between 1.10 and 1.24 mm in diameter. *Melanotaenia eachamensis* was the most fecund, produced the largest eggs of the three species, and consequently exhibited the greatest maternal investment (as measured by gonadosomatic index). The majority of reproductive effort occurred during the dry season, although reproductively active fish were present year-round for each of the species, but particularly so for *M. s. splendida* and *C. rhombosomoides*. No evidence for a role by temperature or photoperiod as environmental cues for reproduction was found, and it was suggested that gonad development was strongly tied to somatic growth. The concentration of reproduction to the dry season ensures that larvae are produced during a period of relatively stable and benign physical conditions. Comparison of temporal changes in gonadosomatic index values suggest that the spawning season of *M. eachamensis*, which occurs in high-elevation streams, is more restricted and commences about 1 month earlier than either other species. A similar phenology was observed for the *M. s. splendida* population found at high elevation and highlights the potential for spatial differences in stream productivity to influence life history.

**B. J. Pusey<sup>1</sup>, A. H. Arthington<sup>2</sup>,  
J. R. Bird<sup>2</sup>, P. G. Close<sup>1</sup>**

<sup>1</sup>Cooperative Research Centre for Tropical Rainforest Ecology and Management and <sup>2</sup>Centre for Catchment and In-Stream Research, Griffith University, Nathan, Queensland, Australia

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B. J. Pusey, Centre for Catchment and In-Stream Research, Griffith University, Nathan, Queensland, Australia 4111

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Un resumen en español se incluye detrás del texto principal de este artículo.

## Introduction

Studies concerned with the reproduction of tropical freshwater fishes reveal a diversity of life-history styles, particularly in regard to reproductive phenology and its relationship to discharge regime. Many lowland species reproduce during the wet season, when the inundation of lateral floodplains ensures an expanded habitat (in area and diversity) and a greater array and abundance of food (Lowe-McConnell 1975; Goulding 1980; Bishop et al. 1981; Fernandes 1997). Tropical streams, in contrast, may not undergo the same seasonal expan-

sion in habitat availability, and elevated discharge may result in conditions unfavourable for reproduction (i.e., physical removal of eggs, larvae and spawning substrate). As a consequence many species of tropical and sub-tropical stream-dwelling fishes spawn during the dry season (Lowe-McConnell 1979; Milton & Arthington 1983, 1984, 1985; Wootton 1990). However, some studies have reported a more fine-grained and protracted partitioning of the spawning calendar irrespective of seasonal changes in flow regime (Kramer 1984; De Silva et al. 1985), suggesting that flow-related mortality of larvae is not necessarily the dominant fac-

tor determining patterns of reproductive seasonality in tropical stream fishes.

Temporal variation in juvenile and adult food availability, interspecific competition for food among juveniles and for spawning sites among adults and selection for reproductive isolation have all been posited as potential factors in the selection of reproductive seasonality in tropical stream fishes (Kramer 1978). Studies of temperate stream fishes have also emphasized the importance of localised productivity and the acquisition of energy in determining the timing of reproduction (Mann et al. 1984; Encino & Granado-Lorencio 1997), and these factors may be of importance in tropical stream fishes also (Roberts 1989). Other life-history parameters such as egg size, fecundity, age at first reproduction and longevity may conceivably vary in response to flow-related variation in food availability and/or mortality of larvae.

Studies of the ecology of Australian tropical freshwater fishes are limited (Bishop & Forbes 1991; Pusey et al. 1995a, b; Pusey & Kennard 1996) and none have examined the reproductive biology of tropical stream-dwelling fishes. The present study was aimed at defining the reproductive biology of three species of rainbowfish that occur in rainforest streams of the Wet Tropics region of northeastern Australia but that have contrasting distributions at the local and regional scales. The *Melanotaenia splendida* (Peters) species complex is widely distributed across northern Australia, with the subspecies *M. s. splendida* occurring on the eastern side of the Great Dividing Range from Cape York Peninsula south to the Burnett River in southern Queensland (Allen 1989). It inhabits a variety of habitats in the study area, including wetlands and lakes, and lotic environments ranging from the lower reaches of large rivers through to small low-gradient rainforest streams (Pusey et al. 1995a, 1997; Pusey & Kennard 1996). In lowland sections of rivers with extensive floodplains, this species has been shown to be short lived (2–3 years), moderately fecund and to breed throughout the year, albeit with the greatest activity occurring during the summer wet season (Beumer 1979; Bishop et al. 1981). The reproductive biology of *Melanotaenia eachamensis* Allen & Cross or *Cairnsichthys rhombosomoides* (Nichols & Raven), in contrast, remain unstudied. Both species are narrowly distributed endemics of the Wet Tropics region of northern Queensland (Pusey & Kennard 1996; Pusey et al. 1997) and are generally limited to small, well-shaded streams. *Cairnsichthys rhombosomoides* does not occur above 100 m elevation (Pusey & Kennard 1996), whereas *M. eachamensis* is rarely found below an elevation of 700 m a.s.l. (Pusey et al. 1997). These latter two species never

co-occur, but each occasionally co-occurs with *M. s. splendida*.

The present study was undertaken as part of a larger program investigating the environmental flow requirements of the freshwater fishes of Queensland involving studies of distribution, relationships between habitat structure and fish assemblage structure, the phenology of larval fish abundance and the microhabitat requirements of adult and larval fishes. The choice of species in the study allows a comparison of life histories within and across genera of closely related species and a comparison of the life history strategies of species with very different patterns of distribution (geographically very wide versus narrow).

## Methods

### Study area and study sites

The Wet Tropics region of northern Queensland, a World Heritage area, contains the largest area of tropical rainforest in Australia (Tracey 1982). The Johnstone River (catchment area=1634 km<sup>2</sup>) is comprised of the North and South Johnstone rivers, both of which descend through steep gorges to flow over a narrow coastal plain before joining and exiting to the sea at Innisfail (Fig. 1). Rainfall is the highest in Australia (2000–3500 mm · year<sup>-1</sup>) and mean annual discharge in the Johnstone River is consequently high (4.7 × 10<sup>6</sup> million liters · year<sup>-1</sup>). The majority of discharge (about 70%) occurs during the monsoonal wet season (January to April), usually as the result of one or two cyclone-associated run-off events, but discharge remains high throughout the year due to

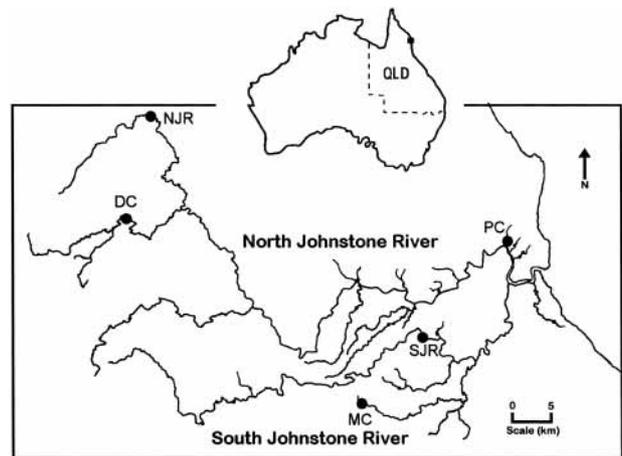


Fig. 1. Location of the study sites in the Johnstone River drainage. Inset shows the location of the Johnstone River with respect to the Australian continent. Site codes are: North Johnstone River – NJR; Dirran Creek – DC; Mena Creek – MC; South Johnstone River – SJR, and Polly Creek – PC

high base flows originating from basaltic groundwater and the orographic effect of the Great Dividing Range on moisture-laden southeasterly winds, which predominate from May to October. Discharge in rivers of this region is highly predictable, with most of the predictability due to constancy (*sensu* Colwell 1974) (Pusey et al. 1995a). Predictably low flows occur during the period from June to November. Daily discharge data for each of the

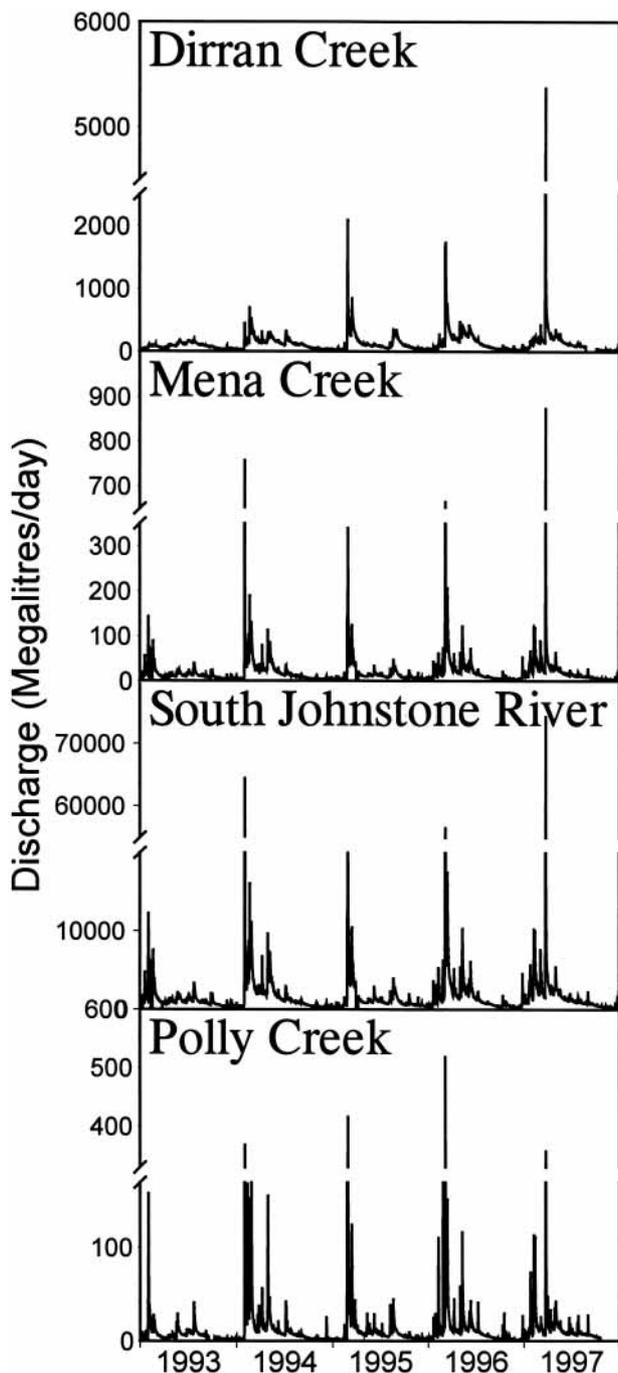


Fig. 2. Daily discharge (million liters · day<sup>-1</sup>) for four of the study sites over the period January 1993 to December 1997

study drainages was supplied by the Queensland Department of Natural Resources, and plots of discharge over the study period reveal a consistent basin-wide pattern of stream flow (Fig. 2). A single large flood occurred each year in February or March, with smaller spates occurring from January to April and occasionally as late as July. Low flows persisted from August to November or December. Polly Creek, probably as a consequence of its small catchment area, tended to have more small spates per year than did other drainages. Overall, the general flow pattern was one of elevated flows during the wet season (January to April), usually accompanied by one major flood per year in February or March, and stable low flows from August to December.

We collected fish from five locations in the Johnstone River basin (Fig. 1, Table 1). Two sites (NJR and DC on the North Johnstone River and Dirran Creek, respectively) were located above 700 m.a.s.l., and the remainder (SJR, MC and PC on the South Johnstone River, Mena Creek and Polly Creek, respectively) located below 100 m.a.s.l.. Upstream of the study sites, the North Johnstone River and Dirran Creek catchments are composed entirely of nutrient-rich Atherton Basalt, whereas the South Johnstone River, Polly and Mena Creek drain catchments are comprised primarily of nutrient poor Tully Granite or Barron River Metamorphics (Dickenson et al. 1993).

#### Sampling procedure

We sampled fish populations by a combination of electrofishing and seine-netting at 4-monthly intervals during the period June 1994 and June 1997 and monthly intervals from July 1997 to April 1998. We retained fish for laboratory examination after anesthetization in a 2-phenoxyethanol solution and fixing in 10% buffered formalin. We measured the water temperature at each site and on each sampling occasion with a mercury bulb thermometer or a max/min thermometer deployed overnight on the day of sampling. We were also fortunate to have been granted access to 12 months (two sites: SJR and NJR) or 6 months (one site: DC) of continuously logged water temperature data (Franklyn & Moritz, unpublished data).

#### Laboratory examination

We measured (SL to the nearest 0.5 mm), weighed (to nearest 0.01 g), sexed and staged (according to the criteria listed in Table 2) each specimen. The gonad was then removed, weighed and the gonadosomatic index (GSI) estimated. Rainbowfish produce eggs in distinct batches and we therefore

Table 1. Characteristics of the study sites and the species present within each. The species list includes only the species included in the present study.

Site No.	Stream	Latitude	Longitude	Elevation (m.a.s.l)	Gradient (%)	Species sampled
High-elevation sites						
1.	North Johnstone – main channel	17°21'36''	145°43'48''	740	0.97	<i>M. s. splendida</i>
2.	Dirran Creek	17°27'00''	145°36'00''	720	0.26	<i>M. eachamensis</i>
Low-elevation sites						
3.	Mena Creek	17°40'12''	145°52'48''	100	2.1	<i>M. s. splendida</i> <i>C. rhombosomoides</i>
4.	South Johnstone – main channel	17°34'48''	145°55'48''	30	0.08	<i>M. s. splendida</i>
5.	Polly Creek	17°28'48''	146°01'48''	5	0.05	<i>M. s. splendida</i> <i>C. rhombosomoides</i>

Table 2. Scheme used for categorizing gonadal development and maturity.

Stage	Description
I	<i>Immature</i> - gonads not visible or small, thin and strap-like.
II	<i>Early developing</i> – ovary pale orange, with few oocytes, visible at $\times 20$ magnification; testes elongate, whitish sac.
III	<i>Developing virgin and resting adult</i> – ovaries orange, often with red flecks, eggs opaque, just visible to naked eye, small oil droplets present in larger oocytes; testes gray-white.
IV	<i>Late developing</i> – ovaries orange, eggs clearly visible, opaque, larger oil droplets present throughout oocyte; testes opaque, white to gray-white, no milt present.
V	<i>Gravid</i> – ovaries yellow-orange with some translucent, round eggs, oil globules forming single, polarized mass; testes white, and extrude milt with pressure.

quantified fecundity in two ways. An ovarian subsample (about one third of the gonad) was removed and, weighed and the number of yolked eggs of all sizes was counted under a stereomicroscope. We estimated total fecundity by scaling the number of eggs counted by the appropriate ratio of subsample to total ovarian weight. We also counted the number of ovulated or near ovulated eggs present in the ovary of stage V fish, and this is hereafter referred to as batch size. We estimated mean egg diameter from a randomly selected sample of ten ovulated or near ovulated eggs from each stage V female using a calibrated ocular micrometer.

#### Statistical analyses

All statistical tests were performed in SPSS for Windows<sup>TM</sup>. We used analysis of covariance (ANCOVA) to test for spatial variation (between-site) and sexual dimorphism in the relationship between body length and weight (after log transformation of each variable) for each species. We used ANOVA to test whether mean size increased as fish matured reproductively after transforming length ( $\log x + 1$ ) to satisfy concerns about data heteroscedasticity. We pooled GSI data across all years from June 1995 to April 1998 to estimate monthly variation in mean GSI. We recognize that information about interannual variation in reproductive effort may

have been lost by doing so but reasoned that the low interannual variation in rainfall and flow regime characteristic of the region (Pusey et al. 1995a; Pusey and Kennard 1996), amply demonstrated in the discharge plots presented in Fig. 2, suggests such variation to be minimal over the period of our study. Moreover, the small sample sizes necessitating data pooling were necessary to minimize the long-term impact of sampling on the fish assemblages under investigation as part of a suite of additional studies. We used ANOVA to test for between-month variation in mean GSI (after  $\log x + 1$  transformation) for each of the sexes. We used ANOVA to test for spatial (between site) variation in female GSI (after transformation) for *M. s. splendida* only but limited the analysis to the peak spawning period only (September to October). We used SNK post hoc tests to identify significant differences between treatment groups

## Results

### Water temperature

Water temperature (both continuously logged and spot records) varied seasonally over the period of study, with maximum temperatures occurring from December to March (the wet season) and minimum temperatures occurring from May to August: the typical austral pattern (Fig. 3). Diurnal fluctuations tended to be minor but were greatest in

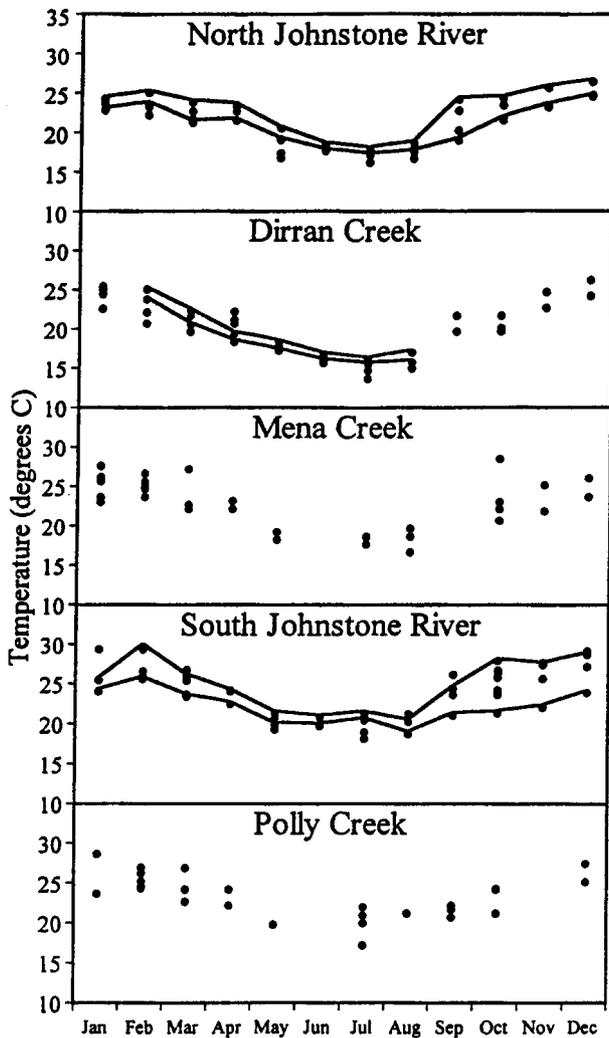


Fig. 3. Temporal variation in water temperatures at each of the study sites. Data shown are compiled from three sources; spot records (1994–1997), maximum and minimum temperatures over the period July 1997 to April 1998 and continuously logged data (shown as the solid lines)

the period September to November, when flows were diminished. Monthly temperatures were below 20°C for 4–5 months of the year at the sites located above 100 m.a.s.l. but rarely fell below 20°C at the two low-elevation sites (South Johnstone River and Polly Creek). The annual temperature range was between 12.1 and 12.8°C for all sites except the North Johnstone River site, where the annual range was 10.7°C.

Interspecific, gender-related and spatial differences in fish size

The three species rarely exceeded 100 mm in length and 10 g in weight, and exhibited very similar changes in weight with increasing size (Table 3).

Although male fish attained greater maximum length than did female fish (Table 4), sexual dimorphism with respect to weight at a given length was not detected (Table 3). Spatial differences in the relationship between body weight at a given length were detected, however, with *M. s. splendida* and *C. rhombosomoides* collected from the highest elevation site at which they were present being significantly heavier for a given length than conspecifics at low elevation sites (Table 3).

Maturation and temporal changes in reproductive stages

Male *M. s. splendida* commenced maturation (stage II) at 30.5 mm, females at 32.5 mm, although many larger fish exhibited no evidence of gonad development (Table 4). Gonad maturation was commensurate with somatic growth, and the mean length at each stage was significantly different from all other reproductive stages (Table 4). The mean length of ripe (stage V) males was 75.5 mm SL. Concomitant sexual and somatic maturation was also evident for females, although females matured to stage V at smaller sizes (mean length of stage V females: 55.2 mm) (Table 4). The majority of fish collected from January to April were at stage III or less, whereas most fish were at stage III or above by July (Table 5). Ripe females were present in all months but most abundant from July to September, and stage IV males were present all year.

Initiation of gonad maturation in *M. eachamensis* also began at small size (27 and 26 mm for male and female fish, respectively). Gonad maturation in both male and female *M. eachamensis* was commensurate with somatic growth and followed a pattern almost identical to that observed in *M. s. splendida*, with significant increases in size being recorded for each maturation stage (Table 4). Ripe females (stage V) were significantly smaller (49.9 mm) than males (54.4 mm) ( $t=4.47$ ,  $n=22; 99$ ,  $P<0.001$ ). Gonad maturation followed a similar temporal pattern in both sexes (Table 5), with the majority of fish collected in the period from January to April being at stage III or less. All fish caught in May were at stage III, and most fish had attained full maturity by August.

*Cairnsichthys rhombosomoides* also commenced gonad maturation at small size (34 and 27.5 mm for female and male fish, respectively) (Table 4). Gonad maturation was similarly commensurate with somatic growth, with significantly larger mean lengths at each successive maturity stage being detected by ANOVA (Table 4), although stage V females as small as 38 mm and males as small as 43.5 mm were observed. Most specimens collected during August to December were at stage

Table 3. Length/weight characteristics of each of the species examined.

Species	<i>n</i>	Mean length	Intercept	Gradient	<i>r</i> <sup>2</sup>	Sexual dimorphism	Spatial polymorphism
<i>M. s. splendida</i>	1965	51.7±0.24 (20–104)	−4.761	2.986	0.967	1.94 <sup>NS</sup>	154.5*** NJR>MC>PC>SJR
<i>M. eachamensis</i>	270	38.5±0.62 (14–62)	−4.914	3.087	0.966	3.48 <sup>NS</sup>	–
<i>C. rhombosomoides</i>	372	43.2±0.34 (24–63)	−4.608	2.878	0.872	0.004 <sup>NS</sup>	25.27*** MC>PC

The total number of fish examined (*n*), the mean length (±S.E.) (in mm) and range in length (in parentheses) are given. Parameters (intercept, gradient and goodness of fit) resulting from linear regressions of log-transformed weight (g) against log-transformed length (mm) are also given. Logs were base 10. *F* values and their associated levels of significance are given for analyses of covariance testing for gender-related (sexual dimorphism) and site-related (spatial polymorphism) differences in body weight. *M. s. splendida*, *M. eachamensis*, *C. rhombosomoides*. Sites are indicated by NJR=North Johnstone River, MC=Mena Creek, PC=Polly Creek, SJR=South Johnstone river, DC=Dirran Creek. NS: *P*>0.05, \*\*\* *P*<0.001.

Table 4. Average length in millimetres for fish within each reproductive stage.

Sex	Reproductive Stage					<i>F</i> value
	I	II	III	IV	V	
<i>Melanotaenia splendida splendida</i>						
Male	43.5 <sup>a</sup> (26–70) 76	48.7 <sup>b</sup> (30.5–85) 336	56.9 <sup>c</sup> (38–96) 294	63.1 <sup>d</sup> (44.5–100) 108	75.5 <sup>e</sup> (48–104) 15	110.1***
Female	38.8 <sup>a</sup> (24.5–53.5) 42	43.9 <sup>b</sup> (32.5–76) 146	51.8 <sup>c</sup> (34–82) 457	53.5 <sup>d</sup> (37–96) 317	55.2 <sup>d</sup> (38–85) 99	59.1***
<i>Melanotaenia eachamensis</i>						
Male	26.7 <sup>a</sup> (20–43) 9	35.3 <sup>b</sup> (27–60) 53	40.0 <sup>c</sup> (30.5–52) 28	47.5 <sup>d</sup> (40.5–60) 23	54.4 <sup>e</sup> (49–62) 14	56.4***
Female	22.1 <sup>a</sup> (17–27) 8	30.2 <sup>b</sup> (26–35) 18	40.3 <sup>c</sup> (30.5–59) 37	46.1 <sup>d</sup> (27–57) 34	49.9 <sup>e</sup> (37–59) 22	46.9***
<i>Cairnsichthys rhombosomoides</i>						
Male	32.7 2	38.9 <sup>a</sup> (27.5–53) 44	43.3 <sup>b</sup> (29–59) 57	46.1 <sup>c</sup> (36–59) 55	52.2 <sup>d</sup> (43.5–63) 16	24.8***
Female		37.9 <sup>a</sup> (34–43) 19	42.0 <sup>b</sup> (32–58) 65	44.2 <sup>c</sup> (35–59) 83	47.6 <sup>d</sup> (35.5–57) 16	13.0***

Range in length given in parentheses. The *F* value resulting from ANOVA test of between-stage variation in mean length is given for each species and sex within species. \*\*\* *P*<0.001. Mean lengths that are significantly different at *P*<0.05 are denoted by different superscript letters. The sample size is listed immediately below the range in lengths for each stage.

III or above, and stage IV and V fish were most abundant in August to October (Table 5).

Temporal variation in GSI

*M. splendida* exhibited highly significant monthly variation in GSI values ( $F_{10, 1008}=51.9$ , *P*<0.001 and  $F_{10, 732}=17.01$ , *P*<0.001 for female and male fish, respectively; Fig. 4a). Mean female GSI values were significantly lower in April (0.92%) and May (0.76%) than at any other time, whereas peak GSI values occurred in September (3.59%) and October (3.74%) and were significantly higher than at any other time. GSI decreased significantly in Novem-

ber and December, relative to the October peak, but still remained significantly higher than for August, January or February. Temporal changes in male GSI closely followed those observed in female fish (Fig. 4a), with peak values occurring in September (0.68%) and minimum values occurring from March through to May. When considered together, data presented in Fig. 4a and Table 5 suggest that, although reproductive activity is greatest from August to October, some individuals continue to spawn throughout the wet season and the colder months of the dry season (May and July).

Significant temporal variation in mean monthly GSI of female and male *M. eachamensis* was de-

## Reproduction in three species of rainbowfish

Table 5. Temporal variation in the abundance of male and female rainbowfish within each reproductive stage over a calendar year.

Month	n	Reproductive stage					Month	n	Reproductive stage				
		I	II	III	IV	V			I	II	III	IV	V
Male						Female							
<i>Melanotaenia splendida splendida</i>													
January	89	2	53	22	22	1	January	94	1	7	53	33	6
February	119	21	60	17	2	0	February	123	2	15	53	29	1
March	88	8	64	24	4	0	March	150	10	27	44	14	5
April	18	0	39	50	11	0	April	41	2	12	72	12	2
May	56	25	55	18	2	0	May	72	8	24	58	6	4
July	109	12	38	36	12	2	July	151	5	21	44	15	15
August	122	10	30	48	11	1	August	143	4	13	31	33	19
September	55	1	18	36	31	15	September	56	5	0	18	50	27
October	70	0	10	64	24	2	October	110	0	1	29	63	7
November	55	2	29	47	20	2	November	59	0	3	42	44	9
December	48	4	26	46	24	0	December	62	2	8	44	44	2
<i>Melanotaenia eachamensis</i>													
January	17	41	47	12	0	0	January	22	36	32	18	5	9
February	17	0	76	24	0	0	February	8	0	12	25	50	12
March	27	4	56	33	7	0	March	23	0	9	82	9	0
April	14	0	50	50	0	0	April	5	0	0	80	0	20
May	1	0	0	100	0	0	May	2	0	0	100	0	0
August	18	0	0	6	66	28	August	15	0	0	7	26	67
October	4	0	0	0	75	25	October	10	0	0	20	80	0
November	9	0	33	0	67	0	November	12	0	8	0	75	17
December	14	7	50	28	15	0	December	22	0	32	14	27	27
<i>Cairnsichthys rhombosomoides</i>													
January	6	0	67	0	33	0	January	5	0	0	20	80	0
February	17	6	53	35	6	0	February	18	0	33	28	39	0
March	21	0	33	33	33	0	March	23	0	22	30	48	0
April	26	0	27	41	27	5	April	21	0	5	18	62	15
May	18	0	33	39	28	0	May	27	0	7	63	30	0
July	12	0	25	33	33	9	July	13	0	15	38	38	9
August	20	0	25	25	50	0	August	32	0	9	44	34	13
September	12	0	8	25	25	42	September	7	0	0	28	58	14
October	16	0	0	0	50	50	October	10	0	0	0	60	40
November	11	9	9	45	28	9	November	14	0	0	29	64	7
December	15	0	7	60	33	0	December	12	0	0	50	34	16

Sample sizes for each month are listed as *n* and reflect pooling of data for each species across all sites in which it was present and over the entire sampling period. Abundances are expressed as a percentage of total monthly sample (rounded to nearest unit).

tected by ANOVA ( $F_{7,116}=26.2$ ,  $P<0.001$  and  $F_{7,118}=37.5$ ,  $P<0.001$ , respectively; Fig. 4b). Mean female GSI values were greatest during the period August to December, with these months being significantly higher than any other month (SNK multiple range test). GSI was lowest during May. (The June, July and September samples each contained less than three fish and were excluded from the analysis.) Significantly elevated males GSI values were detected in August and October (SNK multiple range test).

Temporal (monthly) variation in gonad size was detected for *C. rhombosomoides* also ( $F_{10, 182}=8.75$ ,  $P<0.001$  and  $F_{10,170}=8.72$ ,  $P<0.001$  for females and males, respectively). Female *C. rhombosomoides* exhibited maximum GSI values in October, whereas maximum male GSI values occurred in both September and October. Mean GSI

was lowest during May for females and January for males (Fig. 4c).

### Spatial variation in GSI

Significant spatial variation in female GSI was detected for *M. s. splendida* when data within sites were pooled across the months of September to October (the peak breeding period) (ANOVA;  $F_{3,1008}=32.3$ ,  $P<0.001$ ). The highest mean GSI values were recorded from the South Johnstone site, and the lowest were recorded from Polly Creek (multiple range test:  $SJR>NJR=MC>PC$ ). In addition to these differences, the major spawning period in the high elevation site (NJR) commenced at least 1 month earlier in the high elevation site (NJR) than the low-elevation sites and GSI values started to decrease earlier here than at other sites.

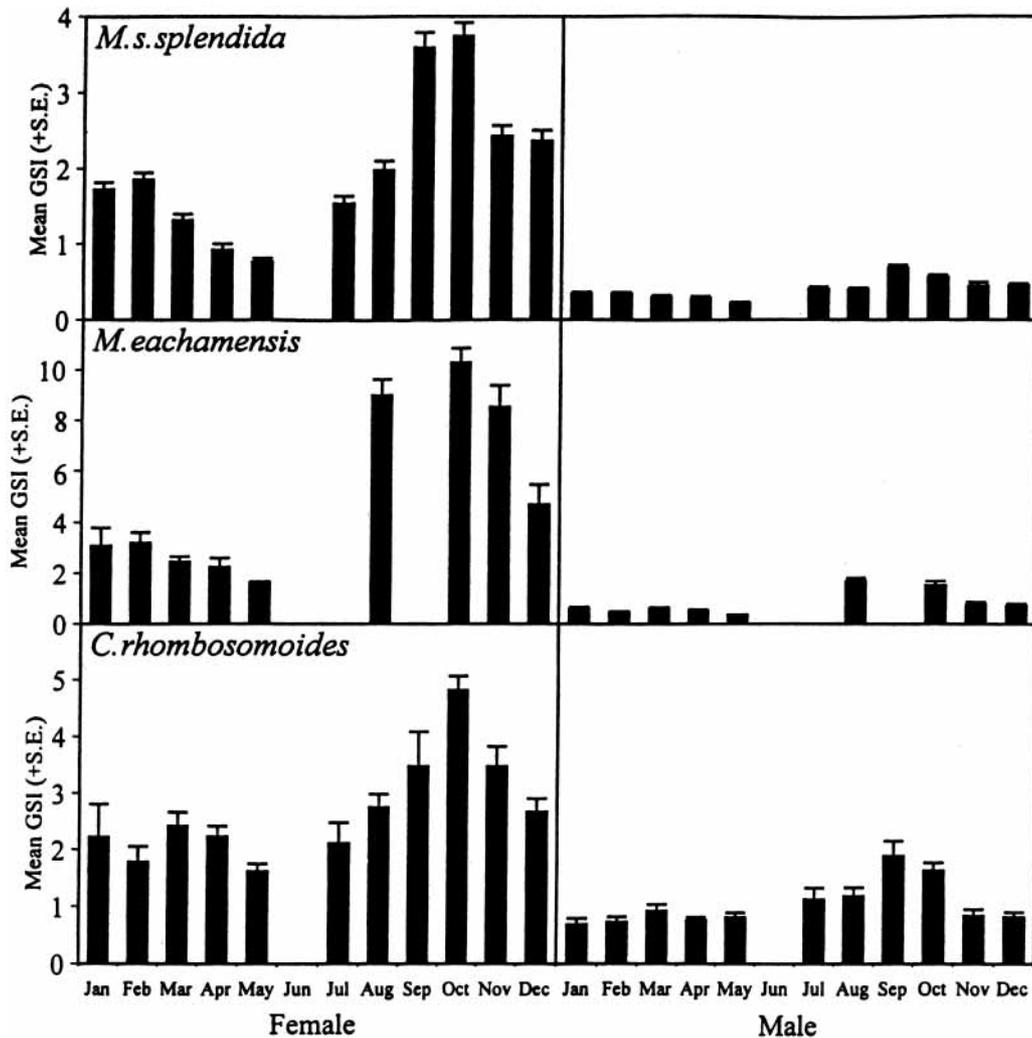


Fig. 4. Monthly changes in mean GSI ( $\pm$ SE) for three species of rainbowfish. Sample sizes were pooled within months across years and sampling locations and mean GSI was estimated from samples restricted to specimens in stage II and above (sample sizes given in Table 5). The apparent absence of error bars, particularly in the figures for males, is a result of very small error

Further, less extensive monthly variation in mean GSI was observed for the population in the lowland tributary Polly Creek, suggesting that reproduction was not as strongly seasonal as observed in the other sites (Fig. 5). No significant between-site difference in female mean GSI over the period of August to November was detected ( $t=0.96$ ,  $n=58,125$ ,  $P>0.05$ ) for *C. rhombosomoides*.

#### Fecundity

Fecundity in *M. s. splendida* was significantly related to fish size;  $\log(\text{egg number})=1.86+0.018(\text{length})$ ;  $r^2=0.415$ ,  $P<0.001$ ,  $n=185$ . Fish of 40 mm length produced about 370 eggs, whereas fish of 70 mm produced about 1655 eggs. Significant spatial variation in fecundity was detected by ANCOVA (main effect of site with length as covariate;  $F_{3,180}=6.33$ ,  $P<0.001$ ), and ANOVA of the site-

specific mean residuals resulting from the pooled regression of egg number against length ( $F_{3,184}=3.52$ ,  $P<0.05$ ) revealed that fish from the Mena Creek site were significantly more fecund than fish at Polly Creek but not fish at the other two sites. Batch size was significantly correlated with fish length (batch size =  $1.78(\text{length}) - 57.4$ ;  $r^2=0.224$ ,  $n=43$ ,  $P<0.01$ ) and varied from 2 to 177 eggs per female (0.9% to 13.8% of total egg number). No spatial variation in batch size was detected (ANCOVA with length as the covariate and site as the main effect for fish in stage V only;  $F_{3,43}=2.172$ ,  $P>0.05$ ), and batch size as a proportion of total number of eggs did not vary significantly between sites (ANCOVA;  $F_{3,36}=1.005$ ,  $P>0.05$ ). The mean diameter of stage V eggs, for females pooled across study sites, was  $1.124\pm 0.008$  mm SE ( $n=49$ ), and egg size did not vary significantly between sites ( $F_{3,48}=1.004$ ,  $P>0.05$ ).

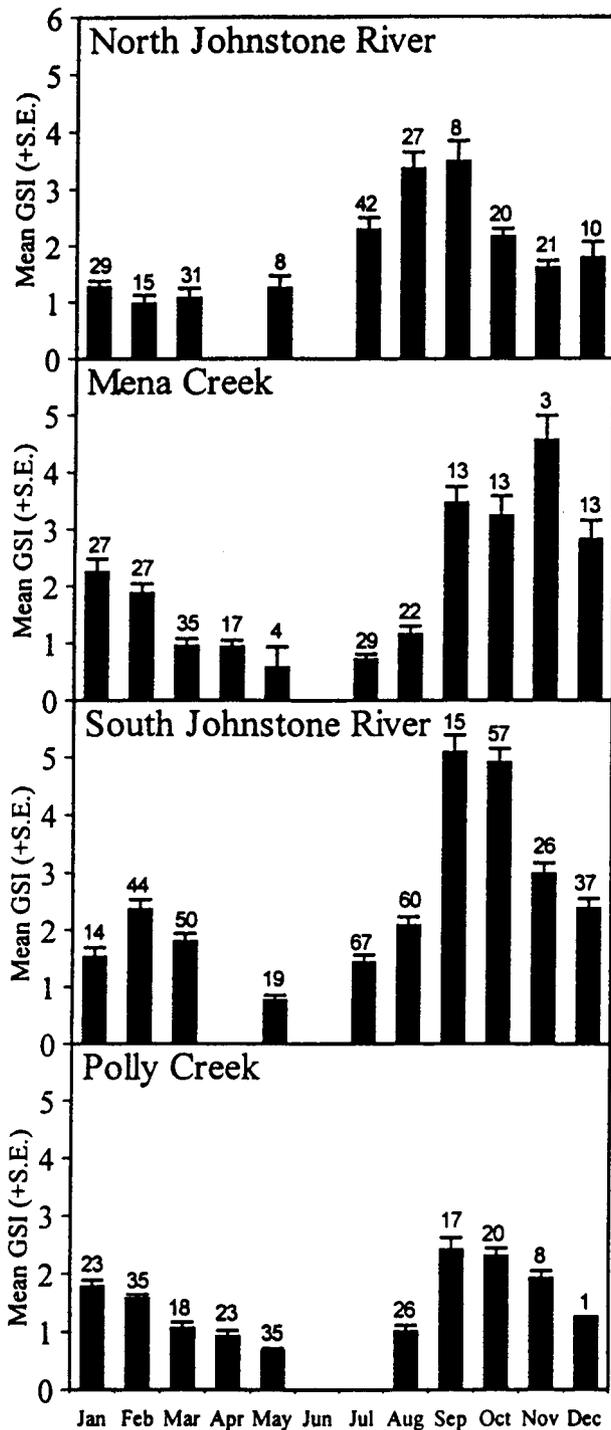


Fig. 5. Spatial variation in temporal variation in mean GSI ( $\pm$ SE) for female *M. s. splendida*. Estimates were based on data pooled within months across years for each of the four sampling locations in which *M. s. splendida* occurred

Fecundity in *M. eachamensis* varied between 206 and 2126 eggs per fish and was significantly related to fish size;  $\log(\text{egg number})=1.735+0.027(\text{length})$ ;  $r^2=0.498$ ,  $P<0.001$ ,  $n=39$ . Batch size for gravid females ranged from 7 to 161 eggs per fish

and was significantly related to fish length; batch size= $6.07(\text{fish length}) - 226$ ;  $r^2=0.513$ ,  $P<0.02$ ,  $n=10$ . Batch size varied between 2.2 and 16.4% of the total fecundity. Egg diameter ranged from 1.207 to 1.324 mm (mean= $1.238\pm 0.022$  mm SE,) and was significantly greater than either *M. s. splendida* or *C. rhombosomoides* ( $F_{2,91}=63.1$ ,  $P<0.001$ ).

Fecundity in *C. rhombosomoides* was also significantly correlated with fish size;  $\log(\text{egg number})=1.88+0.016(\text{length})$ ,  $r^2=0.266$ ,  $P<0.05$ ,  $n=17$ . Fecundity ranged from 131 to 737 eggs per fish, with the majority of fish examined carrying between 300 and 500 eggs. Batch sizes ranged from 8 to 66 eggs per fish for stage V females (2.5 and 13.4% of the total egg number). The mean egg diameter for *C. rhombosomoides* at Polly Creek ( $1.091\text{ mm}\pm 0.019$  SE) was significantly smaller than of fish from Mena Creek ( $1.177\text{ mm}\pm 0.023$  SE) ( $t=2.80$ ,  $n=4, 5$ ,  $P<0.05$ ). The mean egg diameter of eggs pooled across both study sites was  $1.139\pm 0.021$  mm SE and not significantly different from that of *M. s. splendida*.

**Discussion**

Spawning phenology

The presence of small numbers of stage IV or V females in every month for which data were available suggests that a small proportion of the *M. s. splendida*, *M. eachamensis* and *C. rhombosomoides* populations in the Johnstone River are reproductively active year-round. The presence of larval fishes year-round in some of the study sites also attests to a prolonged breeding season (Pusey et al. submitted). Nevertheless, the majority of reproductive activity occurs from August to November (the dry season) and larval fish abundance is greatest during this period also (Pusey et al. submitted). Reduced stream flow and a reduced spate frequency ensure a more benign physical environment than during the wet season, and food may also be more abundant at this time also. Measurable quantities of waterborne chlorophyll *a* in rainforest streams of the Johnstone River occur only during the low flow period from September to November (John Peeters, personal communication). Moreover, wet-season primary production may be reduced because of a combination of increased cloud cover associated with the monsoonal wet season and high suspended sediment loads during periods of elevated discharge, both of which limit light availability for primary producers.

A concentration of reproductive effort in the months in which discharge is at its minimum has also been reported for subtropical Australian

stream-dwelling fishes also, including *Melanotaenia duboulayi* (Milton & Arthington 1983, 1984, 1985), and it was suggested that this phenology was adaptive in the sense that larvae produced at this time of year were less likely to experience physical displacement or high mortality during elevated streamflow. Humphries et al. (1999), in a discussion of breeding styles in the temperate Murray-Darling river system, noted that low-flow spawning species typically produced larvae with a defined larval period, limited mouth gape and limited yolk reserves and a consequent dependence on the presence of an abundant microinvertebrate or algal food at the time of first feeding. These studies emphasise contrastingly different factors (abiotic versus biotic) selecting for the same pattern (low-flow spawning), but there is little reason why they need be mutually exclusive.

The reproductive phenology of various subspecies of *M. splendida* in tropical Australian floodplain rivers shares some similarities with the rainbowfish covered in the present study. Reproduction is protracted, as evidenced by a small proportion of the population being reproductively active throughout the year, but the majority of spawning occurs in a shorter, well-defined period which, in contrast to that reported here, is limited to the wet season (Beumer 1978; Bishop et al. 1984). Bishop & Forbes (1991) suggested that this pattern of reproduction was common to many species and allowed young to take advantage of the seasonal inundation of floodplains. Notably, the flow regime of such rivers is strongly seasonal, resulting in massive differences in the extent and type of aquatic habitat available during the wet and dry seasons. The flow regimes of streams and rivers of the humid tropics of northeastern Queensland, whilst still seasonal, are much less variable, and habitat structure does not show the same degree of seasonal change in extent or type. Where a stream is unconstrained and floodplains extensive, spawning is probably more successful when timed to coincide with elevated discharge and consequent floodplain inundation. In the case where the channel is of high gradient and steeply incised, like most rainforest streams of the Wet Tropics region, the most appropriate strategy is likely to be to coincide spawning with stable periods of low flow.

#### Environmental cues for spawning?

Several authors have suggested that increased water temperature and photoperiod are required to initiate spawning in a range of temperate and tropical Australian freshwater fishes, including rainbowfish (Lake 1967; Beumer 1979; Milton & Arthington 1983, 1984, 1985). Elsewhere, investi-

gators have suggested that the low amplitude of seasonal change in temperature and photoperiod generally observed in tropical regions may mitigate against their value as cues for reproduction (Lam 1983; Bye 1984; Jobling 1995), although the seasonal ranges in temperature and photoperiod in the study area are in fact substantial and may be potentially useful cues (this study, Beumer 1979, MacKinnon & Herbert 1996). Temporal variation in photoperiod in northern Queensland is asynchronous with that of air or water temperature (Beumer 1979, MacKinnon & Herbert 1996), because the high cloud cover occurring in the monsoonal summer limits light penetration but not heat transfer. The seasonal peak in the mean number of sunshine hours occurs during the period of September to November (9.2–10.0 hours per day), whereas the mean number of sunshine hours per day for the remainder of the year is less than 7.5 hours. Despite the obvious potential for both water temperature and photoperiod to cue reproduction, the evidence for either as a proximal control is equivocal. For example, significantly elevated GSI values occur in August in the high elevation (NJR) *M. s. splendida* population, whereas water temperatures at this time and site are not greatly different from those recorded from May to July. A similar pattern was also observed for *M. eachamensis*, which exhibited elevated GSI values at a time of low temperatures (July). Moreover, the presence of reproductively active *M. s. splendida* individuals throughout the year seems to preclude temperature or photoperiod as being an important proximal factor in this species.

Bye (1984) stressed that the cues that stimulate gonad development must anticipate the season suitable for spawning. The species examined in the present study commence gonad maturation at an early size and achieve maturity in their first year of growth. Moreover, the fact that progression from one reproductive stage to the next, at least to stage IV maturity, coincides with increasing somatic growth suggests that the major control on the timing of reproduction is the acquisition of sufficient energy to fuel both growth and reproduction. Food availability has been suggested to be the main determinant of reproductive seasonality in other small tropical freshwater fishes also (Roberts 1989). Thus, the species studied here appear to reproduce at the first opportunity to do so within the constraint of the need to balance size-specific fecundity against high mortality endured by early life-history stages (Pusey et al. submitted). The fact that the highest mean GSI levels occur over an apparently well-defined period may simply reflect high mortality in those larvae that are produced outside of the dry season followed by syn-

chrony of growth within the population of those larvae that do survive to recruit into the adult population.

#### Spatial variation in reproduction

Spatial variation in the timing and duration of the peak spawning period was evident for both inter- and intraspecific comparisons. *M. eachamensis* commenced spawning about 1 month earlier than did *M. s. splendida* or *C. rhombosomoides* (both species pooled across sites). Notably, however, the peak spawning period of the high-elevation *M. s. splendida* population also commenced earlier than did populations at lower elevations. Given that *M. s. splendida* matured at the same length irrespective of location and that a significantly greater weight for a given length was observed for the high-elevation population of this species, we suggest that earlier reproduction in *M. s. splendida*, and probably *M. eachamensis*, is a function of greater growth rates at the high elevation sites. Such fish are thus able to breed earlier than are downstream populations of rainbowfishes. The significantly larger egg size observed for fish from the high-elevation streams may confer a growth advantage on developing larvae. Marsh (1986) reported that increased egg size in the orangethroat darter (*Etheostoma spectabile*) not only resulted in the production of relatively larger larvae at hatching and increased growth but also that it resulted in greater resistance to starvation, particularly at comparatively lower temperatures. Second, differences in catchment lithography within the Wet Tropics region, especially with respect to nutrients, are known to be important in determining spatial variation in terrestrial vegetation types and productivity (Tracey 1982) and may also be important in determining spatial variation in aquatic production also. We suggest that streams draining fertile basalt soils (i.e., North Johnstone River and Dirran Creek) are more productive than streams draining Tully Granite or Barron River Metamorphics, and fish growth rates may be higher as a result.

The *M. s. splendida* population present in Polly Creek appeared to spawn over a longer period, and female GSI was lower than that recorded elsewhere. The factors that combine to produce this condition are unknown, but it does illustrate that reproductive phenology and investment may vary considerably within a species over relatively small spatial scales. It is noteworthy, that although spawning appears protracted, larval abundance at this site is strongly seasonal, being greatest during the dry season months and very low outside of this period (Pusey et al. submitted).

#### Fecundity

*M. eachamensis* was the most fecund and *C. rhombosomoides* the least fecund of the three rainbowfishes, although all species studied produce many eggs despite their small size. The comparatively higher fecundity and egg size in *M. eachamensis* combine to produce higher GSI values than observed in the remaining species (~10% for *M. eachamensis* compared with ~4% and ~5% for *M. s. splendida* and *C. rhombosomoides*, respectively). These differences accord well with the observed differences in the duration of the spawning season. Streams in which *M. eachamensis* occur are typically deeply incised, with little room for lateral expansion of rising streamflow. A high investment in reproduction during a concentrated breeding season may be the most appropriate strategy in such streams and in a climate with well-defined wet and dry periods. In contrast, the spawning strategy and reproductive investment of *M. s. splendida* essentially parallels that observed for this species or closely related subspecies in other northern Australian rivers (Beumer 1979, Bishop et al. 1981), albeit the spawning season occurs earlier in the year during the late dry season.

Substantial congruence in life-history styles has been elsewhere reported for groups of fishes from similar habitats (Schlosser 1990, Winemiller & Rose 1992). For example, many species typical of North American headwater streams are characterized by small body size, early age at first reproduction and a short life span, and Schlosser (1990) suggested that within-river gradients in environmental variability related to discharge regime were responsible for selection for congruent life history styles. However, closely related genera or species may show different life-history styles in the tropical stream environments (Kramer 1978, DeSilva et al. 1985), apparently in response to selection pressures associated with the biotic milieu (Kramer 1978). The three rainbowfishes examined here mature at small size and are fecund batch spawners and, although some individuals are reproductively active throughout the year, the majority of reproductive activity occurs during the dry season. There is little evidence for either temperature or photoperiod as cues for reproduction; instead reproduction appears to commence when fish have reached certain minimum size. The attainment of sexual maturity appeared to occur earlier in the year at the high-elevation sites possibly as a result of faster growth in more productive habitats. Overall, and despite the identification of intra- and interspecific differences in life history attributes, the general pattern exhibited by all three species was for reproduction to occur at an early age and

to occur mostly within the dry season when discharge is reduced, water levels relatively stable and the incidence of spates low.

## Resumen

1. Durante cinco años estudiamos la biología reproductiva de tres especies de Melanotaeniidae en ríos de bosque lluvioso del noreste de Queensland (Australia). *Melanotaenia eachamensis* solo aparece en altitudes superiores a 700 m.s.n.m. y es endémica de la región tropical seca mientras que *Cairnsichthys rhombosomoides*, también endémica de esta región está restringida a altitudes inferiores a 100 m.s.n.m. *M. splendida splendida* está ampliamente distribuida por el noreste de Queensland y se presenta en un amplio rango de altitudes. Las tres especies son de pequeño tamaño, muy fecundas y desovan fundamentalmente durante la época seca, cuando la corriente es reducida y relativamente estable (frecuencia de inundación reducida).

2. No encontramos evidencias que apoyen que la luz diurna o la temperatura del agua son importantes para la fenología de la reproducción. Es más, la maduración gonadal raramente depende de que el individuo alcance un tamaño mínimo. En cualquier mes se podrían alcanzar esos tamaños mínimos y, en consecuencia, pocos individuos aparecen reproductivos durante todo el año. Sin embargo, se postula que la elevada mortalidad que sufren las larvas producidas fuera del período de caudal bajo y estable de la estación seca, seguida por la sincronía en el crecimiento de las larvas nacidas en la estación seca y que tienen una tasa de mortalidad menor con la llegada de la estación húmeda, resulta en una aparente sincronía de la actividad reproductora, es decir, una estación reproductiva bien definida.

3. El gasto reproductivo en términos de índice gonadosomático es mayor en *M. eachamensis* debido a una relativa mayor fecundidad y al mayor tamaño del huevo. Esta especie tiene un estación reproductora relativamente más corta que empieza un mes antes que en otras especies, excepto *M. s. splendida* que muestra un ciclo vital bastante congruente. Se ha postulado que las diferencias litológicas de la cuenca (basalto frente a granitos o rocas metamórficas) y las consiguientes diferencias en la disponibilidad de nutrientes y la producción primaria influyen en las tasas de crecimiento y determinan el comienzo de la reproducción.

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