

Early development and growth of the eastern rainbowfish, *Melanotaenia splendida splendida* (Peters) I. Morphogenesis and ontogeny

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Abstract. This paper describes the ontogeny and morphogenesis of the eastern rainbowfish, *Melanotaenia splendida splendida*, including details on reproduction and the conditions required for laboratory breeding and rearing. *M. s. splendida* is easily bred under standard laboratory conditions using readily available commercial foods. Aquaria with two males and three females can produce between 40 and 200 eggs daily. They can be induced to spawn daily throughout the year through manipulation of light and temperature conditions. The eggs, which ranged in size from 0.93 to 1.20 mm, had a homogeneous yolk and a clear, uniform chorion, making observation of developmental stages possible. Development was telolecithical and division was meroblastic. Development of *M. s. splendida* was similar to that of other *Melanotaenia* species. At 28°C, hatching occurred between 4 and 8 days, with an average larval length of 3.7 mm standard length. Growth was rapid and the fish reached sexual maturity within approximately 90 days. Knowledge of the developmental stages of *M. s. splendida* is important in enabling further work, such as bioassays and environmental monitoring, to be carried out, investigating the ways in which changes in the environment, such as pollution, will impact on Australia's freshwater fishes. *Melanotaenia splendida splendida* is an ideal species for this purpose in the north-eastern tropics of Australia.

Introduction

The eastern rainbowfish, *Melanotaenia splendida splendida*, belongs to a group of small, colourful, freshwater fish of the family Melanotaeniidae, endemic to Australia, New Guinea and the Aru Islands (Allen and Cross 1982; Allen 1989; Coates 1990). There are 66 species in seven genera (McGuigan *et al.* 2000; Allen *et al.* 2002), occupying a diverse variety of habitats from desert springs and intermittent streams of the dry interior to lush tropical rainforests along the Pacific coast of Queensland (Backhouse and Frusher 1980; Milton and Arthington 1984; Allen 1989, 1991; McGuigan *et al.* 2000). *Melanotaenia* is the largest genus in the family with 32 known species, 15 of which are found in Australia (McGuigan *et al.* 2000; Allen *et al.* 2002).

Rainbowfish are perhaps the most ubiquitous of all freshwater fishes occurring in tropical Australia (Crowley and Ivantsoff 1982), occurring in large numbers throughout their range (Pusey *et al.* 2000). They are chiefly inhabitants of the tropical northern half of the Australian continent (Backhouse and Frusher 1980; Allen and Cross 1982). *Melanotaenia splendida*, of which there are four subspecies, occurs across the northern half of the continent from Western Australia to Queensland, and south through central Australia. *Melanotaenia splendida splendida* is restricted to the north-eastern coastal fringe of Queensland from near Gladstone north to

Cape York Peninsular (Allen and Cross 1982). These fish are a major source of food for waterfowl and important commercial fish species such as barramundi and mangrove jack (Russell and Garrett 1985; Allen 1989; Pusey *et al.* 2000) and play an important role within tropical freshwater ecosystems.

There are few published descriptions of the development of rainbowfish embryos and larvae. Reid and Holdway (1995) described the development of *Melanotaenia fluviatilis*, a temperate melanotaeniid, found from southern Queensland to South Australia. This marks the southern limits of rainbowfish distribution (Allen and Cross 1982). Development has been described for *Melanotaenia nigrans* (Crowley and Ivantsoff 1982; Ivantsoff *et al.* 1988), *Melanotaenia splendida australis* (Ivantsoff *et al.* 1988), and *Melanotaenia splendida inornata* (Crowley and Ivantsoff 1982; Ivantsoff *et al.* 1988). All of these are tropical species.

Relatively little is known about the early life history of the majority of rainbowfish species in their natural habitat. There are only brief notes published on the reproduction of some species (Sterba 1963; Breder and Rosen 1966; Lake 1978; Munro 1980; Ivantsoff *et al.* 1988). The life history of *M. s. splendida* in northern Queensland was studied in more detail by Beumer (1979) and Pusey *et al.* (2001). A number of *M. s. splendida* were found to be reproductively active throughout the year. Spawning activity peaked during

pre-flood and flood periods (November to May) according to Beumer (1979), but Pusey *et al.* (2001) found peak spawning activity during the dry season (August to November) when flow rates were reduced and the likelihood of spates was significantly reduced. *Melanotaenia splendida splendida* spawned in slow flowing waters and the backwaters of flooded areas, where eggs were attached to aquatic vegetation by adhesive filaments originating at one point on the egg membrane (Beumer 1979; Ivantsoff *et al.* 1988). Spawning events occurred predominantly in the early morning, with one to three eggs deposited at a time, and lasted 10–14 days, during which time 40–200 eggs were produced (Beumer 1979). Sexual maturation occurred at 30–34 mm standard length (SL) for both sexes (Beumer 1979; Pusey *et al.* 2001).

Interest in the development and early biology of the rainbowfish has increased since they were first used to determine the toxicity of copper and zinc by Skidmore and Firth (1983). Rainbowfish have been extensively used as laboratory test organisms for assessing the toxicity of chemicals and pollutants (e.g. Holdway *et al.* 1988; Holdway *et al.* 1994; Barry *et al.* 1995a, 1995b; Reid *et al.* 1995; Kumar and Chapman 1998) and for the monitoring of wastes (e.g. Neilsen and King 1995). Better use of rainbowfish as test organisms in the laboratory and in field studies requires knowledge of their biology, including reproduction, ontogeny and life history. Thus, a number of studies have described the early development and biology of various species of rainbowfish, including methodologies for breeding and husbandry, namely Crowley *et al.* (1986) and Reid and Holdway (1995) (*M. fluviatilis*), Crowley *et al.* (1986) (*M. duboulayi*) and Ivantsoff *et al.* (1988) (*M. s. australis*, *M. nigrans* and *M. s. inornata*). These species inhabit many of the major waterways of Australia, with the exception of the coastal lowlands of northern Queensland, an area of increasing population growth and agricultural development. The distribution of *M. s. splendida*, from Gladstone north to Cape York Peninsula, makes this subspecies an ideal addition to the previously described rainbowfish that can be used for ecotoxicological studies. This study describes the early ontogeny and morphogenesis of *M. s. splendida*, which has not been previously described.

Material and methods

The fish stocks used in this study were collected in 1995 from Bluewater Creek, 15 km north of Townsville, Queensland (19°05'S, 146°35'E). The collected fish were identified as *M. s. splendida* through knowledge of their known distribution and meristic counts according to Allen and Cross (1982). The fish were transported to the laboratory where they were maintained in 2000-L, flow-through, outdoor tanks. Fish were moved into an indoor aquarium where they were allowed to acclimatize for 2 weeks before the beginning of the study. Second-generation laboratory-bred fish were used in all experiments.

Breeding tanks were set up in 36-L, glass, flow-through aquaria, each with two males and three females. Tanks were completely bare and water temperature was maintained at $28 \pm 1^\circ\text{C}$. Photoperiod was

16 h light : 8 h dark. Spawning substrate was supplied to each tank and consisted of approximately 50 strands of 10–15-cm green nylon wool tied together at the top to form a mop. Spawning eggs were attached to this substrate by fine filamentous threads.

The eggs were left attached to this substrate to minimize handling stress and were transferred to 15-L glass aquaria in a constant temperature room maintained at $28 \pm 1^\circ\text{C}$ and gently aerated. Larvae were transferred to 25-L static glass tanks within 4 h of hatching. Gentle aeration was not provided until 4 days after hatching so as to prevent damage to the small larvae. Fifty per cent of the water was exchanged every second day.

Newly hatched larvae were fed four times a day on cultured 'green water' (which contained various invertebrates including rotifers, paramecium, nematodes and silicates), TetraMin E baby fish food, and newly hatched *Artemia* nauplii, which had been ground up in a Potter-Elvehjem tissue homogenizer. Larvae older than 14 days were fed live *Artemia* nauplii, ground commercial flake food and ground up adult *Artemia*. This feeding regime generally resulted in greater than 90% larval survival. The bottom of each tank was siphoned daily to remove any uneaten food and faeces.

Eggs were collected at 30-min intervals for the first 24 h and then hourly until hatching. After hatching, 10 larvae were removed daily for observation and measurement. Eggs and larvae were selected randomly from different chambers to ensure that observations included individuals from different parental stock. Observations and measurements (to the nearest 0.01 mm) were made with a Zeiss stereo-binocular microscope fitted with a calibrated graticule eyepiece. Total length of larvae is a problematic measure because the caudal fin can be easily damaged, so standard length (SL) was the preferred measure. Myomere counts were aided by the use of a cross-polarized light source. Measurements and observations were made from live specimens, anaesthetized with Tricaine (3-aminobenzoic acid ethyl ester). At least five eggs were used to determine the timing of each developmental event and the average time was rounded to the nearest half hour. Eggs and larvae used for observation were discarded after use to avoid possible effects on development as a result of stress. Drawings and observations on developmental details were made with the aid of a camera lucida, photographs and on fixed specimens. General terminology follows Blaxter (1988), pigmentation follows Russell (1976), and the developmental stages are based on descriptions of rainbowfish by Crowley and Ivantsoff (1982), Ivantsoff *et al.* (1988) and Reid and Holdway (1995).

Results

Hatching behaviour

Sexual dimorphism became apparent when the fish reached 37–42 mm SL. Generally, the larger fish tended to be males, which were identified from the elongation of posterior rays in the second dorsal and anal fins. Spawning behaviour typically began half an hour after the lights came on in the aquarium room at 0830 hours, with a peak of activity at about 1000 hours. Activity then gradually declined until all spawning activity had finished at 1500 hours. The number of eggs collected daily from each spawning tank ranged from 40 to 250.

Growth and development

The eggs had a homogeneous yolk and a clear, uniform, spherical chorion. A number of fine filaments (30–40), 3–8 mm in length, originated from a small area on the chorion at the animal pole. Numerous oil droplets (35–60) occurred directly

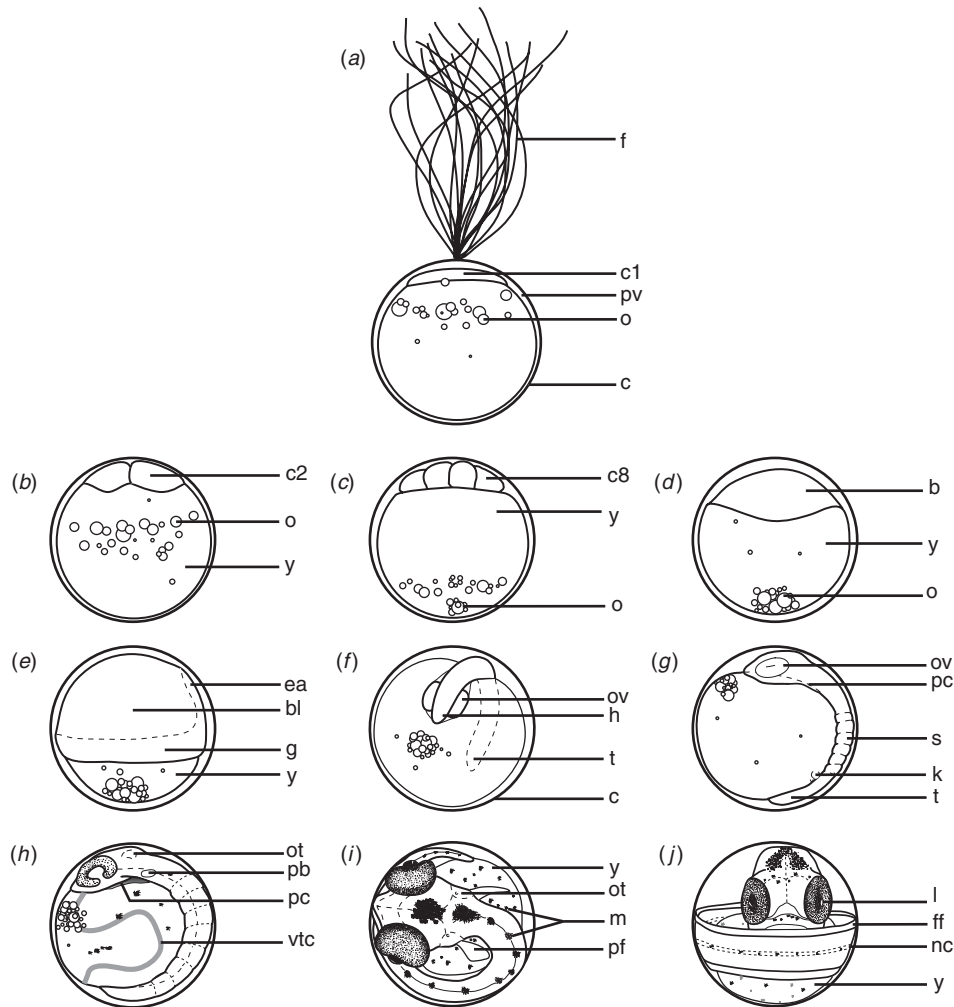


Fig. 1. Embryonic development of *Melanotaenia splendida splendida*. Development stages and time elapsed: (a) 0.5 h; (b) 1 h, first cleavage; (c) 2 h; (d) 10.5 h; (e) 12.0 h, embryonic axis and germ ring; (f) 17.5 h; (g) 20 h; (h) 48 h; (i) 74 h; (j) 96 h. b: Blastodisc; bl: blastoderm; c: chorion; c1: first cell; c2: two-cell stage; c8: eight-cell stage; ea: embryonic axis; f: filaments; ff: marginal fin fold; g: germ ring; h: head; k: Kupffer's vesicle; l: lens; nc: notochord; o: oil droplets; ot: otic vesicle; ov: optic vesicle; pc: pericardial cavity; pb: pectoral fin bud; pf: pectoral fin; pv: perivitelline space; s: somites; t: tail; vtc: vitelline circulatory system.

below the filaments and ranged in size from 0.01 to 0.12 mm. Egg diameter was 0.93–1.20 mm, the mean being 1.04 mm ($n = 80$). The perivitelline space was 0.02–0.08 mm. Development of *M. s. splendida* eggs was telolecithal, cleavage was discoidal and restricted to a small disc directly below the filaments. Stages and timing of development were as follows.

Timing of development

- 0 h. Fertilization. A variable number of oil droplets grouped together at the animal pole directly below the filaments, which over the course of development, migrated in an orderly band to the vegetal pole (Fig. 1a–b).
0.5 h. First cell formed.

- 1 h. First polar cell division resulted in two cells of the same size (Fig. 1b).
1.5 h. Second polar division at right angles to the first division resulted in four cells.
2.0 h. Eight-cell stage resulted from a vertical division parallel to that of the first division (Fig. 1c).
2.5 h. Sixteen-cell stage. Cells were generally not uniform in shape or size.
3.5 h. First horizontal division took place resulting in 32 cells. Cells much smaller, occupying the same area on the yolk as the initial single cell.
4.25–7.0 h. Continuous division resulted in the formation of the blastodisc (Fig. 1d).

- 7.0–11.30 h. Division continued until the onset of epiboly. The blastodisc began to flatten and spread downwards over the surface of the yolk to form the subgerminal cavity. The embryonic shield and germ rings became visible and the embryonic axis was clearly seen in the mid-area of the embryonic shield (Fig. 1e).
- 12.30 h. Yolk plug formed when the embryonic ectoderm covered most of the yolk. Neural groove became visible.
- 13.25–15.00 h. Outline of the head of a small embryo visible close to the oil droplets.
- 17–18.25 h. Optic vesicles the most prominent feature of the embryo. Embryo little less than half way around the yolk (Fig. 1f).
- 20 h. Tail well defined and Kupffer's vesicle appeared as two or three globules sitting on the tail of the embryo.
- 22 h. Embryo well defined and the caudal somites appeared as fine lines on the tail of the embryo (Fig. 1g).
- 24 h. Optic cup formed, appearing as a slit in the optic vesicle. Lens not yet visible. Melanophores began to appear on the yolk sac, lateral surfaces of the embryo and the dorsal surface of the head.
- 27 h. Lens visible in the optic cup, which was open on the ventral side. Optic vesicles pigmented. Kupffer's vesicle no longer present.
- 30.5 h. Heart visible and undifferentiated, appearing as a straight tube. Pericardial cavity present. Vitelline circulatory system developed though no red blood cells present. Otic capsules began to form. Tail bud extended free of the yolk sac (Fig. 1h).
- 37.25 h. Pause in the blood flow indicated differentiation of the heart into separate chambers. Red blood cells were present.
- 46 h. Two pairs of otoliths, the sagittae and lapillus, present in the otic capsule. Change in the orientation of the tail from the polar to equatorial plane.
- 48 h. Pectoral fin buds appearing as slight bulging protuberances just posterior to the head region. Optic cups well pigmented although still not joined ventrally.
- 55 h. Branchial arches apparent. Small membranous pectoral fins appeared as small discs. Optic cup joined ventrally. Swim bladder appeared as a small vesicle in the peritoneal cavity below the junction of the head and tail. This region was heavily pigmented (Fig. 1i).
- 62 h. Liver appeared, initially as a pale yellow/gold vesicle within the abdominal cavity, just

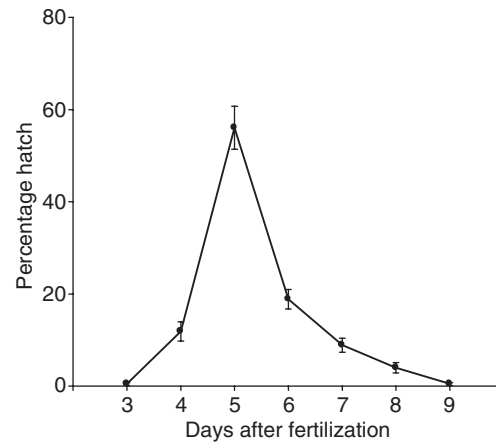


Fig. 2. Percentage hatch of *Melanotaenia splendida splendida* larvae, days after fertilization (mean \pm s.e.).

- below and posterior to the left pectoral fin. Outline of the mandibular arch as an inverted crescent as seen from the anterior aspect. Chromatophores present along the anal pore.
- 74 h. Meckel's cartilage lying directly below the upper jaw outlined by a row of melanophores.
- 96 h. Mouth fully formed (Fig. 1j).
- 105 h. About 0.5–1 h before hatching, the chorion became flaccid. Hatching was very rapid. With a flick of the tail the embryo freed itself.

Hatching details

Hatching began on Day 4 and continued for 4 days, with a peak on Day 5 (Fig. 2). At hatching, the larvae measured 3.7 ± 0.03 mm (mean \pm s.e., $n = 30$), and were well developed. They were strong swimmers with well-developed pectoral fins and a continuous median fin fold, beginning dorsally at the first or second pre-anal myomere and continuing around the tail, ending at the anal pore. There were 6–7 pre-anal myomeres and 29–32 post-anal myomeres. The larvae swam at the surface of the water, generally within the top 75 mm. As the swim bladder was inflated upon hatching, larvae did not sink when they stopped swimming. The mouth was well developed and functional, the gut coiled, and larvae began feeding within hours of hatching. The yolk sac was greatly reduced, although still contained a number of oil droplets, which were situated posterior to the mouth. The eyes were large and well pigmented and the sagittal and lapillal otoliths were prominent, though the asteriscii were not present (Fig. 3a).

Growth and development

Growth rates of the larvae were slow, with little variation until the Day 12, and showed relatively little variation in size (Fig. 4). After Day 12, growth rates increased. As the larvae increased in age, the variation in length between individuals

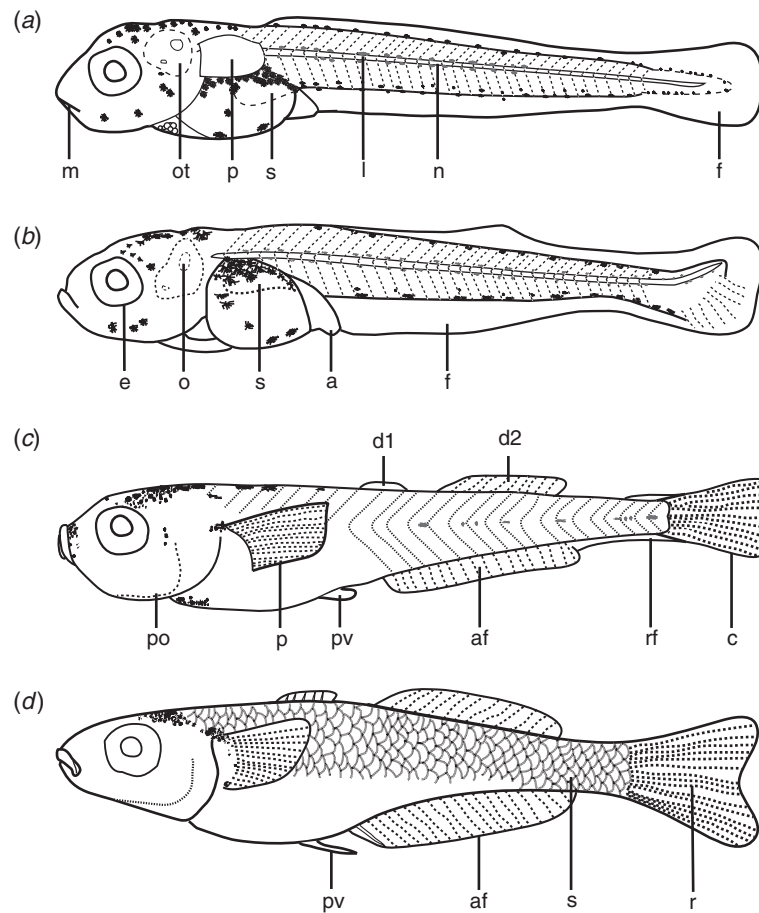


Fig. 3. Development of fins and scales in *Melanotaenia splendida splendida*. (a) Newly hatched larva (3.7 mm standard length); (b) 14-day-old larva (6.74 mm standard length) (pectoral fin not drawn to enable visualization of swim bladder and pigmentation); (c) 21-day-old larva (9.32 mm standard length); (d) 40-day-old larva (20.75 mm standard length). a: Anal pore; af: anal fin; c: caudal fin; d1: first dorsal fin; d2: second dorsal fin; e: eye; f: marginal fin fold; l: lateral melanophore; m: mouth; n: notochord; o: otolith; ot: otic vesicle; p: pectoral fin; pv: pelvic fin; r: rays; rf: residual marginal fin fold; s: scales.

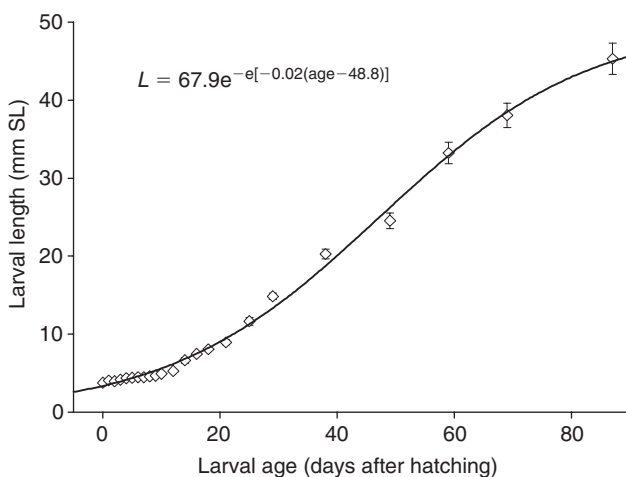


Fig. 4. Growth of *Melanotaenia splendida splendida* after hatching up to 87 days (mean \pm s.e.). SL: Standard length.

also increased, with the coefficient of variation increasing from 3% to 6%, up to 12 days after hatching, to 15% at 87 days after hatching. The Gompertz model was fitted to the data with the equation $L = 67.9 \times e^{-e(-0.02(t-48.8))}$, the best description of early growth.

Fin development

The pectoral fins developed as a rayless membrane before hatching. Pectoral fin rays began to develop, along with the rays of the second dorsal and anal fins, at 6.8–7.3 mm SL. They developed sequentially from dorsal to ventral, and a full complement was present at 9.2–9.9 mm SL. There were 13–14 pectoral fin rays, although occasionally 11 rays were observed.

A thickening of the tissue on the ventral side of the urostyle indicated the initial development of the caudal fin when the larvae were 5.4–5.7 mm SL. The formation of

Table 1. Characteristics of newly hatched larvae from the genus *Melanotaenia*

	<i>Melanotaenia splendida splendida</i> ^a	<i>Melanotaenia splendida australis</i> ^b	<i>Melanotaenia splendida inornata</i> ^b	<i>Melanotaenia nigrans</i> ^b
Pre-anal myomeres	6–7	6–7	6–7	6–7
Post-anal myomeres	29–32	25–27	26–28	28–29
Dorsal origin fin fold	1–2 (pre anal)	1 (post anal)	1 (post anal)	5–7 (pre anal)
Dorsal midline melanophores	27–36	19–29	29–45	40–48
Notochordal melanophores	17–24	10–24	14–29	22–30
Ventral midline melanophores	26–34	21–43	14–25	34–43

^aThis study; ^bIvantsoff *et al.* 1988.

cartilaginous hypural elements began when the larvae were 5.8–6.1 mm SL and the first rays of the caudal fin developed from these elements (Fig. 3b). Flexion of the notochord began between 5.9–6.2 mm SL and was usually complete by 7.2 mm SL. The epurals began to form on the dorsal side when the larvae were 6.0–6.5 mm SL. The tip of the notochord was gone and the tip of the urostyle and the base of the hypural elements had begun to ossify by the time the larvae reached 7.3–8.4 mm SL. All rays in the caudal fin were completely ossified by 16.8 mm SL. The caudal fin was initially rounded, and then became forked as the outermost rays increased in length. Development of the caudal fin was completed by 17.2–18.6 mm SL.

A thickening of the tissue in the primordial marginal fin fold indicated the area of the future anal and second dorsal fins at 6.5–7.0 mm SL. The rays of both fins developed shortly afterwards and grew through the marginal fin fold towards the distal edge. Spines of both fins developed after the development of the rays at 7.2–7.7 mm SL. The fin fold between the anal fin and the anus persisted until all rays and the spine were completely formed by 8.6–10.7 mm SL (Fig. 3c). The fin fold anterior to the second dorsal was resorbed before the development of the first dorsal fin. All fin elements were formed and ossification complete by 16.6–17.2 mm SL. The second dorsal fin had one spine and 10–12 rays, while the anal fin had one spine and 18–20 rays.

The first dorsal fin did not have its origin in the marginal fin fold, unlike the caudal, anal and second dorsal fins. The fin fold degenerated when the fish were 8.6–10.7 mm SL and the dorsal fin became emergent at this stage. The spines and membrane of the first dorsal developed and grew together. The spines had fully developed by 12.3–13.9 mm SL. The spines of the dorsal fin were fully ossified when the larvae reached 15.7–16.5 mm SL. There were six to seven spines present in the first dorsal fin.

The pelvic fins were the last to develop. Rudimentary fins developed just anterior to the anus and appeared as two small ridges parallel to the longitudinal axis of the body (Fig. 3c). They were first seen at 5.8–6.1 mm SL. Initially, these fins were membranous. As they developed, the origin of the fin curved around to assume the adult form and rays and the spine began to develop when the larvae were about

9.0–10.5 mm SL. Development of this fin was complete when the fish reached 12.2–13.6 mm SL.

Pigmentation

Melanophores developed during the embryonic period. At hatching, there was dark pigmentation on the dorsal surface of the head and the dorsal surface of the peritoneal cavity, just above the swim bladder, while there were numerous melanophores on the abdomen. A single row of melanophores occurred along the dorsal midline, beginning on top of the head and continuing to the end of the notochord. A double row of ventral contour melanophores began post-anally, ending at the last myomere, and continued as a single row around the notochord to join the melanophores on the dorsal side. A row of internal melanophores occurred along both the dorsal and ventral margins of the notochord. The number of melanophores ranged from 27 to 36 along the dorsal midline, 17 to 24 internally along the notochord and 26 to 34 along the ventral midline (Table 1).

The melanophores on the head increased in number, eventually forming a Y-shaped pattern when the fish attained 10.2–10.4 mm SL, and persisted into adulthood. The abdominal melanophores gradually disappeared until none remained once the fish reached 15.3–16.0 mm SL. Melanophores developed along the dorsal margins of the rays as the rays developed in the fins. Chromatophores developed in the second dorsal, anal and caudal fins from about 10–15 mm SL and were more distinctive in male fish.

Development of the gill filaments and operculum

The branchial arches began to develop before hatching and were visible from the ventral surface of the larvae after hatching. Each of the branchial arches had a series of small nodules, which were the precursors of the filaments and lamellae. As the filaments grew, the dermal opercular bones also developed to eventually completely cover the filaments. By the time the fish were 15.3–16.1 mm SL, the gill filaments and operculum were completely formed.

Squamation

Squamation did not begin until the fish attained a length of 10.1–10.4 mm SL. The first scales were those along the

mid-lateral line, followed shortly by development of scales on the dorsal surface and then those on the ventral surfaces. The interorbital and opercular surfaces were the last to be covered with scales, which began when the larvae were 11.0–11.4 mm SL. Squamation was complete by the time the larvae were 17.5–19 mm SL (Fig. 3d).

Discussion

In this study, egg production rates of *M. s. splendida* in the laboratory were 15–100 per day per female, and similar to that of *M. nigrans*, *M. s. australis* and *M. s. inornata* (Ivantsoff *et al.* 1988). In the field, *M. s. splendida* had lower egg production rates (one to three eggs) according to Beumer (1979). Lower rates under field conditions may be owing the presence of extensive substrate enabling rainbowfish to ‘spread the risk,’ spawning small numbers of eggs over a large area. The artificial conditions in the laboratory with limited area and substrate may conversely induce the female to spawn all eggs at the one time. The number of eggs shed by a single female increased with the size of the female, which supports the finding of Pusey *et al.* (2001) that fecundity was significantly related to fish size. Females were observed to spawn only once each day; however, males often spawned with more than one female in one day.

The eggs of *M. s. splendida* at fertilization were similar in appearance to those of other members of the species and genus. All were spherical, with a number of adhesive filaments arising from a small area of the chorion at the animal pole. Mean egg diameter (1.04 mm) was slightly larger than that of *M. fluviatilis* (0.94 mm), *M. duboulayi* (0.91 mm), and *M. s. inornata* (0.88 mm), but slightly smaller than *M. s. australis* (1.07 mm) and *M. nigrans* (1.05 mm). The perivitelline space of the eggs in this study (mean 0.043 mm) was within the range found for other members of the genus (0.03–0.06 mm). Crowley *et al.* (1986) suggested using the relationship between the perivitelline space and egg diameter as a feature to differentiate the eggs of melanotaeniids, but these data indicate that this relationship is not species or subspecies specific.

Development of *M. s. splendida* eggs followed a pattern similar to that previously described for *M. fluviatilis*, *M. s. inornata*, *M. s. australis* and *M. nigrans* (Crowley and Ivantsoff 1982; Ivantsoff *et al.* 1988; Reid and Holdway 1995). A difference was the rate of development, based on hatching time. A shorter embryonic period was observed compared with previous studies (Beumer 1979), but these differences were probably a result of different rearing temperatures.

In the present study, mean embryonic period, was 5 days at 28°C. Mean embryonic period for *M. fluviatilis* was 7 days at 24°C (Backhouse and Frusher 1980), 7–9 days at 25°C (Reid and Holdway 1995) and 4.5 days at 27°C (Crowley *et al.* 1986). The mean embryonic period for *M. s. inornata*

and *M. nigrans* was approximately 6.5 days at 25°C and 4.5 days at 27°C (Crowley and Ivantsoff 1982; Ivantsoff *et al.* 1988). Therefore, temperature must be considered as one of the major factors in determining embryonic period in the genus *Melanotaenia*.

The mean larval length of *M. s. splendida* at hatching (3.78 mm SL) was considerably larger than that measured by Beumer (1979) (2.10–3.47 mm total length), but similar to other *Melanotaenia* species (3.4–4.2 mm total length) (Crowley and Ivantsoff 1982; Ivantsoff *et al.* 1988; Reid and Holdway 1995). Initial slower growth followed by rapid growth, as observed in this study, might have been related to either absorption of the yolk sac or change in diet to *Artemia*, both of which occurred simultaneously.

Munro (1980) suggested that *M. fluviatilis* larvae were poor swimmers. For several days after hatching they clung to plants and only swam freely 9 days after hatching. In this study; however, hatched larvae were well developed, strong and competent swimmers, which is supported by Backhouse and Frusher (1980), Beumer (1979), Crowley and Ivantsoff (1982) and Ivantsoff *et al.* (1988).

Larval morphology was similar to previous descriptions of other members of the genus, relative to reduced yolk sac, a well-developed mouth, and a membranous fin fold (Crowley and Ivantsoff 1982; Crowley *et al.* 1986; Ivantsoff *et al.* 1988; Reid and Holdway 1995). Myomere counts, and pigment patterns and numbers of melanophores observed in this study were similar to those of other members of the genus (Ivantsoff *et al.* 1988) (Table 1). *Melanotaenia* have six to seven pre-anal myomeres, and the number of post-anal myomeres ranged from 25–27 for *M. nigrans* to 29–32 in the present study. The general pattern of pigmentation of other *Melanotaenia* was similar to that of *M. s. splendida*; however, the number of melanophores was highly variable. Meristic counts and pigmentation patterns play an important role in the identification of fish at the specific or generic level (Kendall *et al.* 1984), but it has been noted that laboratory-reared larvae are frequently more heavily pigmented than wild-caught specimens and may show greater meristic variation (Houde and Potthoff 1976; Lau and Shafland 1982).

The sequence of fin development of *M. s. splendida* is similar to that described for other *Melanotaenia* species (Crowley *et al.* 1986; Ivantsoff *et al.* 1988; Reid and Holdway 1995). To compare development at size is difficult because the present study used SL, whereas others used total length. However, development appears similar when corrected for total length.

Growth rates for *M. s. splendida* were considerably higher than those reported for other members of the genus. One possible reason for this is that the fish in this study were reared at a temperature higher than any other study (28°C, 2°C higher). This higher temperature was chosen because peak spawning activity of *M. s. splendida* occurs during November to March when temperatures range from 25°C to 35°C (Beumer

1979). Under normal conditions of adequate food, increasing temperature (within the tolerance limits of the fish) generally results in higher growth rates (Blaxter 1992; Kucharczyk et al. 1998).

Food is another important factor affecting growth, especially in the early larval stages. The preferred size of prey for larval fishes increases as mouth size and feeding competency increase (Hunter 1984). Providing natural 'green-water' with resident zooplankton as food for the newly hatched fish has several advantages. The larvae are easily able to switch to different sized prey, a feature not present in monocultures of organisms such as rotifers or *Artemia*. 'Green water' also enables the zooplankton to feed on resident algae and microbes, thus retaining their nutritional value for greater periods of time. It also acts as a buffer, maintaining higher water quality (Tucker 1992; Whyte et al. 1994).

In conclusion, the eastern rainbowfish, *M. s. splendida*, has many useful characteristics for a laboratory-test organism. It is easy to maintain under standard laboratory conditions and will develop on standard commercial foods. The fish can be induced to spawn by manipulation of water temperature and photoperiod throughout the year. Breeding tanks of mature fish can be easily set up. *M. s. splendida* are relatively fast growing, reaching sexual maturity within approximately 3 months at 30–40 mm SL. Under appropriate conditions, survival rates >90% can be attained. All these factors combined ensure a regular supply of eggs and larvae in the laboratory. The clear chorion of the eggs ensures that the different stages during development are easily observed and thus the impact of contaminants or changing environmental conditions on development can be clearly elucidated while conducting bioassays. This includes such studies as investigating developmental defects in embryos following exposure to environmental contaminants (e.g. Klumpp and von Westernhagen 1995; Hose et al. 1996; C. A. Humphrey and D. W. Klumpp, unpublished data). Together with the widespread geographical distribution of rainbowfish species and their wide distribution through catchments, these factors make this fish an extremely useful tool in any freshwater biomonitoring laboratory.

References

- Allen, G. R. (1989). 'Freshwater Fishes of Australia.' (T. F. H. Publications, Inc.: Melbourne.)
- Allen, G. R. (1991). 'Field Guide to the Freshwater Fishes of New Guinea.' Christensen Research Institute, Publication No. 9. (Christensen Research Institute: Mandang, Papua New Guinea.)
- Allen, G. R., and Cross, N. J. (1982). 'Rainbowfishes of Australia and Papua New Guinea.' (Angus and Robertson Publishers: Melbourne.)
- Allen, G. R., Midgley, S. H., and Allen, M. (2002). 'Field Guide to the Freshwater Fishes of Australia.' (Western Australian Museum: Perth, Australia.)
- Backhouse, G. N., and Frusher, D. J. (1980). The crimson-spotted rainbowfish, *Melanotaenia fluviatilis* (Castelnau 1878). *Victorian Naturalist* **97**, 144–8.
- Barry, M. J., Logan, D. C., Ahokas, J. T., and Holdway, D. A. (1995a). Sublethal effects of endosulfan and oxygen concentration on embryos of the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Australasian Journal of Ecotoxicology* **1**, 71–6.
- Barry, M. J., O'Halloran, K., Logan, D. C., Holdway, D. A., and Ahokas, J. T. (1995b). Sublethal effects of esfenvalerate pulse-exposure on spawning and non-spawning Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Archives of Environmental Contamination and Toxicology* **28**, 459–63.
- Bestgen, K. R., and Williams, M. A. (1994). Effects of fluctuating and constant temperatures on early development and survival of Colorado Squawfish. *Transactions of the American Fisheries Society* **123**, 574–9.
- Beumer, J. P. (1979). Reproductive cycles of two Australian freshwater fishes: the spangled perch, *Therapon unicolor* Gunther, 1859 and the East Queensland rainbowfish, *Nematocentris splendida* Peters, 1866. *Journal of Fish Biology* **15**, 111–34.
- Blaxter, J. H. S. (1988). Pattern and variety in development. In 'The Physiology of Developing Fish. Part A: Eggs and Larvae'. (Eds W. S. Hoar and D. J. Randall.) pp. 1–58. (Academic Press Inc.: New York.)
- Blaxter, J. H. S. (1992). The effect of temperature on larval fishes. *Netherlands Journal of Zoology* **42**, 336–57.
- Breder, C. M., and Rosen, D. E. (1966). 'Modes of Reproduction in Fish.' (The Natural History Press for the American Museum of Natural History: New York.)
- Coates, D. (1990). Biology of the rainbowfish, *Glossolepis multisquamatus* (Melanotaeniidae), from the Sepik River floodplains, Papua New Guinea. *Environmental Biology of Fishes* **29**, 119–26.
- Crowley, L. E. L. M., and Ivantsoff, W. (1982). Reproduction and early stages of development in two species of Australian rainbowfishes, *Melanotaenia nigrans* (Richardson) and *Melanotaenia splendida inornata* (Castelnau). *Australian Zoologist* **21**, 85–95.
- Crowley, L. E. L. M., Ivantsoff, W., and Allen, G. R. (1986). Taxonomic position of two crimson-spotted rainbowfish, *Melanotaenia duboulayi* and *Melanotaenia fluviatilis* (Pisces: Melanotaeniidae), from Eastern Australia, with special reference to their early life-history stages. *Australian Journal of Marine and Freshwater Research* **37**, 385–98.
- Holdway, D. A., Wiecek, M. M., and Brown, V. M. (1988). 'OSS Test Protocols for the Biological Testing of Waste Waters for Release into Magela Creek. III. Larval Rainbow-fish test (*Melanotaenia splendida*).' Supervising Scientist for the Alligator Rivers Region, Open File Record No. 53. (Australian Government Publishing Service: Canberra.)
- Holdway, D. A., Barry, M. J., Logan, D. C., Robertson, D., Young, V., and Ahokas, J. T. (1994). Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Aquatic Toxicology* **28**, 169–87.
- Hose, J. E., McGurk, M. D., Marty, G. D., Hinton, D. E., Brown, E. D., and Baker, T. T. (1996). Sublethal effects of the Exxon Valdez oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989–1991. *Canadian Journal of Fisheries and Aquatic Science* **53**, 2355–65.
- Houde, E. D., and Potthoff, T. (1976). Egg and larval development of the sea bream *Archosargus rhomboidalis* (Linnaeus): Pisces, Sparidae. *Bulletin of Marine Science* **26**, 506–29.
- Hunter, J. R. (1984). Synopsis of culture methods for marine fish larvae. In 'Ontogeny and Systematics of Fishes.' American Society of Ichthyologists and Herpetologists, Special Publication No. 1. (Eds H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr, and S. L. Richardson.) pp. 24–7. (Allen Press Inc.: Lawrence, USA.)

- Ivantsoff, W., Crowley, L. E. L. M., Howe, E., and Semple, G. P. (1988). 'Biology and Early Development of Eight Fish Species from the Alligator Rivers Region.' Supervising Scientist for the Alligator Rivers Region, Technical Memorandum 22. (Australian Government Publishing Service: Canberra.)
- Kendall, A. W. Jr, Ahlstrom, E. H., and Moser, H. G. (1984). Early life history stages of fishes and their characters. In 'Ontogeny and Systematics of Fishes'. American Society of Ichthyologists and Herpetologists, Special Publication No. 1. (Eds H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr, and S. L. Richardson.) pp. 11–22. (Allen Press Inc.: Lawrence, USA.)
- Klumpp, D. W., and von Westernhagen, H. (1995). Biological effects of pollutants in Australian tropical waters: embryonic malformations and chromosomal aberrations in developing fish eggs. *Marine Pollution Bulletin* **30**, 158–65.
- Kucharczyk, D., Luczynski, M., Kujawa, R., Kaminski, R., Ulikowski, D., and Brzuzan, P. (1998). Influences of temperature and food on early development of bream (*Abramis brama* L.). *Archiv fuer Hydrobiologie* **141**, 243–56.
- Kumar, A., and Chapman, J. C. (1998). Profenofos toxicity to the eastern rainbowfish (*Melanotaenia duboulayi*). *Environmental Toxicology and Chemistry* **17**, 1799–806.
- Lake, J. S. (1978). 'Australian Freshwater Fishes and Rivers of Australia: An Illustrated Field Guide.' (Nelson Publishers: Melbourne.)
- Lau, S. R., and Shafland, P. L. (1982). Larval development of snook, *Centropomus undecimalis* (Pisces: Cenrtopomidae). *Copeia* **3**, 618–27.
- McGuigan, K., Zhu, D., Allen, G. R., and Moritz, C. (2000). Phylogenetic relationships and historical biogeography of melanotaeniid fishes in Australia and New Guinea. *Marine and Freshwater Research* **51**, 713–23.
- Milton, D. A., and Arthington, A. H. (1984). Reproductive strategy and growth of the crimson-spotted rainbowfish, *Melanotaenia splendida fluviatilis* (Castelnau) (Pisces: Melanotaeniidae) in South-eastern Queensland. *Australian Journal of Marine and Freshwater Research* **35**, 75–83.
- Munro, I. S. R. (1980). Family Melanotaeniidae. In 'Freshwater Fishes of South-Eastern Australia'. (Ed. R. M. McDowell.) pp. 17–23. (A. H. and A. W. Reed: Sydney.)
- Nielsen, D. L., and King, H. M. (1995). Biomonitoring of paper mill effluent using fish ventilatory signals. *Australasian Journal of Ecotoxicology* **1**, 113–16.
- Pusey, B. J., Arthington, A. H., and Read, M. G. (2000). The dry-season diet of freshwater fishes in monsoonal tropical rivers of Cape York Peninsula, Australia. *Ecology of Freshwater Fishes* **9**, 177–90.
- Pusey, B. J., Arthington, A. H., Bird, J. R., and Close, P. G. (2001). Reproduction in three species of rainbowfish (Melanotaeniidae) from rainforest streams in northern Queensland, Australia. *Ecology of Freshwater Fish* **10**, 75–87.
- Reid, H. P., and Holdway, D. A. (1995). Early development of the Australian crimson-spotted rainbowfish, *Melanotaenia fluviatilis* (Pisces: Melanotaeniidae). *Marine and Freshwater Research* **46**, 475–80.
- Reid, H. P., Ahokas, J. T., and Holdway, D. A. (1995). Use of cyanazine and malathion pulse-exposure toxicity to estimate the age of onset of functional liver metabolism in larval Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Water Research* **29**, 2010–13.
- Russell, D. J., and Garrett, R. N. (1985). Early life history of barramundi, *Lates calcarifer* (Bloch), in north-eastern Queensland. *Australian Journal of Marine and Freshwater Research* **36**, 191–201.
- Russell, F. S. (1976). 'The Eggs and Planktonic Stages of British Marine Fishes.' (Academic Press: London.)
- Skidmore, J. F., and Firth, I. C. (1983). 'Acute Sensitivity of Selected Australian Freshwater Animals to Copper and Zinc.' Australian Water Resources Council, Technical Paper No. 81. (Australian Water Resources Council: Canberra.)
- Sterba, G. (1963). 'Freshwater Fishes of the World.' Translated and revised by D. W. Tucker, British Museum. (Vista Books: London.)
- Tucker, J. W. Jr (1992). Feeding intensively-cultured marine fish larvae. In 'Proceedings of the Aquaculture Nutrition Workshop, Salamander Bay'. (Eds G. L. Allan and W. Dall.) p. 248. (NSW Fisheries, Brackish Water Fish Culture Research Station: Salamander Bay, Australia.)
- Whyte, J. N. C., Clarke, W. C., Ginther, N. G., Jensen, J. O. T., and Townsend, L. D. (1994). Influence of composition of *Brachionus plicatilis* and *Artemia* on growth of larval sablefish (*Anoplopoma fimbria* Pallas). *Aquaculture* **119**, 47–61.

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