Technical Memorandum 22

Biology and early development of eight fish species from the Alligator Rivers Region

W. Ivantsoff, L.E.L.M. Crowley, E. Howe and G. Semple

Supervising Scientist for
the Alligator Rivers Region

ERRATUM

On page 32, the caption to Fig. 5 should read:

Scanning electron micrographs (SEM) of the chorion morphology of: (a) *P. tenellus* (magnification approx. x23); and (b) *P. gertrudae* (x34 approx.)

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ABSTRACT

Ivantsoff, W., Crowley, L.E.L.M., Howe, E. & Semple, G. (1988). Biology and early development of eight fish species from the Alligator Rivers Region. Technical Memorandum 22, Supervising Scientist for the Alligator Rivers Region.

The following fish were studied: *Melanotaenia nigrans, Melanotaenia splendida australis, Melanotaenia splendida inornata, Pseudomugil gertrudae, Pseudomugil tenellus, Craterocephalus* nov. sp., *Craterocephalus stercusmuscarum stercusmuscarum*, and *Ambassis macleayi.*

Coloration, other specific attributes, sexual dichromatism and dimorphism were recorded whenever possible so that the sexes could be easily recognised. Mating and other behaviour patterns were noted to facilitate recognition of breeding pairs. Spawning patterns and occurrence of cannibalism were recorded, as was the effect of the presence of more than one breeding pair in a tank.

Egg numbers at each spawning were recorded and the main breeding season for each of the species was determined. No single stimulus to spawning could be determined. Spawning appeared to be sporadic but continuous throughout the year, with a peak during late spring/early summer.

Detailed records of embryonic development were kept for all species except *Ambassis macleayi*. Descriptions and drawings of stages were made and where possible, distinguishing features noted so that the embryos of different species could be recognised. Time of development to hatching, at constant temperature, was recorded.

Larval development of rainbow-fishes was recorded in detail and times to particular developmental stages noted and compared. For other species, the times to particular developmental stages were recorded and representative larval stages described and drawn.

General recommendations are made in regard to the requirements to breed and maintain fish, the numbers that can be produced for further study and the constraints that may be present when working with selected fish.

1 INTRODUCTION

Lake (1971) listed eight species of native Australian fish which could be bred in dams specially constructed for breeding but found that only four of the species studied could be bred in any one habitat. All of the fish in his list are relatively large when mature. A review of the literature on Australian fish indicates that very little, if any, work has been done on breeding small fish species; most of the reports are anecdotal (for example, McCulloch 1913; Hamlyn-Harris 1929; Whitley 1958) and these have been perpetuated in subsequent literature (Ivantsoff 1980; Bishop et al., in press). Llewellyn (1979) studied a species of *Craterocephalus* and described its early stages of development, up to the formation of the fin fold and the neural tube. McKay (1973, 1974) examined the reproductive cycle of another small fish, an eleotrid. There are many excellent studies on small fish in the field (Lake 1967; Llewellyn 1971, 1974; Milton & Arthington 1983*a*, 1983*b*; Bishop et al., in press) but they have very little relevance to the breeding of fish in the laboratory. Only scattered items of information on breeding can be found in scientific publications. For example, Sterba (1963) recommended that breeding may by induced in certain species of fish by increasing the salinity of the water; Allen & Cross (1982) advocate regular maintenance of aquaria. Beumer (1979) suggested that day-length may be a factor which might induce breeding in rainbow-fishes, and change in water levels at appropriate temperatures might be regarded as a necessary stimulus to induce spawning in certain fishes (Lake 1971).

Amateur publications frequently report success in breeding small fish, especially those which are prized highly by aquarists. Rainbow-fish and blue-eyes (family Melanotaeniidae) are often mentioned together with an appropriate method on how to keep and breed them. Hardyheads (Atherinidae) have also been reported as having been bred but no detailed description of the process has been found. Hearsay reports appear from time to time about other small Australian fish, for example, glass perchlets (Ambassidae).

The objective of this study was to establish methodologies for the breeding of fish and other aquatic species, such as *Macrobrachium* spp., for use in toxicological and other studies by the Office of the Supervising Scientist. Terms of the contract stipulated a report on the study, including a discussion of equipment requirements, culture conditions, nutrition and life cycle data.

The following species were successfully bred and are the subject of this report:

Fishes

Family Melanotaeniidae:	*Melanotaenia nigrans*
	M. splendida australis
	M. splendida inornata
	Pseudomugil gertrudae
	P. tenellus
Family Atherinidae:	*Craterocephalus* sp. nov.
	C. stercusmuscarum stercusmuscarum
Family Ambassidae:	*Ambassis macleayi*

2 BREEDING OF THE FISHES STUDIED

2.1 Collecting of fish

Stocks for use in these studies were collected in the Magela Creek system during a field trip in November-December 1981. Localities for the collection of different species were selected with the help of Mr K. Bishop and Mr P. Ward, who had an excellent knowledge of the local distribution of the different species. The localities visited and species collected were:

Family Melanotaeniidae

Melanotaenia nigrans:	Koongarra, 12°36'S 132°52'E Twin Falls, 13°19'S 132°47'E Radon Springs, 12°45'S 132°54'E Ranger, Retention Pond 1, 12°40'S 132°52'E
M. S. australis:	Twin Falls, 13°19'S 132°47'E
M. S. inornata:	Coonjimba Billabong, 12°40'S 132°54'E Ja Ja Billabong 12°32'S 132°54'E Ranger, Retention Pond 1, 12°40'S 132°52'E
Pseudomugil gertrudae:	Gulungul Billabong, 12°38'S 132°53'E Radon Springs, 12°45'S 132°54'E
P. tenellus:	Coonjimba Billabong, 12°40'S 132°54'E Radon Springs, 12°45'S 132°54'E Ranger, Retention Pond 1, 12°40'S 132°52'E

Family Atherinidae

Craterocephalus nov. sp.:	Twin Falls, 13°19'S 132°47'E
C. stercusmuscarum stercusmuscarum:	Ja Ja Billabong, 12°32'S 132°54'E

Family Ambassidae

Ambassis macleayi:	Island Billabong 12°33'S 132°54'E

Specimens were collected either with a one-man 'Japanese' pole seine net or with a 10 mm mesh, 7.5 m seine net. On capture, the fish were placed into large plastic garbage bins lined with plastic bags filled with water taken at the site of capture and were transported to the laboratory at Jabiru East where they were transferred into small aquaria (up to 10 animals in each). Each tank was topped up with water and each day, one third of the water was exchanged, until the fish were transported to Sydney. The tanks, which were in the open air, were aerated to ensure an adequate oxygen supply. The water temperature was not regulated during the holding period. The fish were not fed on the first day, after which a regimen of feeding twice a day with dry fish food (TetraMin Staple Food) was established until the fish were ready to be transported.

2.2 Transportation of fish

One day prior to departure, the fish were packed in small plastic bags (40 mm x 25 mm) which were slightly less than half filled with water. A maximum of 3-4 large (> 80 mm) fish or up to 6 small fish (< 40 mm) were placed in each bag. The procedure was partly based on advice received from a fish importer who suggested that small fish, approximately 30 mm SL (standard length), would need no more than 150 mL of water and that the water-to-air-space ratio should be 1:4.

The bag holding the fish and water was then placed inside another bag to minimise risk of leakage or puncture. The inner bag was then inflated with pure oxygen and tied off with a rubber band, the outer bag was sealed in the same manner. A number of bags with fish were then placed into large polystyrene containers with lids, packed tightly with any available light, soft packing material and sealed with adhesive masking tape. The containers were marked to indicate the contents and to ensure that the fish were air-freighted as livestock.

2.3 Equipment

Initial set up of the laboratory

On arrival in Sydney, the animals were immediately transported to the University laboratory and released into previously prepared aquaria containing Sydney tap-water.

All tanks used initially were of uniform size, holding 200 L of water. The water was allowed to stand for a week prior to use; temperature was set at 26 ± 1°C using thermostatically controlled, fully submersible heaters (any brand sold by aquarium shops). Circulation and filtration of water were combined by using subsand filters which were covered by 25-35 mm of very coarse river sand. These tanks were regarded as holding tanks for initial acclimation with no more than 5-10 fish per tank.

On the day of arrival at the laboratory, the fish were offered frozen brine shrimp. The following day, commercial dried food (TetraMin) was also introduced. Once the food was readily accepted, a regime of three feeds a day was established, with the dried food being given twice a day and the frozen brine shrimp once a day. Occasionally, when the diet was supplemented with live food (mosquito and may-fly larvae), the frozen brine shrimp was withheld. Sometimes, the frozen brine shrimp was unavailable and a mixture of freshly minced prawns and liver set in gelatine was offered instead. This preparation was given to the fish three times a week. The regimen maintained the fish in a healthy condition.

At first, only those fish which showed any sign of disease were isolated and treated until infection disappeared. Subsequently, the procedure was changed to deliberate prophylaxis. This will be discussed in the relevant section.

Setting up of the laboratory for breeding

A house with three large rooms was converted to a laboratory to maintain and breed fish. Several modifications such as the installation of a large stainless steel sink in the main room, provision of sufficient power points to supply power to each tank, water drainage outlets in the floor and the installation of banks of fluorescent lights over the rows of tanks, were regarded as the necessary requirements for successful maintenance and breeding. Another room was equipped with a household refrigerator and a small chest freezer for storage of food and other perishable items.

Tanks

Several sizes of tanks were found to be very suitable for breeding and maintenance.

50 x 30 x 25 cm: these were selected for breeding or holding a small number (5-10, depending on size) of fish. Also good for isolating sick fish.

70 x 38 x 30 cm: for holding a small number of fish (up to 10 adult rainbow-fish). Also used for sorting fish into various sizes.

125 x 35 x 45 cm: for holding large number of fish (up to 40) ready for experimental work.

Several other sizes were also available. Three tanks (27 x 152 x 33 cm) were specially made up with partitions (with small holes to allow circulation of water) so as to divide the tank into three sections, if necessary. It was thought, initially, that spawning fish in one section could induce non-spawning fish to breed in an adjacent section. Subsequent work showed that such induction was unnecessary and the tanks were used for holding large numbers of adult fish (about 30). Another large tank donated by a member of the School of Biological Sciences (100 x 50 x 45 cm) held up to 40 fully grown rainbow-fish and at least 5 times that number of young fish of up to 10 mm length. The maximum number of fish per unit surface area of water can be calculated (a fish 50 mm long would require 130 cm^2 surface area, Emmens 1962): it was, however, considered safer to underpopulate the tanks to avoid stress and disease. The tanks were set out as indicated in Fig. 1.

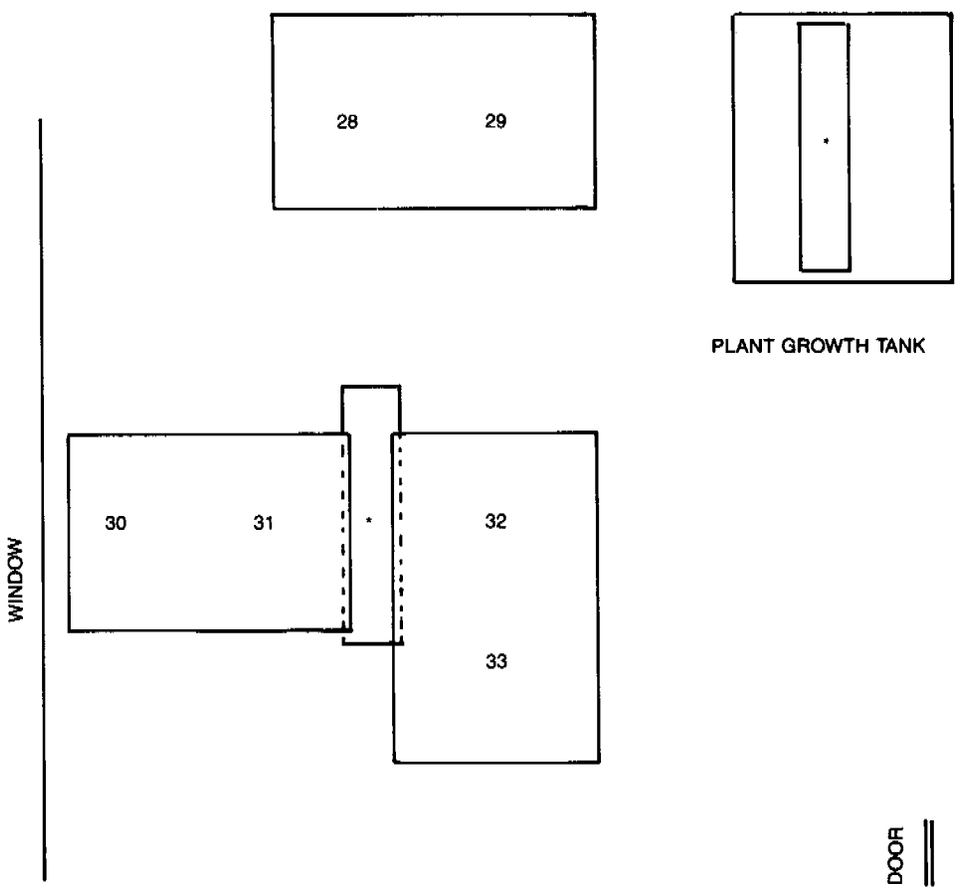
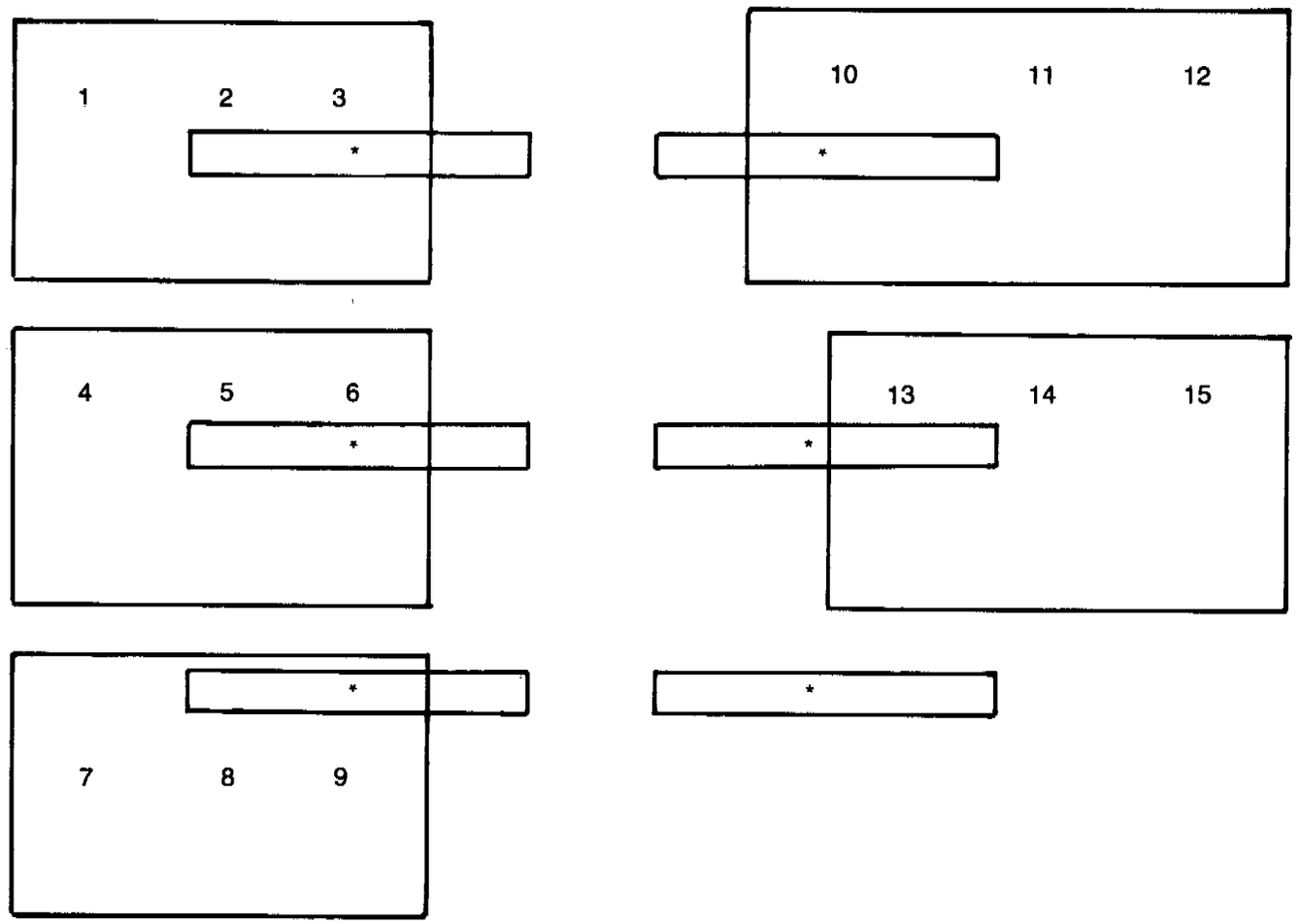


Figure 1. *(Above and opposite):* **Layout of the rooms where the fish were kept. The numbers indicate the tank positions on the tables (large rectangles). The approximate positions of the fluorescent lights (long thin rectangles) are also indicated.**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Maintenance of tanks

Usually, tanks were cleaned at two to three week intervals. Walls of the tank cleaned with 'Filwel' polyester fibres to remove any algal growth and then rinsed with tap-water; subsand filters were treated with household bleach to eliminate any algal growth and then thoroughly rinsed with tap-water. Filters and sand were then replaced and the tanks filled with freshly-drawn tap-water. Tanks were then left without fish until required. External filters were cleaned and the Filwel fibres replaced.

Alternatively, in the large tanks, half the water was replaced with fresh tap-water if there was no algal growth on the walls of the tank and no sign of residual food on or in the gravel at the end of a fortnight.

Several tanks set up for immediate use were always available in case of an emergency or for transfer of fish or eggs if spawning had occurred.

Tank equipment

Filters. The filtration system used depended on the size of the tank. The largest tanks were filtered and aerated with 'Eheim' power filters with a capacity of about 400 L/h. Medium sized tanks were filtered with either 'Maxi-flo' external filters or subsand gravel filters and the smallest tanks exclusively with the subsand filters. The subsand filters were powered by a 'Rena 101' air pump which provided aeration of the water at the same time. The filter medium for subsand filters was coarse sand (grain size > 1 mm) which was removed and washed at least once a month. The filtering medium for the other filters was Filwel polyester fibre which was discarded and replaced at least once a week.

Temperature control. Initially, the laboratory was to be air-conditioned to maintain constant air temperature, and therefore, water temperature (26 ± 1°C) throughout the year. Such an expenditure proved to be unwarranted as it was found that the summer temperatures in Sydney rarely exceeded 30°C in the laboratory, thus being always lower than the maxima in the Magela Creek drainage region. Each tank was equipped with a thermostat, either 'Jaeger' or 'Exotic de Luxe', which was set by trial and error to 26 ± 1°C. Once set, the heaters maintained the set temperature but were checked daily for malfunction or failure. The temperature was read off a standard type of floating aquarium thermometer.

Lights. Ordinary household fluorescent lights (double, 20 W) were placed over the tanks as indicated in Fig. 1. These were turned on by staff on arrival (about 9.00 a.m.) and turned off at 5.00 p.m. The main rooms of the cottage received direct sunlight during the morning and afternoon.

Vegetation. Only breeding tanks were planted with weed. Several varieties were tried of which the most successful were *Ceratopteris* sp. and Java moss (*Taxiphyllum barbieri*). These plants proved to be easy to maintain and handle. Other plants tried were *Nitella* sp., *Vallisneria* sp. and *Elodea* sp.; these were often eaten by the fish, or did not survive under the given conditions. The *Ceratopteris* was used both floating on the water surface (4-6 plants depending on size of plant) and planted in the gravel (numbers as for floating). Java moss, which is filamentous, was usually planted in the gravel, but occasionally twined along the air hoses. This plant can block the subgravel filtration if placed too close to the edges of the filter, as the filaments can be sucked down through the gravel. It is, however, an excellent medium on which to collect the eggs of rainbow-fishes, blue-eyes and hardyheads.

A large tank was set up near a window to grow additional water plants as required. The two main plants were always kept in surplus amounts.

2.4 Diet

The main food given to the fish was 'TetraMin' brand complete flake food. Fish larger than 50 mm standard length (SL)[1] received the flakes as commercially available. Smaller fish, from about 20 mm SL, received the same flakes but crushed to particle size of about 1 mm diameter. Fish between 8 mm and 20 mm received dry TetraMin 'Baby Food' and the new-born fry received a commercial liquid preparation known as 'Liquifry'. Larger fish were fed three times per day, the amount of food given was enough for the fish to eat within about 3 minutes. With smaller fish, several shakes from the TetraMin Baby Food container or three drops of Liquifry were considered sufficient; food was given five times per day.

The above diet was supplemented in a number of ways. At least twice a week commercially available frozen brine shrimp was substituted for the dry food for large fish. Shrimps were thawed and placed in a fine mesh strainer and rinsed several times with tap-water before being given to the fish. From time to time, when brine shrimp was not available, a home made preparation of 50% liver and 50% prawns, macerated in a blender and set in gelatin to prevent water clouding, was used instead: the preparation was kept frozen till use. Fish smaller than 20 mm total length (TL), were fed with live brine shrimp nauplii, *Artemia salina*, available as eggs and cultured in the laboratory by placing a teaspoon full of eggs into a 2 L jar full of well aerated sea water at 26 ± 1°C. At that temperature, the nauplii hatched at the end of 24-36 hours. Eggs were separated from nauplii by stopping the aeration for ten minutes to allow the eggs to settle and the nauplii to concentrate in the water column. These were then siphoned off into a bag of very fine muslin, washed well under fresh water, placed into 200 mL of fresh water and given to the smaller fish at least 2 or 3 times a week.

Young fry too small to take live brine shrimp were occasionally fed with plankton collected from a pond in the University grounds containing cladoceran, and other crustacean, larvae. May-flies, mosquito, chironomid and other larvae, if present, were saved for larger fish.

Variations in the diet as indicated above are discussed in chapters concerning the breeding and rearing of particular fish.

2.5 Water quality

The quality of Sydney water appears to be relatively constant (Skidmore & Firth 1983). For this reason, and because the fish to be bred were known to be hardy, no attempt was made to regulate the pH, to deionise or to control the hardness of the water. It was assumed that maintaining tanks as clean as possible, would eliminate the usual problem of water contamination and pH changes. It is possible that breeding fish in water of different quality to that of Sydney water might require determination of optimal conditions in that water before embarking on a breeding program.

2.6 General care and prevention of disease

Fish were maintained in a healthy condition by ensuring that the aquaria were clean and the water unpolluted. The fish were inspected daily for any physical change or disease. Sick fish were immediately isolated and treated if a known treatment was available. The tank where disease occurred was cleaned immediately and the unaffected fish were transferred into one of the spare tanks.

1. Standard length: from tip of snout to end of caudal peduncle (along lateral line); total length: from tip of snout to distal end of caudal fin

2.7 Comments on methods and equipment used

Capture of fish

Small dip nets or one-man Japanese pole seine nets with 2-5 mm mesh appear to be better for capturing small fish than larger (7.5 metre, 10 mm) seine nets. The fish are caught quickly and transferred into bags with minimum handling, presumably with less stress to the fish. This is most important if the fish are collected in the middle of the day, when the surface temperatures may reach 36°C. The slower method of dragging a seine net through the hot layer of water invariably leads to great mortality of fish.

Collecting in the morning is preferable to any other time of the day, because of cooler water temperatures.

The fish collected must be transported in the water from which they came. Several attempts to carry them in 'clean' laboratory water ended in total mortality within several hours.

Aerating the water during transportation of fish to the laboratory is unnecessary as adequate aeration occurs as a result of jolting of the vehicle carrying the fish.

Fish delivered to a holding area before being transported elsewhere should be kept in tanks sufficiently large to avoid overcrowding. In this case overcrowding may be defined as insufficient volume and surface area for the number of fish; or the occurrence of stress through close confinement after capture and the lack of suitable concealment for escape from dominant fish. The onset of fungal infection or other disease is frequently caused by the stress of overcrowding. Fish which are diseased or stressed are usually unsuitable for transportation as high mortality rates result. The laboratory set up at Jabiru is well designed to hold fish for any length of time. Initially the water in the holding tanks must come from the collecting area and can then be diluted with the laboratory water gradually, about 10% to 20% can be changed daily, without affecting the fish.

Transportation over long distances

A plastic bag, filled with water and oxygen, has now become the standard container for transporting fish. Provided the fish are well insulated and protected against jarring, the mortality rates of transported fish are low, usually close to zero. Overcrowding must be avoided at all costs and 1-2 mL of 1% methylene blue solution added to each bag will help to prevent fungal infection.

As well as the species outlined in section 2.1, several other species were also brought back to Sydney. These were: *Nematalosa* sp., *Denariusa bandata, Glossamia aprion, Toxotes chatareus* and a number of gudgeons (Family Eleotridae). The bony bream survived the journey but died within a day of arrival. The others all lived for different periods of time but could not be used in the breeding program because of the lack of time and space.

Laboratory set up and equipment

Any large area well equipped with power points, adequate water supply, drainage and a separate area for washing of equipment, can serve as an aquarium room.

Small tanks (50 x 30 x 25 cm) are the easiest to use. Small fish like melanotaeniids and atherinids, do not appear to require large volumes of water. The tanks can be washed very easily, the sand can be quickly replaced and the whole aquarium can be in use within a short period of time, provided cured water is available. Larger tanks are more difficult to clean and move because of their weight but they can hold more fish and are therefore

important as holding tanks for fish not currently being used for breeding or other experimental work.

Larvae are best grown in small tanks (30 x 30 x 25 cm) which should be equipped in the same way as other tanks. The eggs can be transferred into these from the breeding tanks with the weed, to which the eggs adhere at spawning, and left till they hatch and grow to at least 10-15 mm TL. Very small larvae are often difficult to observe in larger aquaria.

Subsand filters are both the cheapest and easiest to maintain. Larger and more sophisticated filters are generally expensive and require thorough cleaning and the replacement of the filtering medium at frequent intervals. Such filters are also unsuitable for tanks containing larvae, as the larvae are frequently sucked into the inlet tube. Very large tanks function best with Eheim type filters which move the water rapidly thus providing both the necessary filtering and aeration.

Temperature control

Standard aquarium heaters were found to be quite adequate to control temperatures at the desired level. Although the temperatures were always set at 26°C, it was found (Crowley 1984) that hatching time and development to maturity were shortened at elevated temperatures. The results of this work will be discussed in a later section.

Light

Light is sometimes considered to be a potential stimulus to spawning in melanotaeniids (Beumer 1979). For this reason, a mature pair of *M. nigrans* and *M. s. inornata* were subjected to a 12-h photoperiod using a 40 watt 'Grolux' fluorescent tube, suspended 200 mm above the tanks. Results suggested that the longer light period was not a stimulus to spawning. Several other experiments in the same laboratory (Konagai 1984) also indicated that light is not necessarily an important physical parameter which affects the rate of growth or the frequency of spawning. As the experimental work was done in the earlier part of this study, it was decided not to pursue this line of inquiry further. The light regime was subsequently maintained as described previously.

Vegetation

Several species of aquatic plants were tried to ascertain the one most suitable as a spawning medium. These included: Java moss, *Ceratopteris* and *Nitella* spp.; in addition, it was sometimes possible to obtain *Ceratophyllum, Elodea* and *Vallisneria* spp. but the latter plants presented some difficulty. Some were hard to obtain in large quantity while others failed to grow sufficiently to be of use. All these plants, however, were acceptable as spawning sites as long as the eggs could adhere to the roots or other parts of the plant. It is also possible to use 'spawn mops' (Terciera 1983) instead of plants, if the latter are unavailable. These mops may be constructed from nylon yarn either attached to polystyrene to trail down from the surface, or attached to small stones to be secured in the gravel substrate, the yarn then floats upwards.

Diet

Towards the closing stages of this study, it was found that the commercial preparations of TetraMin adult and baby foods, together with Liquifry preparation were adequate to rear and maintain fish at all stages of their life history. However, frozen brine shrimp, brine shrimp nauplii or live mosquito larvae were still given when available.

Halfway through the study, a small quantity of particulate food manufactured by Mars Confectionery proprietors was made available to test its potential as a food to rear larval fish. The food was available in 3 particle sizes (all < 100 μm). This preparation appeared to

be no better than that already available commercially and was therefore not pursued as another potential food source for fish breeding. It is obvious, therefore, that provided the food is available at an appropriate particle size, it may be accepted by the fish as a food item.

Water quality

The pH in the tanks in which the fish were kept was that of the Sydney water supply (pH 7.6-7.8). As the tanks were usually cleaned regularly (about every two weeks), the pH fell below 6.5 on only two or three occasions (pH 4.5-5.5). It appeared that no adjustment to the pH was necessary, although the fish came from an area where the pH is 3.9-6.5 (K. Bishop, pers. comm.). It was evident that the fish could tolerate a variety of conditions without suffering any ill effects.

Disease

The fish were kept relatively free from disease throughout the whole of the study. The most common ailment apparent was fin and mouth fungus (probably *Saprolegnia* sp.). Whenever infection occurred, the affected fish was removed to a separate tank and treated with 1% methylene blue solution. The remaining fish were treated in exactly the same manner and the process was repeated after 24 hours. If necessary, the methylene blue treatment was continued (up to 2 weeks) until there was no sign of fungal infection and the damaged fins commenced regrowth. Treatment time usually varied between 1 and 7 days. Continued infection over a longer period (more than 14 days) was considered chronic and any afflicted fish were preserved in formalin.

It was found that dirty tanks (caused by excessive feeding) increased the probability of fungal infection as did handling of fish with bare hands. Avoidance of the above assured maintenance of a healthy stock.

No other diseases were diagnosed during the study although an excellent guide to fish diseases was available (Kingsford 1975). Whenever a fish appeared to be ill, it was immediately isolated and allowed to recover in isolation. Fish which darken in colour, lose ability to control their movements, remain apart from other fish, swim close to the surface or lie upside down on the bottom are rarely saved and should therefore be removed from the tank as soon as possible.

3 BIOLOGY OF THE FISHES STUDIED

3.1 Northern Territory rainbow-fishes

General description

Colour. In their natural habitat, rainbow-fishes display a range of colour variation within a single species (Allen & Cross 1982; Munro 1980; Lake 1978). This appears to depend on a number of factors such as locality, water temperature, age, stress or breeding condition and can make correct identification difficult (Lake 1978).

In the laboratory, it was found that the colour of the males of each species and subspecies studied was readily recognisable. The females of all the species (except *M. nigrans*) could, however, be confused as their appearance and colouration were similar, particularly during non-breeding periods or when stressed. The following descriptions are of the colours assumed by the males and females of each species and subspecies during breeding activity.

M. nigrans - males

Body: a very dark mid-lateral band is present, running from the snout to the caudal peduncle, about two scales in width. Anteriorly, above the mid-lateral band, the body is silvery with a lavender iridescence. Caudally, below the mid-lateral band, there is a thin red stripe. Along the belly, there is a narrow red band from the isthmus to one third along the caudal fin. The nape is pale luminous orange and a bright red opercular spot is apparent.

Fins:

Pectorals -	pale luminous orange;
Ventrals -	spine, outer rays and membrane dark;
First dorsal -	spine and membranes dark;
Second dorsal -	golden with a dark distal border;
Anal -	golden with a distal red and black border, the red being continuous with the red of the belly from isthmus to the caudal fin;
Caudal -	deep golden to sulphurous yellow with orange to red colour ventrally.

M. nigrans - females

Body: the colour is similar to that of the males but not so intense. There is no red stripe below the mid-lateral band caudally and no red band on the belly. The nape is not coloured but the opercular spot is apparent.

Fins:

Pectorals -	colourless to pale straw;
Ventrals -	colourless;
First dorsal -	colourless;
Second dorsal -	pale yellow;
Anal -	pale yellow;
Caudal -	yellow.

M. s. australis - males

Body: There are alternating longitudinal stripes of orange-red and silver-grey above a dark, thick zig-zag mid-lateral band which runs from the posterior edge of the operculum to the caudal peduncle. Caudally, below the mid-lateral band, short, thick zig-zag stripes alternate with silver bands. The belly is pale. The whole body has a sheen of iridescent purple. In some males, the nape becomes very dark. A faint red opercular spot is present.

Fins:

Pectoral -	colourless;
Ventrals -	spine, outer rays and membrane very dark;
First dorsal -	spines and membrane very dark to black;
Second dorsal -	rays, reddish orange; membrane, yellow with dark distal border;
Anal -	as second dorsal;
Caudal -	dark blood-red, uncheckered.

M. s. australis - females

Body: resembles the male's but is much paler and lacks the dark mid-lateral band and zig-zag stripes below it. There is usually no iridescent purple sheen. The opercular spot is faint red.

Fins:

Pectoral -	colourless;
Ventrals -	colourless;
First dorsal -	colourless;
Second dorsal -	colourless to pale yellow-orange;
Anal -	colourless to pale yellow-orange;
Caudal -	orange to light red.

M. s. inornata - males

Body: there are alternating longitudinal stripes of yellow-orange and dusky silver and an intensely dark mid-lateral band from about half way along the body to the caudal peduncle. This band is paler anteriorly as far as the posterior edge of the orbit. An iridescent purple sheen is present over the body. The belly is pale. The nape darkens to be almost black. The opercular spot is a glowing red.

Fins:

Pectoral -	colourless;
Ventrals -	spine, outer rays and membrane dark to black;
First dorsal -	spines and membrane dark to black;
Second dorsal -	bright yellow and black checkered markings, dark to black distal border;
Anal -	as second dorsal but may have red streaks along rays anteriorly in very large specimens. Distal border is dark to black;
Caudal -	as for second dorsal and anal but may or may not have a dark distal border.

M. s. inornata - females

Body: There are alternating longitudinal yellow and silvery grey stripes which appear darker caudally. Larger specimens may develop a dark mid-lateral band caudally, but this is not apparent in smaller specimens. The belly is pale. The opercular spot is bright red. The nape does not darken.

Fins:

Pectoral -	colourless;
Ventrals -	colourless;
First dorsal -	colourless;
Second dorsal -	pale to dark yellow, larger specimens may have checkered appearance with dusky distal border;
Anal -	as second dorsal;
Caudal -	as second dorsal, but may appear checkered even when anal and second dorsal are not.

Sexual dimorphism. The rainbow-fishes of the Magela Creek are sexually dimorphic and dichromatic. In males, the spines of the first dorsal fin are usually extended and may lie well past the origin of the second dorsal when not erect. The posterior rays of the second dorsal and anal fins are extended caudally and in some species may extend past the origin of the caudal fin. In females, the first dorsal spines are short, not reaching the origin of the second dorsal. The posterior rays of the anal and second dorsal fin are not extended. The spines and outer rays of the ventral fins of some males are also extended and may reach past the vent and the origin of the anal fin. The secondary sexual characteristics of fin development are not usually so pronounced in *M. s. australis* as in the other species studied.

With increasing age and length, the males may develop a very deep body with a distinct nuchal hump and angulate breast which increases the proportional body depth in some species to more than 40% of SL. However, this characteristic is not as marked in *M. nigrans* where the greatest proportional body depth of males is about 32% of SL.

Behaviour. The mating behaviour of rainbow-fishes has been discussed by a number of authors (Allen & Cross 1982; Crowley & Ivantsoff 1982; Backhouse & Frusher 1980 and others). Most of these authors give broad descriptions of behaviour rather than the specific courtship pattern peculiar to one or several species.

The behaviour pattern during mating appeared to be similar for all the species studied. This was not a fixed action pattern and could be discontinued at any time. The interruption to the behaviour was usually caused by an outside disturbance such as intrusion by other fish in the tank. The courting male would break off to chase the intruder and then return to the female and resume at the point where the courtship behaviour was interrupted.

With a single pair of rainbow-fish, the prelude to mating behaviour was visual. In this initial phase body and fin colours in both members were intensified. A second visual component was a display by the male, the fins erected as he swam beside or at right angles to the female. Chasing followed, with the male swimming below the female, brushing the vent area with erect fins or butting the female with his head near the pectoral fins. This was a tactile component. The third component of mating behaviour was 'head flicking'. In rainbow-fishes, this movement resembled a shake of the entire body and was performed by the male as it swam below or close to the female. The culmination of the behaviour pattern was reached when the pair lay parallel with their heads touching and bodies vibrating rapidly. As the eggs and milt were shed, the pair separated and the male swished its tail, dispersing the eggs and sperm.

Where there were several pairs of fish in a large tank, there was usually a dominant male, not always the largest, but the most aggressive and colourful. The dominant male selected a female and behaved in the manner already described, displaying, chasing, butting and shaking but these activities were frequently interrupted to drive away intruders of both sexes. Finally, the male drove the female into the weed and swam along side, both vibrating as soon as their heads touched and the bodies came to lie parallel. Even during the vibrating phase, the male would break away to drive off intruders. The female usually waited motionless in the weed. The vibrating resumed as soon as the male returned and the shedding of milt and eggs followed. Occasionally, a second male swam unnoticed to the far side of the female, away from the courting male and without any preliminaries began vibrating with the pair so that two males participated in the final phase of mating. After spawning with one female, the dominant male would start the pattern again with another female.

Females, or even a single female, kept in a tank with no male present, were observed shedding eggs, having performed the vibratory action unaccompanied. Males were never observed displaying, head shaking or vibrating unless a female was present. It would therefore appear that the presence of a female was necessary to initiate the reproductive behaviour in males, while for the females, the presence of a male was unnecessary to stimulate the behaviour which led to the shedding of eggs.

Egg numbers. Under stable conditions in the laboratory, large females (> 50 mm TL) of all melanotaeniids studied spawned for 3-5 months, producing more than 50 eggs per day (possibly more than 100 eggs per day for *M. s. inornata*) at the peak of the season. Smaller females (28-35 mm SL) which were only just sexually mature shed fewer eggs, 20-30 per day and spawning did not occur daily.

Eggs were counted (by eye) without being removed from the tank and counted again at the end of 24 hours. Those that were infertile were white and opaque. The counts were then compared with the numbers of hatching larvae and the egg mortality was thus calculated (see Table 2).

Stimuli to spawning. The main spawning period was from October to early May. Spawning occurred sporadically early and late in the season but occurred each day during the peak of the season (usually mid to late summer). All the eggs were shed at one time. Females in peak reproductive condition continued to spawn for many days, even without the stimulus of a male as described above. In the large holding tanks with many (30-40) fish, spawning occurred throughout the year. With a single pair, spawning also occurred occasionally during the off season.

From June to September, when daylight hours were short but increasing, an experiment was carried out to ascertain if light were a stimulus to spawning as suggested by Beumer (1979). Crowley (1984) showed that there was no significant difference[1] between the spawning activities of the controls and the pairs subjected to different light regimes (Table 1).

Aquarists have suggested that changing the water would induce rainbow-fishes to spawn (Schmida, pers. comm.; Axelrod et al. 1971). Sterba (1963) suggested that it was necessary to add a little common salt to the water to induce *M. nigrans* to spawn. In this laboratory, spawning was sporadic during the winter months and was not governed by water

Table 1. Number of spawnings during June to September for *M. s. inornata* and *M. nigrans* as a function of photoperiod

	12 h light		10 h light	
Week	*M. s. inornata*	*M. nigrans*	*M. s. Inornata*	*M. nigrans*
1	1	1	2	1
2	4	3	1	1
3	1	3	1	-
4	2	1	1	1
5	1	2	-	-
6	3	-	2	-
7	1	2	1	2
8	2	-	1	-
9	-	-	-	-
10	1	2	2	1
11	2	1	1	1
12	3	2	2	3
13	-	1	2	1
14	2	3	3	2
15	3	2	3	3
16	3	4	4	3
Total spawnings	29	27	26	18
Total days	112	112	112	112

1. Statistical test used to determine significance was:

$$z = (p_1 - p_2) / \sqrt{p(p - 1)(1/n + 1/m)};\ H_o: p_1 = p_2,\ \alpha = 0.05$$

change. From December to late April, when spawning was a daily occurrence for most species, and particularly for large females, spawning continued daily even when the water was not changed for more than two weeks. An exception was *M. nigrans* where spawning activity decreased after 2.5-3 weeks. Daily spawning was re-established with fresh water, a slight drop in temperature (to 23°C) and a rise in pH from about 6.5 to 7.8. It was unnecessary to add salt to the water to induce *M. nigrans* or any other species of rainbow-fish to spawn. The pH in the tanks was that of the Sydney water supply (pH 7.6-7.8), yet the fish thrived and bred well under laboratory conditions although they had been collected from waters with pH of 3.9-6.5.

Eggs and embryonic development

Although some work on the embryology and subsequent development of rainbow-fishes has been reported (Munro 1980; Beumer 1979; Lake 1978) no detailed descriptions have been recorded untill now. It was found that the pattern of development of melanotaeniids is the same as normally seen in teleosts and as described by Cooper (1980) and many other workers in the field.

Materials and methods. Eggs from the fish being studied were removed with weed from the tanks immediately spawning had occurred. The eggs and a small amount of the weed were placed into 90 mm covered plastic petri dishes with some water from the tanks in which they had been shed. Each petri dish contained 15-20 eggs of one species. As soon as the eggs and weed were placed in the petri dish, measurements were taken using a binocular microscope with a graticule eyepiece. The petri dishes were then placed in a water bath at 26 ± 1°C. The eggs were examined frequently to observe the development of the embryo.

Results. At spawning, the eggs of the rainbow-fishes studied were extruded in a mass but quickly separated due to the movement of the water caused by the male swishing its tail. The eggs were spherical and initially opaque, but cleared quickly to become colourless. There was a tuft of filaments, 20-30 mm in length, originating from a small area of the chorion above the animal pole. The filaments were contractile and adhesive and held the eggs firmly on to the weed or other rough surface. Below the filaments and enclosed in the yolk were numerous small oil droplets which possibly help in flotation during the first few seconds after spawning. The number, colour and size of the oil droplets differed between the species (Table 2). After fertilisation, the oil droplets moved in an ordered manner down the periphery to the opposite, or vegetal pole, where they remained during development. In unfertilised eggs and those eggs in which cell development and cleavage failed to occur, the oil droplets became randomly scattered. The chorion was clear, smooth and colourless, the yolk was non-granular and colourless.

The oil droplets did not coalesce during the development of the embryo and remained at the vegetal pole, to which they had migrated during the initial cleavages; this position became the anterior of the yolk sac and the oil droplets lay close to the developing heart and circulatory system. They became less numerous as the embryo developed and were no longer visible one to two hours after hatching.

Melanotaeniid eggs, like those of other fish are telolecithal. Division is meroblastic and restricted to a small disc directly below the filaments. The yolk material remains undivided and is later covered by embryonic ectoderm.

Growth of the embryo is a continuous process but the description of this process is artificially divided into stages based on work published by Crowley & Ivantsoff (1982). Table 3 gives comparative times of the stages for these fish. The drawings (Fig. 2) of the different stages are diagrammatic and non-specific but give an outline of the way the embryos develop:

Table 2. Egg diameters, width of perivitelline space, number and size of oil droplets, approximate numbers of eggs per spawning and mortality of eggs of rainbow-fishes

	M. nigrans	*M. s. australis*	*M. s. inornata*
Diameter (mm)	1.08-1.00[a] (1.05)[b]	1.02-1.11 (1.07)	0.87-0.92 (0.88)
Perivitelline space (mm)	0.04-0.06 (0.05)	0.04-0.05 (0.047)	0.04-0.06 (0.05)
Number of oil droplets	45-55	50-70	55-65
Droplet diameter (mm)	0.025-0.05	0.025-0.05	0.025-0.05
Colour	chartreuse	pale gold	pale gold
Number of eggs/spawning	> 50	> 50	> 60
% of eggs surviving to hatching	90-95	0-90	95-100

[a]Range; [b]mean

Stage 1. The eggs are fertilised and chorion becomes clear. The oil droplets are directly below the filaments (Fig. 2a).

Stage 2. The first cell is formed. The oil droplets begin ordered movement towards the mid-polar periphery of the yolk (Fig. 2b).

Stage 3. The first cell divides forming two cells of equal size. The oil droplets migrate as far as the mid-polar periphery (Fig. 2c).

Stage 4. Division of cells continues in the vertical plane resulting in eight equal sized cells. Most of the oil droplets reach the vegetal pole where they remain uncoalesced throughout the subsequent development of the embryo.

Stage 5. First horizontal division takes place resulting in 32 cell stage. The cells are quite small and occupy the same area on the yolk as the original single cell. All oil droplets are now at the vegetal pole.

Stage 6. Continuous division results in a blastodisc. The onset of epiboly is seen when the blastodisc appears to flatten and spread downwards over the yolk surface, covering an increasing area (Fig. 2d).

Table 3. Timing of successive stages during embryonic development of rainbow-fishes

Stage 1 is given in seconds, all other stages are given in hours; temperature = 26 ± 1°C

	Species		
Stage	*M. nigrans*	*M. s. australis*	*M. s. inornata*
1	0-5	0-5	0-5
2	0.67	0.75	0.67
3	1.08	1.17	1
4	2.5	2.5	2.5
5	4	3.25	4.5
6	7.5	8.75	10
7	12.75	12	13
8	15	13.7	14.25
9	19	16.5	17
10	21	18.75	19
11	23	21	23
12	26	24.5	25.5
13	27.5	25.75	26
14	30.25	29.5	30
15	38	32	35.75
16	46.5	44	43.75
17	52	51	53
18	60.5	58.75	63
19	71.3	59.5	64
20	80	78	80
21	109	103	102.5
22	110-113	107-108	107-107.5

Stage 7. The embryonic shield and germ ring become visible. The embryonic axis is clearly visible in the mid-area of the embryonic shield (Fig. 2e).

Stage 8. When the embryonic ectoderm covers the entire yolk except for a small area, the yolk plug, the neural groove becomes visible.

Stage 9. A small embryo with the head clearly defined is seen lying on the surface of the yolk sac, which is now entirely enclosed within the embryonic ectoderm. The head lies close to the oil droplets but the tail is still undefined.

Stage 10. The optic vesicles become the most prominent feature of the embryo. Towards the posterior end, Kupffer's vesicle is clearly visible (Fig. 2f).

Stage 11. The tail becomes well defined and the first caudal somites are seen. The pericardial sinus is visible below the head (Fig. 2g).

Stage 12. The optic cup has formed but no lens is visible. The first melanophores appear on the yolk sac and lateral surfaces of the body.

Stage 13. Melanophores begin to darken the optic cup. The lens is now apparent. Melanophores appear on the head, body and in the peritoneal cavity.

Stage 14. The onset of circulation is seen as the pericardial cavity begins to pulsate rhythmically. Plasma, but no red blood cells, is present at this stage in the yolk sac vessels and heart. The heart appears as a straight tube. The pulse rate is slow but increases with the development of the embryo.

Stage 15. Movement is seen as the embryo flexes its body and tail. The tail extends free from the yolk sac and the embryo is about two thirds of the way around the yolk sac, coiled in the polar plane.

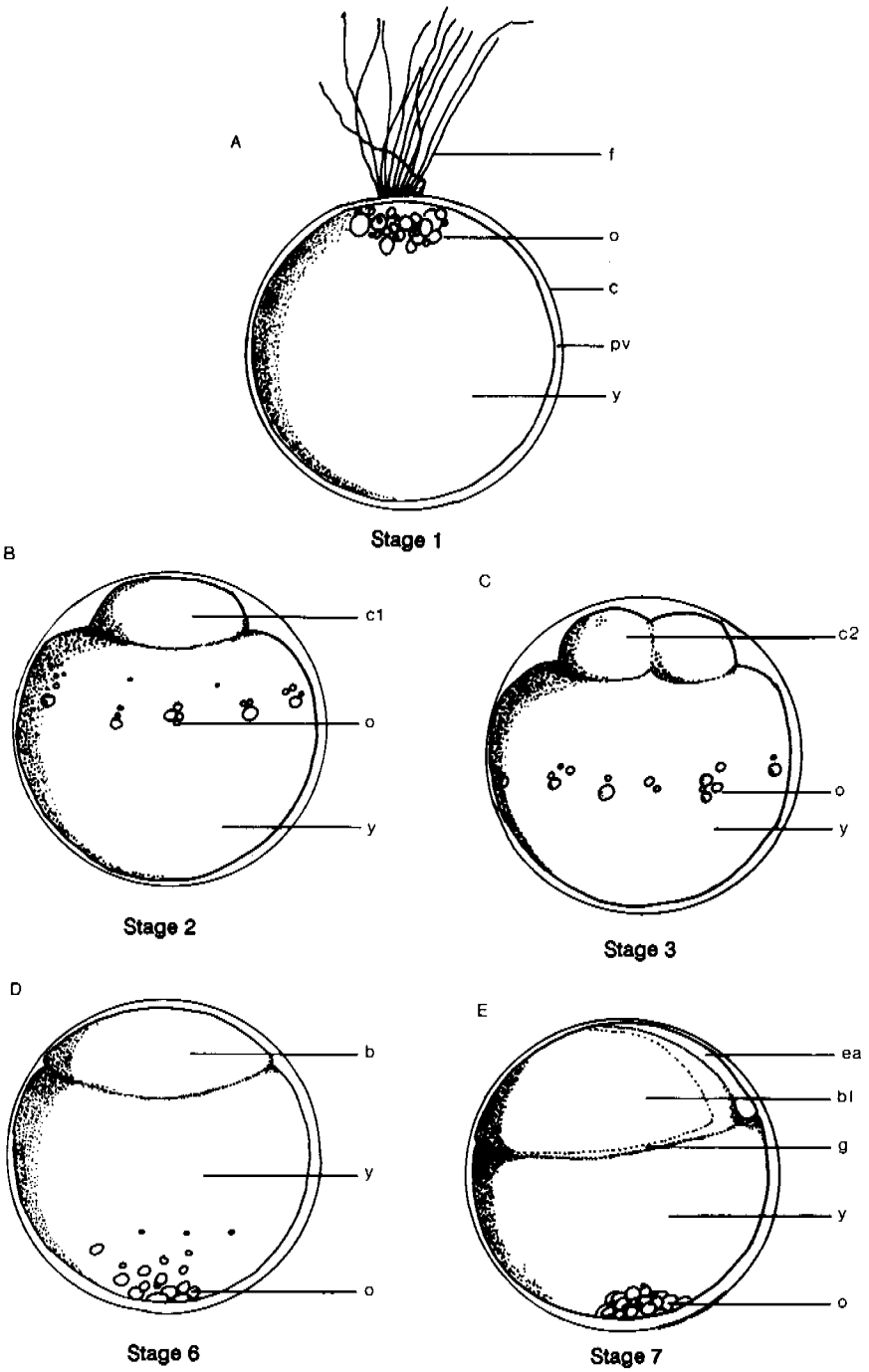
Stage 16. Otic vesicles become apparent. Red blood cells are discernible in the circulating plasma. The colour gradually darkens from very pale pink to red as more red blood cells enter the blood stream. As the blood moves through the heart, a pause in the flow indicates that the heart is divided into chambers although it is still a straight tube (Fig. 2h).

Stage 17. The pectoral fin buds are seen as small semicircular ridges laterally, just behind the head. The optic cups do not appear to be closed ventrally but the lenses are clearly visible with the retinal pigment behind them.

Stage 18. The branchial arches are seen. The yolk sac is becoming smaller and the embryo is increasing in length. The tail is now coiled in the equatorial plane rather than in the polar plane. Movement of the tail from side to side, passing below the yolk sac is frequent. The swim bladder appears as a small vesicle in the peritoneal cavity below the junction of the head and tail. The peritoneal cavity has dense pigmentation on its inner dorsal surface (Fig. 2i).

Stage 19. The liver, which appears initially as a yellow vesicle within the abdominal cavity, is seen just below and posterior to the left pectoral fin. The outline of the mandibular arch appears as an inverted crescent when the embryo is seen from the anterior aspect (Fig. 2j).

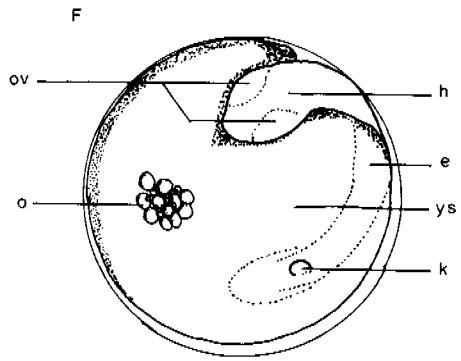
Stage 20. Meckel's cartilage is seen lying directly below the upper jaw which is usually outlined by a row of melanophores. The pectoral fins are increasing in length and moving freely.



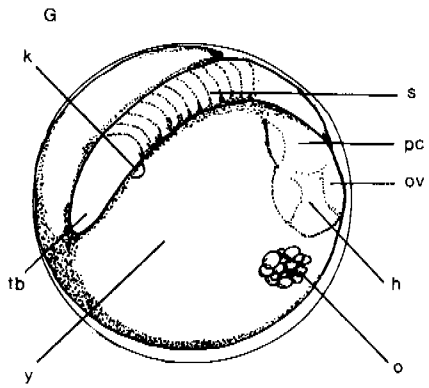
Key

b: blastodisc; bl: blastoderm; br: branchial arches; c: chorion; c1: first cell; c2: two-cell stage; e: embryo; ea: embryonic axis; f: filaments; ff: fin fold; g: germ ring; h: head; he: heart; k: Kupffer's vesicle; l: lens; li: liver; lm: lateral melanophores; m: melanophores; me: Meckel's cartilage; o: oil droplets; oc: optic cup; ot: otic vesicle; ov: optic vesicle; pb: pectoral fin bud; pe: pericardial cavity; pf: pectoral fin; pv: perivitelline space; s: caudal somites; sw: swim bladder; tb: tail bud; t: tail; u: upper jaw; vm: ventral melanophores; y: yolk; ys: yolk sac.

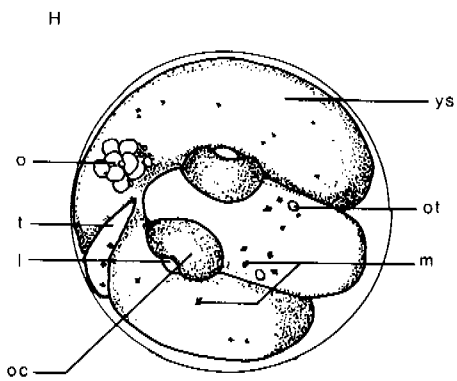
Figure 2. *(Above and opposite)* **Stages in embryonic development of rainbow-fishes**



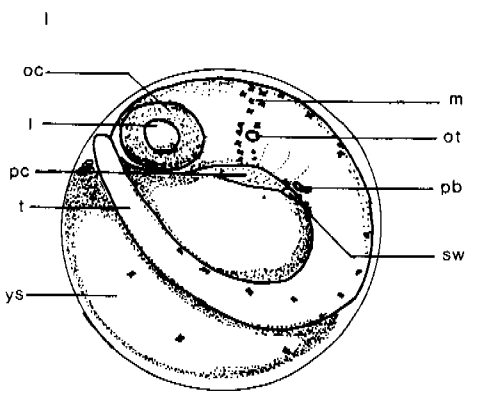
Stage 10



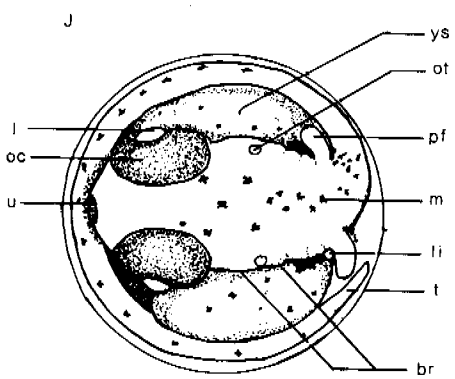
Stage 11



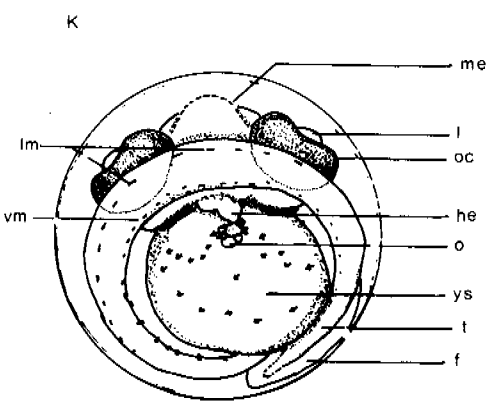
Stage 16



Stage 18



Stage 19



Stage 21

Stage 21. The mouth, now fully formed, opens frequently. The whole embryo often turns within the chorion. The dorsal surface turns yellow, spreading from the head to the body and tail. The tail has a single row of melanophores dorsally and a double row of melanophores ventrally which meet at the tip of the tail. There is a mid-lateral row of melanophores along each side of the tail. The chambers of the heart are well defined. A small number of oil droplets remain in the anterior of the yolk sac, close to the heart. The yolk sac is very reduced and barely visible from the dorsal aspect. The tail is still coiled in the equatorial plane (Fig. 2k).

Stage 22. For several hours before hatching, the tail again moves to the polar plane but to one side of the embryo. About half- to one-hour before hatching, the chorion becomes flaccid. In *M. s. australis* and *M. s. inornata*, it was possible to see a small protuberance in front of the mouth a few seconds before hatching. This was not seen in *M. nigrans*. Hatching is very rapid: a flick of the tail and the larva is free.

Change in the pulse rate during the embryonic development. The pulsation of the heart was slow initially but the rate increased as the embryo developed. There was a high positive correlation between the increase in the pulse rate and age (Table 4); therefore, apart from the recognition of the stage, at a given temperature it should be possible to age the embryo by its pulse rate.

Discussion. During their development, the embryos of the fish studied resembled one another very closely. There were differences in the eggs, however, particularly in the colour, number and size range of the oil droplets (see Table 1). The width of the perivitelline space also differed. The different species could therefore be distinguished by the combination of the characteristics (particularly the colour and number of oil droplets) presented in Table 2.

Table 4. Heart rate in rainbow-fish embryos

r = Pearsons product moment correlation;
p = probability; temperature = 26 ± 1°C

	Heart rate (beats/min)		
Time[a]	*M. nigrans*	*M. s. australis*	*M. s. inornata*
Initial	97	93	94
40 h	120	136	109
50 h	144	140	118
60 h	147	184	130
70 h	151	176	139
80 h	156	206	134
90 h	141	188	170
100 h	153	188	176
Final	190	231	235
r	0.83	0.73	0.78
p	> 0.001	> 0.001	> 0.001

[a]Hours from fertilisation except that the initial rate refers to the first observation of pulse in the pericardial cavity and the final rate is the rate immediately prior to hatching.

The increases in heart rates with time were similar for all the species with no significant differences observed between species. Whilst the pulse rates were not diagnostic of the species, they are certainly considered to be useful to stage the developing embryos provided that the temperature at which the development takes place is accounted for. The time taken to completion of hatching is governed by temperature. At lower temperatures, development takes longer: the difference for *M. nigrans* and *M. s. inornata* at 25 ± 2°C and at 27 ± 1°C was about 40 hours for each (Crowley & Ivantsoff 1982). This line of inquiry was not pursued further but it has been determined by Konagai (1984), in the same laboratory, that temperature changes in either direction alter the rate of development and the survival rate of a gudgeon, *Hypseleotris galii*.

Larval development

Whilst the size at sexual maturation of most rainbow-fishes (Allen & Cross 1982) and something of the growth rate under natural conditions of *M. nigrans* and *M. s. inornata* (K. Bishop, pers. comm) are known, a detailed study of the development of the external features has not been made.

Studies of planktonic larvae (Brownell 1979; Russell 1976; Berry & Richards 1973) have shown that some characteristics may be used to differentiate between species in the larval stages. These characteristics include myomere counts, melanophore patterns, sequence of ray and scale development and variations in proportions during growth. Fuiman & Baker (1981) suggested that myomere count is the most positive character for separating freshwater cyprinid larvae. Cooper (1980) found that scale formation and development of fins could also be used to differentiate between the species.

Terminology for the stages of larval development was introduced by Hubbs (1943). Since then, several authors have modified or expanded Hubbs' definition (Snyder et al. 1979; Robertson 1975; Balon 1975). In this study, the terminology follows that defined by Berry & Richards (1973).

Materials and methods. Adult fish of the different species were separated into breeding pairs. They were placed in tanks which had been planted with fine-leafed weed. Immediately after spawning, the adult pair were removed from the tank to prevent predation of the eggs.

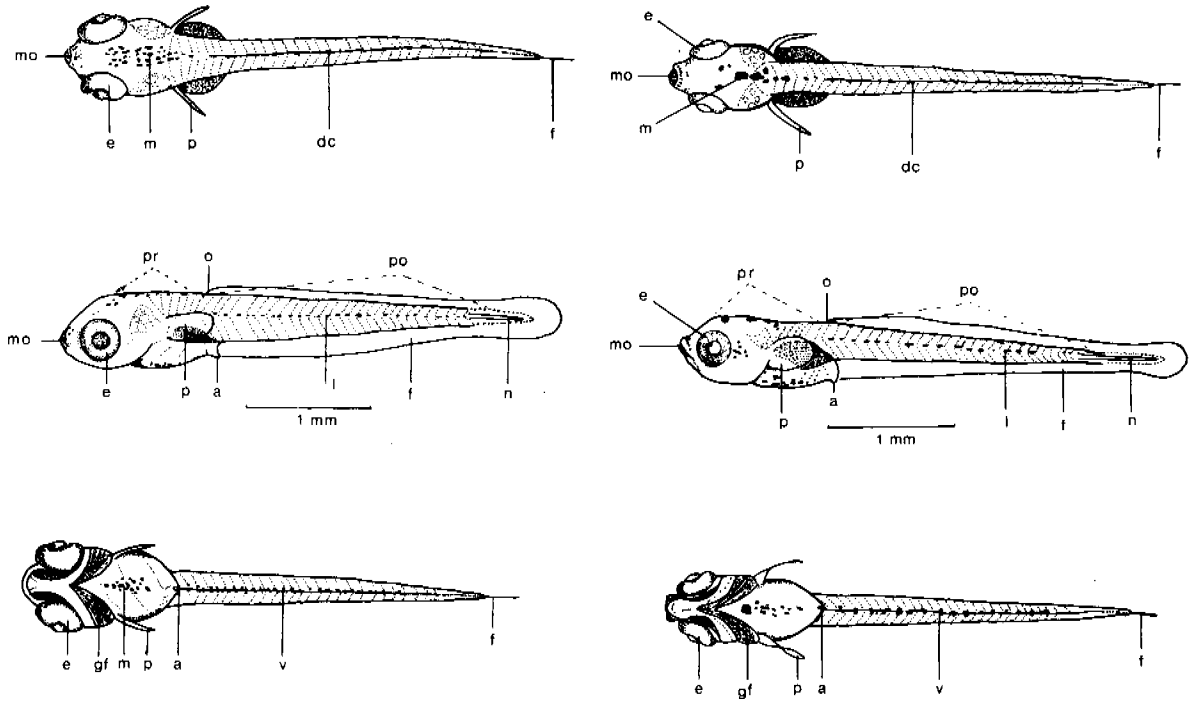
The tanks were maintained at 26 ± 1°C and the young hatched over a 12 hour period 5-6 days after spawning. From the first day of hatching, the larvae were fed 2-3 drops of Liquifry No. 1 and TetraMin E five times a day. From day 14, the Liquifry was discontinued and the diet was supplemented with live brine shrimp nauplii. When the larvae reached a mean total length of 10 mm, finely crushed TetraMin Staple Food replaced the TetraMin E. From 15 mm TL, mashed frozen brine shrimp or sieved raw liver (when the brine shrimp was unavailable) was introduced and the live brine shrimp nauplii were discontinued.

After 24-28 days, the tanks were cleaned and half the water replaced with fresh water. Thereafter, at 14 day intervals, the larvae were moved to clean tanks in which the water had been warmed to 26 ± 1°C and allowed to stand for 24 hours.

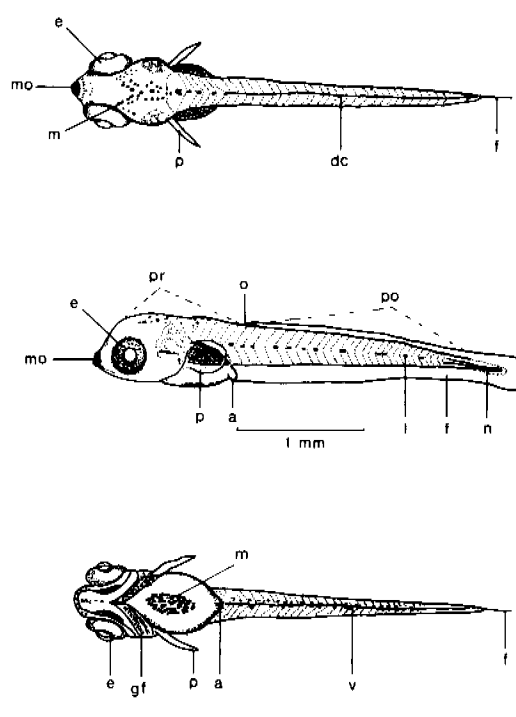
At least 10 larvae were measured every second or third day. The fish were caught in a fine scoop net, placed in a 500 mL plastic container and then transferred, using plastic tubing (5 mm i.d.), with about 1 drop of water into a glass culture dish (40 x 40 mm, 30 mm diameter, 7 mm well depth) or into one concavity of a double concave microscope slide. Measurements were made using a binocular microscope with a graticule eyepiece for which the correction factor was known. By this method, with a minimum amount of handling, the fish were measured as rapidly and accurately as possible and returned to the tank by submersing the culture dish or microscope slide in the water.

Results and discussion. At hatching all the larvae are similar in that they all have well developed mouths, pectoral fins and swim bladder (Fig. 3). The yolk sac is quite reduced. The gut is already coiled and food is taken within a few hours of hatching.

In the *splendida* subspecies, the dorsoventral fin fold extends from the first postanal myomere dorsally and to the anus ventrally. There is no preanal fin fold. In *M. nigrans*, the dorsal origin of the fin fold extends from the preanal myomeres. Details of means and the ranges of TL and numbers of myomeres are given in Table 5.



M. nigrans *M. s. australis*



M. s. inornata

Key

a: anus; dc: dorsal contour (melanophores); e: eye; f: fin fold; gf: gill filament buds; l: lateral contour; m: melanophores; mo: mouth; n: notochord; o: origin of fin fold; p: pectoral fin; po: post-anal myomeres; pr: pre-anal myomeres; v: ventral contour.

Figure 3. Dorsal, lateral and ventral views of newly-hatched larvae of rainbow-fishes

Table 5. Characteristics of newly-hatched larvae of rainbow-fishes

N = 25 for each species

Feature	*M. nigrans*	*M. s. australis*	*M. s. inornata*
Pre-anal myomeres	6-7	6-7	6-7
Post-anal myomeres	28-29	25-27	26-28
Dorsal origin fin fold[a]	5-7 (pr)	1 (po)	1 (po)
Dorsal melanophores	40-48	19-29	29-45
Lateral melanophores	22-30	10-24	14-29
Ventral melanophores	34-43	21-43	14-25

[a]pr = pre-anal; po = post-anal

Melanophores developed during incubation and were obvious on the head, dorsal, ventral and lateral surfaces of the body and tail of the newly hatched larvae. Melanophores were too variable (Table 5) in numbers to be of any diagnostic value in identifying the species. The yellow colour which developed on the dorsal surface of the head and body prior to hatching, persisted for some 24-36 hours after hatching.

The newly hatched larvae swam strongly and remained near the surface of the water. There was no tendency to sink to the bottom or to tilt when swimming ceased.

Munro (1980) reported that larvae of *M. fluviatilis* clung to weeds for several days and were not free swimming for more than a week. This behaviour was not observed for any of the species studied.

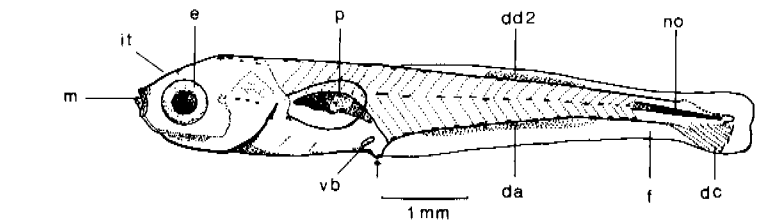
Fin development. Development of fins and scales in melanotaeniids is outlined in Fig. 4.

Pectoral fins: membranous fins are present at hatching. Movement is well co-ordinated. The rays develop first along the dorsal side of the fin. The rays appear at about the same time as the rays for the second dorsal and anal fins.

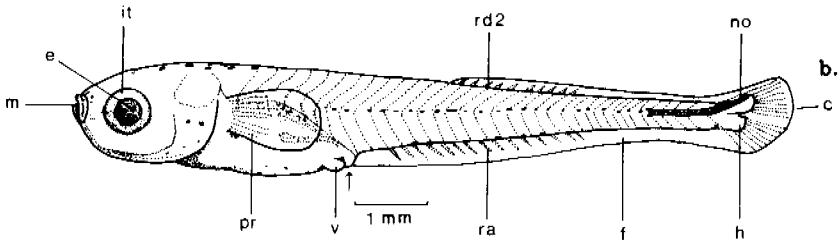
Caudal fin: a break down of the ventral line of melanophores just posterior to the waisted area of the dorsoventral fin fold, and a thickening of the tissue in this area indicates the initial development of the caudal fin. The first rays of the fin develop in the thickened tissue and thence dorsally. The notochord flexes upwards at the same time. The caudal fin is rounded at first but then becomes forked as the outermost rays increase in length.

Ventral fins: These fins are first seen as small ridges just anterior to the anus. They appear almost parallel to the longitudinal axis of the body. As they grow, the origin of the fins curves around so as to assume the adult form. The inner rays are joined to the abdomen by a thin membrane. Initially the fins are membranous, the rays and the spine do not develop until the larvae are about 10-10.5 mm TL (Fig. 4d).

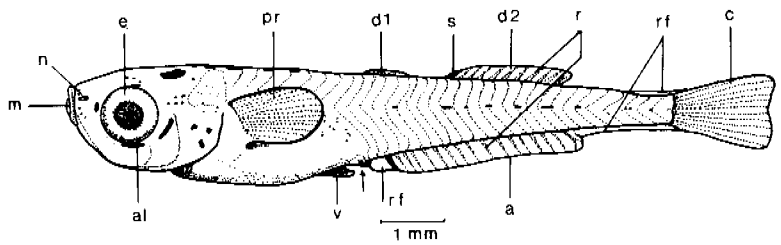
Anal and second dorsal fins: as the first rays of the caudal fin develop, thickening of the tissue of the fin fold appears in the area of the future anal and second dorsal fins. The rays are seen soon afterwards, growing through the thickening tissue towards the distal edge. The spine is the last of the members to develop. The fin fold persists between the anal fin and the vent until all the rays and the spine are complete. Dorsally, the fin fold is resorbed anterior to the second dorsal fin.



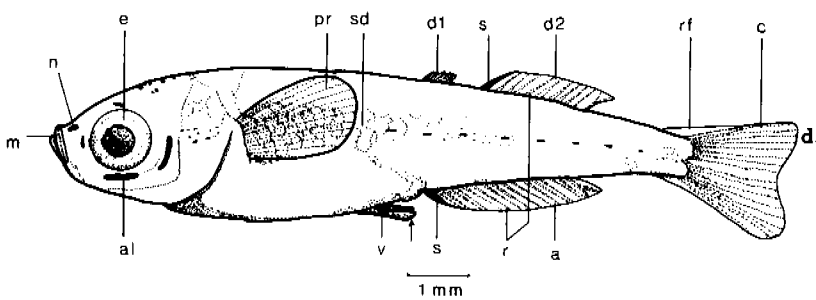
a. Development of the caudal fin and thickening of fin fold tissue for anal and second dorsal fins. Pectoral fin bud is apparent.



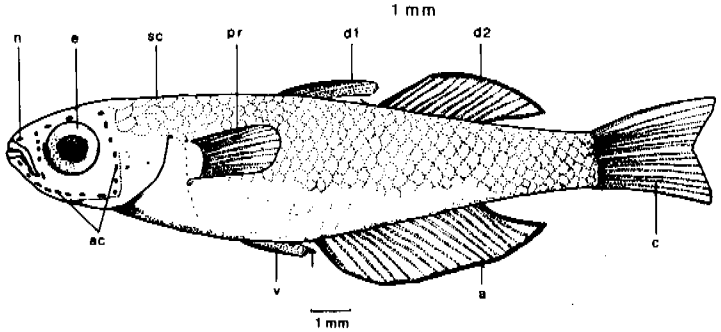
b. Rays developing in anal and second dorsal fin, most rays of caudal fin complete, rays developing in pectorals.



c. First dorsal fin emerges, spines present in anal and dorsal fins. Pores of the acoustico-lateralis system visible on head. Outer rays of caudal fin start to elongate.



d. Fins are completed. Melanophores under pectoral fins indicate the onset of squamation. The fish are now young adults or juveniles.



e. Completion of squamation in *Melanotaenia* spp. At this stage, the opercular area is still without scales. The male secondary characteristics are already apparent in the anal and second dorsal fins.

Key: a: anal fin; ac: acoustico-lateralis pores; al: acoustico-lateralis groove; c: caudal fin; d1: first dorsal; d2: second dorsal; da: developing anal fin; dc: developing caudal fin; dd2: developing second dorsal fin; e: eye; f: fin fold; h: hypural developing; it: interorbital trough; m: mouth; n: nostril; no: notochord; p: pectoral fin; pr: pectoral rays; r: rays of fin; ra: anal rays developing; rd2: second dorsal rays developing; rf: residual fin fold; s: spine; sd: developing scales; vb: ventral fin bud; v: ventral fin.

Figure 4. Development of fins and scales in rainbow-fishes

First dorsal fin: unlike the caudal, anal and second dorsal fins, this fin does not appear to have its origin in the fin fold tissue, since the fold degenerates before the fin becomes apparent. In contrast to the other fins, where the rays and spine develop in and appear to grow through the existing membranous tissue, the spines and the membrane of the first dorsal develop and grow together. This fin does not always develop in *M. s. inornata* and specimens lacking this fin have been collected from the natural habitat.

Further development of fins. The rays become branched only when the fish reach about 20-30 mm in total length. The first ray of the anal and the second dorsal fins are the last to become branched and this does not occur until the fish are greater than 60 mm TL.

Development of the gill filaments and operculum. At hatching, the branchial arches are apparent from the ventral surface. Each arch has a series of nodules (filament buds). These grow quite rapidly to form the filaments and lamellae. As the filaments grow, the dermal opercular bones develop as well. By the time the filaments are all well developed, they are protected by the opercular bones.

Development of scales. The onset of squamation is heralded by the appearance of melanophores immediately posterior to the dorsal origin of the pectoral fin (Fig. 4d). The first scales to be seen are those along the mid-lateral line, followed by development on the dorsal and ventral surfaces, with the development of the dorsal scales being slightly in advance of those on the ventral surface. The interobital and opercular surfaces are the last to be covered. Scales above the mid-lateral band are outlined by melanophores on the posterior edge; as a rule, those below the line are not.

Development of the acoustico-lateralis pores on the head. The pores become visible at the 8.0-8.5 mm TL stage. Initially they become apparent as a series of grooves on the preopercle, the lachrymal, frontal and nasal bones (Fig. 4e). The grooves are not continuous externally and each bone has a separate groove. Later, the grooves become enclosed to leave a series of pores (Fig. 4e).

Pigmentation. Melanophores develop during the incubation period. Newly-hatched larvae are dark on the dorsal surface of the head and body and on the belly. The eyes and the peritoneum above the swim bladder are also heavily pigmented. There is a single dorsal and double ventral body contour of melanophores, the latter extending from about the anus. Where the fin fold is waisted, the melanophores converge to form a single contour extending around the tip of the notochord to join the dorsal contour. In both *splendida* subspecies, the ventral contour later extends to the developing ventral fins.

The abdominal melanophores gradually disappear. Those on the head on the other hand, gradually increase in number to form a Y-shaped marking which appears to be typical for the genus *Melanotaenia.* The yellow colour which is apparent prior to hatching, fades 24-36 hours after hatching. The number of dorsal, lateral and ventral melanophores are not constant for any species but the counts tend to be higher for *M. nigrans* (Fig. 3). As rays develop in the fins, melanophores become apparent, lying along each of the rays. Chromatophores develop in the caudal, anal and second dorsal fins of males from about 14-20 mm TL and the dark distal border of the anal and dorsal fins becomes quite obvious at about the same time. The mid-lateral band in *M. nigrans* becomes apparent from about 8.0 mm TL.

Rates of development and growth. The growth rates of the larvae studied showed a wide variation between individuals from a single spawning. This difference has been noted previously (Allen & Cross 1982). As the larvae grow, the difference becomes progressively more and more apparent.

Bishop et al. (in press) had noted that no detailed measurements of growth rates had ever been made on the rainbow-fishes of the Magela Creek system. From their work they

noted that during a period of one year, the modes of length-frequency distributions increased by approximately 30 mm and that the growth of *M. nigrans* and *M. s. inornata* young appeared fastest during the 'Early-wet' to the 'Mid-wet' season, with the median size of 48 mm reached by the next Early-wet season. Data also indicated that sexual maturity was reached early, between 27 and 39 mm for both sexes. No real differences were observed for the other species of rainbow-fishes in the onset of sexual maturation. Beumer (1979) had obtained similar results and this study does not contradict the findings obtained in the field. At hatching, in this laboratory, the mean length for *M. s. inornata* was 3.46 mm TL. Thirty one days later, the mean length was 7.45 mm, at two months 13.8 mm and 19.5 mm at three months. *M. s. australis* hatched at mean length of 4.0 mm, grew to 9.2 mm in one month, 18.7 mm at two months and 24.6 mm at 78 days. *M. nigrans* hatched at mean length of 4.04 mm TL, reached 14.5 mm in one month and 20.9 mm TL in 52 days (see Table 6 for details).

Since rainbow-fish larvae do not show distinct metamorphosis, it is appropriate to regard as larvae those specimens in which the squamation is not complete (complete by 17-21.5 mm TL in this study). This stage is followed by the juvenile phase which Bishop et al. (in press) define as the stage preceding sexual maturity. However, the sexes can be differentiated during the juvenile phase as the secondary sex characteristics become quite apparent, especially in the male, from about 14-20 mm TL.

Table 6. Lengths of the rainbow-fishes studied in relation to age

Day 0 = day of hatching; all measurements are in mm; temperature = 26 ± 1°C.

N. nigrans		*M. s. australis*		*M. s. inornata*	
Day	TL	Day	TL	Day	TL
0	4.0	0	4.00	0	3.4
2	4.55	2	4.23	3	4.08
5	4.68	4	4.51	5	4.17
6	4.72	6	4.69	7	4.28
10	5.15	8	4.95	10	4.61
12	5.86	10	5.10	12	4.74
14	6.07	12	5.51	14	4.88
17	7.88	14	5.70	16	4.93
20	8.67	16	6.10	19	5.31
22	10.80	18	6.56	22	5.67
24	11.50	20	6.92	26	6.13
27	13.18	22	7.4	28	6.40
31	14.35	26	8.50	34	7.14
34	14.56	28	9.21	36	7.32
36	15.97	33	10.51	38	8.10
38	16.96	35	13.35	41	8.45
43	17.90	38	13.80	50	10.65
46	18.30	40	15.02	55	12.32
52	20.90	42	16.16	58	13.59
		47	16.99	64	13.83
		51	17.06	70	14.10
		54	17.53	73	14.30
		56	18.46	77	16.10
		58	18.66	81	17.93
		63	20.97	86	19.55
		68	21.83		
		78	24.60		

The development of other external features of the larvae can be associated with a certain size reached and the variation in time of development of a particular feature is not great. These features, therefore, may be used as a rough guide in determining the age of the larva examined (Table 7).

There was little apparent difference between members of the *splendida* group when first hatched, with the exception that the larvae of *M. s. inornata* were consistently smaller (3.1-3.7 mm TL) than the others. The size range for *M. nigrans* was 3.7-4.2 mm TL. The dorsal origin of the fin fold also distinguished the *splendida* spp. from *M. nigrans*. In the former, the origin was from the first post-anal myomere, while in the latter the origin was from the pre-anal myomeres.

Except for *M. nigrans*, the myomere counts did not contribute to identification as there was an overlap between all the species studied. Variations in body growth that were examined (Crowley 1984) in the larvae and juveniles were not sufficiently different to be used reliably to identify any of the species studied.

Discussion. The present findings confirm both Bishop et al. (in press) and Allen & Cross (1982) that growth in melanotaeniids is rapid and that the onset of sexual maturation occurs early. If necessary, young fish, no larger than 30-40 mm, can be used for breeding. This may not be necessary, as the rainbow-fishes studied will spawn throughout the year, apparently confirming Beumer's (1979) and Bishop et al.'s (in press) conclusion. Fecund individuals can be retained as 'breeders' and used as frequently as required. Rainbow-fishes tend to be long-lived, with some specimens surviving and breeding in laboratory tanks for well over 4 years. Growth continues for at least this period and the fish reach sizes of upto 120 mm TL, which appears to be in excess of the maximum size observed in natural conditions.

Table 7. Lengths of larvae of rainbow-fishes at which major developmental features become evident

All measurements are in mm TL.

Feature	*M. nigrans*	*M. s. australis*	*M. s. inornata*
Pectoral fins			
Rays and spine	8.0-8.2	8.2-8.5	8.2-8.4
Caudal fin			
First rays visible	6.0-6.4	6.2-6.5	6.0-6.2
Rays completely developed	8.0-8.2	7.5-8.0	7.6-8.2
Branched rays	18.9	22.0	17.0
Ventral fins			
Buds	6.0-6.4	6.2-6.5	6.0-6.2
Rays	9.0-9.3	10.0-10.5	9.5-10.0
Anal and second dorsal fins			
Rays half grown	7.5-8.0	7.0-7.2	7.4-7.7
Full rays	8.5-9.0	8.5-9.0	8.2-8.6
Branched rays	20.00	22.5	23.0
First dorsal fin			
Emergent	8.5-9.0	8.5-9.0	8.2-8.4
Squamation			
First scales	12.5-12.7	12.0-13.0	11.4-12.0
Complete	17.5	22.0	21.0

Recommendations

Because of their high fecundity and high survival rate, large numbers of rainbow-fishes can be bred over a short period. Since at no time during this study were the fish bred to their maximum potential, the maximum numbers capable of being bred cannot be estimated with a great degree of accuracy. However, it would not be difficult, with a battery of 20-30 breeding tanks and 3-4 large holding tanks, to produce 100 *M. s. inornata* or *australis* per week. *M. nigrans* on the other hand, appears to be less fecund and would be difficult to produce in large quantities. Bishop et al. (in press) surmised that this species was probably a continuous or opportunistic breeder. In the laboratory, *M. nigrans* was less inclined to breed than the other species of rainbow-fishes studied and, although it too bred throughout the year, it was not possible to determine with any accuracy when the fish would spawn. It is thought, therefore, that 1000-2000 rainbow-fish per annum could be produced with the facilities which were available in this study.

Survival rates of the fish produced can be improved by not overcrowding, classing the fish into size groups to prevent competition and aggressive behaviour. Maintaining the tanks in scrupulously clean condition will also assure a greater survival.

3.2 Northern Territory blue-eyes

Little, if anything is known about the range of colours and reproductive biology of the Northern Territory blue-eyes. *Pseudomugil gertrudae* was originally described from New Guinea and was first recorded in Australia by Munro in 1958, from Queensland. In 1964 Taylor reported its occurrence in the Northern Territory. *P. tenellus*, which appears to be restricted to the Northern Territory, was first described in 1964 by Taylor; Bishop et al. (in press) have given the only detailed biology of this species.

Numerous but anecdotal items of information about *P. gertrudae* have been recorded in aquarium journals, but there has been nothing about *P. tenellus*, which is yet to find its way into the aquaria of fish breeders.

The two species of blue-eyes are readily recognisable in the laboratory because of the distinctive pigmentation of *P. gertrudae* during all stages of its life history.

General description

P. gertrudae

Colour. *Body:* all scales except those of the mid-dorsal and mid-ventral rows have a spot varying in size and intensity from specimen to specimen and from time to time in the same specimen, forming about 6 rows of discontinuous lines, except for the mid-lateral stripe, which tends to be continuous from just below and in line with the origin of first dorsal fin to the caudal peduncle. The general body colour is pale yellow to gold, the muscle tissue is translucent, with the swim bladder clearly visible. The pectoral base is often coloured by a red or orange spot. The eyes are silver blue. Dorsal and ventral contours, beginning at the origin of second dorsal and anal fins, are black.

Fins: second dorsal, anal and caudal fins are marked with distinct black oval spots. Membranes are whitish or have a golden hue. First dorsal, pectoral and ventral fins have a hyaline membrane, frequently with whitish or yellowish tinge. Membrane between the last two spines of the first dorsal may be black. Edges of all of the fins are outlined in black. Dorsal rays of pectorals are orange or reddish.

Sexual dimorphism. Sexual dichromatism is only moderately marked. The shape of the fins, however, is quite different. In males, the dorsal and the anal fins are larger and the second to fourth spines of the first dorsal are extended into filaments. The outer rays of the ventral fins are also extended into filaments.

Because of the very small size of the adult fish, which scarcely reaches 30 mm TL when fully grown, colours and distinguishing features of this species are difficult to see unless a magnifying glass is used.

Behaviour. The mating behaviour of *P. gertrudae* has never been reported in a scientific publication. In this study, it had been noted that spawning always occurred during daylight hours, with fish going through a visual display. The spawning behaviour began with a full extension of fins. The male displays a small black spotted first dorsal fin, the pectoral fins become bright orange and are rapidly extended in and out, creating an effect of flashing. The ventral fins become more definitely creamy-yellow. The spots on the body become more intense. The female's pectoral fins remain clear, and flashing (as described above for the male) was not observed, the fins remaining unchanged. During spawning, the movements of the males become very rapid. Chasing begins and continues for a variable period of time, usually starting early in the morning and going on till midday. In contrast to the rainbow-fishes (Crowley & Ivantsoff 1982) the male never touches the female during courtship.

During spawning, the pair shake violently and push their way deep into the plant (Java moss) to shed the eggs. Spawning is not inhibited if more than one pair of fish are present in the tank. Several incursions may be made into the moss.

Egg numbers. Spawning in *P. gertrudae* appears to be a sporadic phenomenon, but nonetheless continuous throughout the whole year. In general, only one to three eggs are shed at each incursion into the moss but as many as seven have been observed on some occasions. Under aquarium conditions in this study, *P. gertrudae* spawned continuously from March to January. The rate of egg survival diminished considerably during the winter period.

Frequently, the larvae were observed schooling together with the parents and were left to develop to maturity without being separated, although it appears that immediately after spawning, eggs may be eaten.

Stimuli to spawning. Although spawning was continuous under laboratory conditions, the viability of eggs diminished rapidly as winter approached. As the temperature of water was kept constant throughout the study, temperature cannot be regarded as a stimulus to spawning.

Mrs Effie Howe, a Ph.D. candidate working in the same laboratory, had noted that light intensity did alter the behaviour of *P. gertrudae*. Tanks positioned in front of a window had fish displaying every morning when the sun was shining directly onto the tank. The tank was then moved away from the window and the displaying activity was considerably diminished. This line of investigation was not pursued further and would need more study should *P. gertrudae* be considered as a useful monitor species.

Occasional disturbances such as water change or introduction of new plants would cause spawning to take place. Salt (NaCl, 0.5% concentration or more), change of pH, drop in water level in the tank or other 'inducements' seem to have a variable effect on spawning activity and are therefore not regarded as effective initiators of mating behaviour.

Pseudomugil tenellus

Colour. *Body:* rich golden yellow above the mid-lateral stripe and yellow with a silver sheen below it. The edges of scales are outlined by melanophores but this is scarcely visible unless the fish are viewed through a magnifying glass. The body tissue is opaque and the swim bladder is not visible. The mid-lateral stripe consists of a series of discontinuous silvery spots which become larger as the fish grows. The opercule is silvery and the eyes blue.

Fins: sexual dichromatism is very apparent. In females the fins are light yellow and clear, and distinctly smaller than in the males. In males the fins are a darker colour than the rest of the body. Second dorsal, anal and caudal are dark yellow with outer edge of their membranes outlined in black. Dorsal and ventral tips of caudal have an additional white edge outside the black. First dorsal and pectorals also dark yellow with the membrane over the first two or three elements coloured black.

Sexual dimorphism. The differences in colour of the body and especially the fins make the sexes easily distinguishable. The size of the fins in the males, especially the second dorsal and the anal, make the distinction unequivocal. The fins, however, are not extended into filaments as they are in *P. gertrudae*. The males appear to be slightly larger than the females and more slender, but this impression has not been substantiated by a meristic analysis.

Behaviour. The mating behaviour (as well as the description of colouration when live) has never been reported in scientific literature. As for *P. gertrudae*, spawning appears to be restricted to daylight hours. Occasional checking of the fish in the early hours of the morning (12.00-4.00 a.m.), invariably found the fish in a quiescent state, frequently close to the bottom of the tank.

Mating activity begins with a visual display. The male selects and approaches his mate from the side, then swims parallel to her as she either tries to evade him or locate a spawning site amongst the weeds, close to the surface. The male erects all his fins but does not hold them erect for more than a few seconds at a time. The body becomes a darker sulphurous yellow. The dorsal and pelvic fins become brighter yellow and the black edges appear to be more accentuated. The second dorsal and the anal fins acquire a row of white dots across the centre of their rays. A row of white-gold iridescent spots develops along the side of the body. The female tilts to one side and releases the eggs with her body quivering during the process, with the male releasing the sperm at the same time. Her colours are much more muted than those of the male. Release and fertilisation of eggs is very rapid, taking no more than a few seconds to complete.

Egg numbers. Spawning in *P. tenellus* is sporadic, but probably continues throughout the year in aquaria. In the first study, in 1982-3, the fish spawned from October to late June. The following year spawning was continuous but the egg mortality in winter was almost 100%. Bishop et al. (in press) concluded that under natural conditions, while most spawning occurs in early Wet season, a certain amount occurs at other times. The number of eggs released at spawning is small, usually between one and five, especially when the females are smaller than 25 mm SL. The eggs are large, with filaments which adhere to weed. This species prefers to lay its eggs in the roots of floating plants such as *Ceratopteris* sp. or any other species with fine roots extending into the water. The eggs adhere to these roots a short distance apart, sometimes as close as 10 mm. Plant species other than *Ceratopteris*, however, were less preferred and the incidence of mortality of eggs laid on other plants was greater. Java moss appears to be a totally unsuitable spawning medium for this species.

Stimuli to spawning. The behaviour of *P. tenellus* and its response to the environment does not appear to differ from that of *P. gertrudae*. No one stimulus could be determined as a factor which would induce spawning. The morning light did, however, seem to stimulate the fish. At this time, both sexes were more active and the males displayed more aggressive

behaviour towards each other, constantly erecting their fins, drawing parallel to one another, and chasing each other in circles but never actually making contact unless the tank was overcrowded.

Eggs and embryonic development

Although a considerable body of literature is now available on the taxonomy and systematics of the genus *Pseudomugil* (for a summary see Ivantsoff & Allen 1984) only one known study on population biology of *P. tenellus* is available (Bishop et al., in press). No detailed embryological study has ever been made on any of these species. The study of the embryology of the two species from the Northern Territory was made in some detail by a postgraduate student, Mrs Effie Howe and a Technical Officer, Mr Greg Semple, in this laboratory.

Materials and methods. Adult specimens of each species of blue-eyes were settled in separate tanks holding 30 L of water. The temperature was maintained at 26 ± 1°C. One pair of *P. tenellus* and 3 pairs of *P. gertrudae* were found to be an optimal number to produce a sufficient number of eggs for subsequent examination. The newly-spawned eggs were removed from the plant, placed in water in petri dishes which were then floated in an aquarium with the temperature maintained at 26 ± 1°C. The water in the petri dishes was partly changed at least once a day. The eggs were examined regularly to observe development.

To ascertain differences in fine structure of the chorion and filaments, some of the eggs were prepared for the SEM by fixing them in 3% gluteraldehyde in 0.025M phosphate buffer, pH 6.8-7.0, for 1-2 days. The eggs were then rinsed several times in the phosphate buffer over the next two days. The fixed eggs were dehydrated in an alcohol series from 10% to absolute (10% increments) for 30 minute at each concentration. After several changes of absolute alcohol, the eggs were dried in a critical point drier, mounted and gold coated before being photographed under the SEM.

Results. The eggs of both species were spherical but there were distinct differences in the size of the oil droplets within the egg and the rate of embryonic development leading to hatching. *P. tenellus* eggs were smaller than those of *P. gertrudae*. The filaments of *P. tenellus* (Fig. 5) were grouped in one tuft on the egg's surface, those of *P. gertrudae* were in two tufts at opposite poles of the egg. The filaments of both species have a collar at the point of attachment to the egg's surface and the filaments are extremely sticky and elastic. It was noted that, whenever they came in contact with a plant, adhesion was very strong.

A large number of oil droplets was observed in the eggs just after fertilisation. In fertile eggs, these globules moved to the vegetal pole within the first four cleavages. Infertile eggs quickly became opaque. During subsequent development of fertilised eggs, the oil droplets diminished in number. Only two to three large oil droplets remained by the time the young developed a pericardial cavity (see Table 8 for the summary of some physical parameters).

Cell division was meroblastic in all species and restricted to a small disc. The yolk material remains undivided and was later covered by the embryonic ectoderm (Fig. 6).

As in melanotaeniids, the continuous process of embryogenesis is artificially divided into stages. Table 9 gives comparative times of the stages for the blue-eyes. The drawings in Fig. 6 are diagrammatic and non-specific but give an outline of the way the blue-eye larvae form.

Discussion. Pre-spawning behaviour was essentially similar in both species but spawning occurs near the surface for *P. tenellus* and near the gravel substrate within the filaments of such plant as Java moss for *P. gertrudae*. Under aquarium conditions, *P. gertrudae* spawned

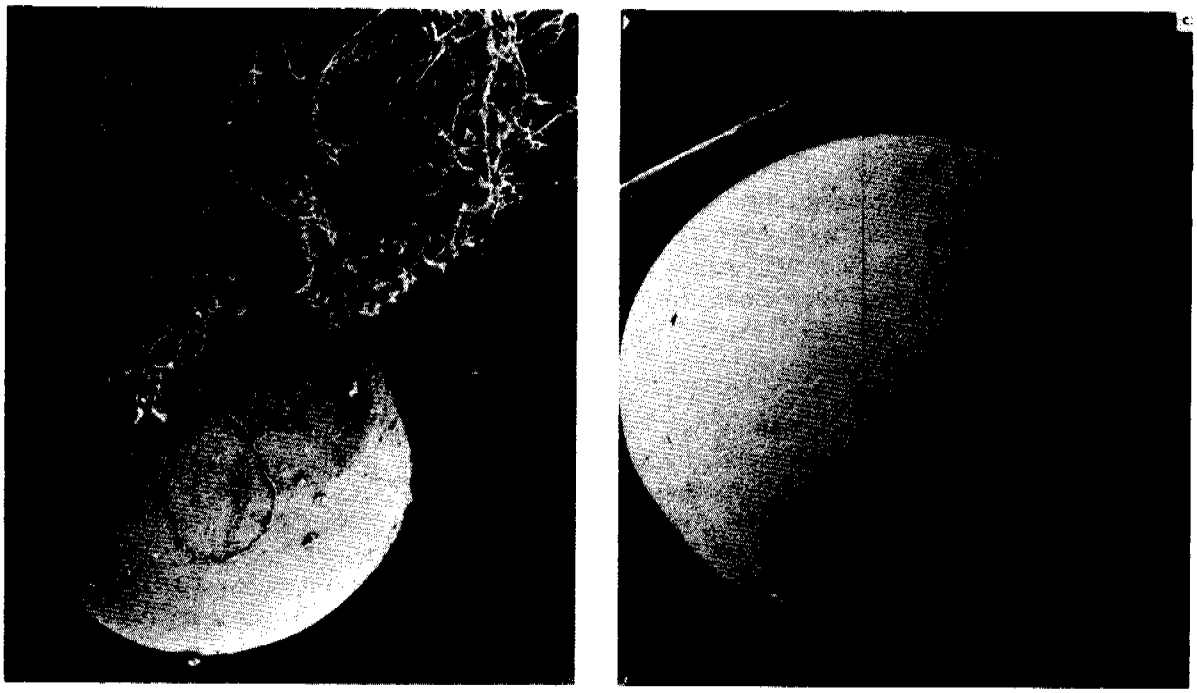
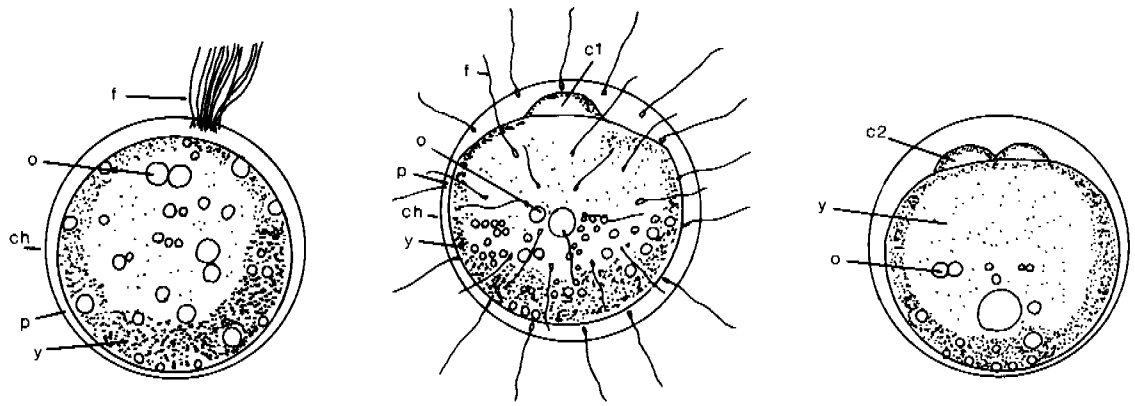


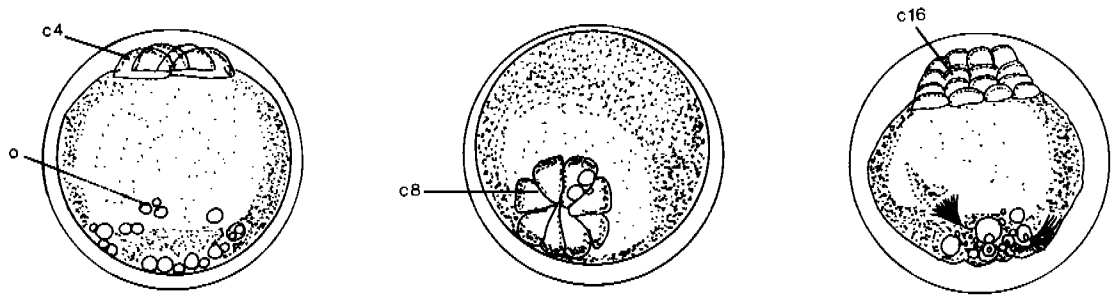
Figure 5. Scanning electron micrographs (SEM) of the chorion morphology of: (a) *P. gertrudae* (magnification x34 approx.); and (b) *P. tenellus* (approx. x23)



Fertilisation: 0-2 seconds
Filaments as in *P. tenellus*

One-cell stage
50 min - 1 h 5 min
Filaments as in *P. signifer*
(E. Howe, unpublished)

Two-cell stage:
1 h 20 min - 1 h 45 min

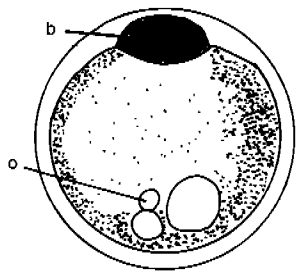


Four-cell stage:
1 h 30 min - 2 h 50 min

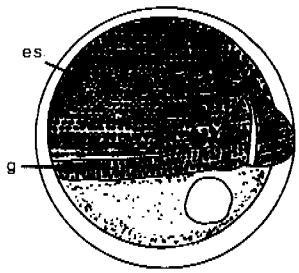
Eight-cell stage:
2 h 30 min - 3 h 35 min

Sixteen-cell stage:
3 h 30 m - 4 h 5 m
Distortion of yolk in *P. tenellus*

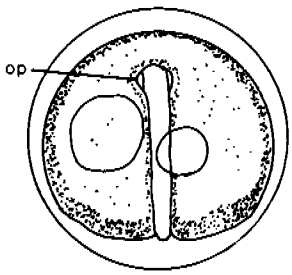
Figure 6. *(Above and opposite)* Diagrammatic representation of the embryonic development of several species of *Pseudomugil*, including *P. tenellus* and *P. gertrudae* at 26 ± 1°C



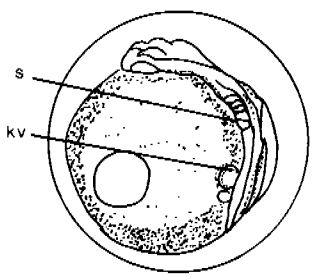
Successive cleavages, increase in the number of cells with a reduction of cell size; 8-14 h



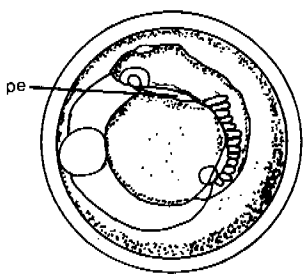
Gastrula has expanded to cover half of the yolk
22-23 h



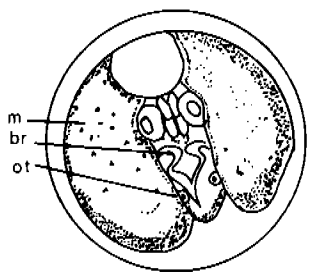
Optic vesicles present
29-32 h



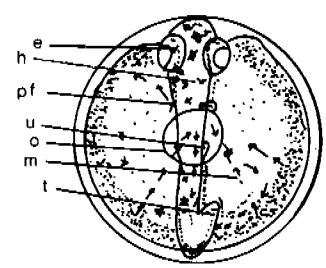
Several caudal somites and Kupffer's vesicle present
34-43 h



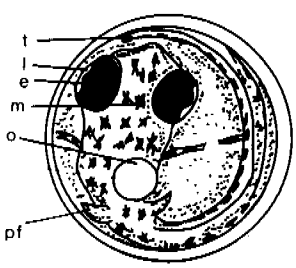
Pericardial cavity visible, optic cup present
49-50.5 h
yolk sac of both species
48-67 h



Otic vesicles present
Brain well defined
Light melanophore pattern on



Heart, swim bladder and pectoral fins present
70-90.5 h



Eye pigmented and lens formed
134-211 h

Key

b: blastoderm; br: brain; c1: one cell; c2: 2 cells; c4: 4 cells; c8: 8 cells; c16: 16 cells; ch: chorion; e: eye; ea: embryonic axis; es: embryonic shield; f: filaments; g: germ ring; h: heart; kv: Kupffer's vesicle; l: lens; m: melanophores; o: oil droplets; op: optic vesicles; ot: otic vesicles; p: perivitelline space; pe: pericardial cavity; pf: pectoral fin; s: somites; t: tail; u: bladder; y: yolk.

Table 8. Characteristics of eggs of *P. tenellus* and *P. gertrudae*

Feature		*P. tenellus*	*P. gertrudae*
Egg shape		spherical	spherical
Average diameter of egg (mm)		1.06	1.30
Approximate no. of eggs spawned at a time		1-5	1-7
Filament formation		one tuft	two tufts
Width of perivitelline space (mm)		0.05	0.1
No. of oil droplets after fertilisation		>100	30-40
No. of oil droplets:			
at:	4 cell-stage	70	25-30
	8 cell-stage	55-60	25-30
	16 cell-stage	45-50	25-30
	32 cell-stage	30-35	20-25
	64 cell-stage	25-30	15-20
	128 cell-stage	20-25	10-15
when:	central area of blastoderm elevated	10-12 large (0.01-0.31 mm) 4-6 small (<0.01 mm)	4-8
	gastrula covers the yolk	2-3 large 10 small	3-5 large
	pericardial cavity first observed	1 large	1-2 large

most of the year but the egg mortality in winter was very high. *P. tenellus* appeared to have a more limited spawning season but produced a greater number of eggs. The eggs could be clearly distinguished by the tuft arrangement on the chorion and by the number of oil droplets in the yolk. Recognition of stages upto the 128 cell stage also appeared possible for both species by counting the remaining oil globules.

There are distinct differences in the duration of embryonic development between the species. Development in *P. tenellus* was more rapid (10-12 days to hatching) whilst in *P. gertrudae* hatching took place between 12 and 13.5 days at 26 ± 1°C. The blue-eyes studied take about twice as long to develop as the *Melanotaenia* spp. studied.

Larval development

It appears that the larval development of the genus *Pseudomugil* had not previously been studied; growth rates of blue-eyes only could be inferred from the only known work on the biology of *P. tenellus* (Bishop et al., in press). From the work with the blue-eyes in this laboratory, it is apparent that the larval stages of different species can be distinguished only with some difficulty.

Materials and methods. Adult fish of *P. tenellus* and *P. gertrudae* were placed in breeding tanks either in single pairs or, in order to produce a greater number of eggs, up to 9 pairs. Java moss was introduced into tanks with *P. gertrudae* and floating fine-leafed weed with a well developed root system to tanks with *P. tenellus*. Immediately after spawning, the adults were usually removed from the tank to prevent predation, although with *P. gertrudae* predation appeared to be a less frequent phenomenon than with the other species studied. If the tanks had numerous plants the larvae found refuge amongst the leaves and roots.

Table 9. Embryogeny of *P. tenellus* and *P. gertrudae*

Times are given to the nearest half hour after 128 cell stage.

Stage of development	Time	
	P. tenellus	*P. gertrudae*
1. Fertilisation	0-2 s	0-2 s
2. 1 cell	1 h 5 min	50 min
3. 2 cells	1 h 45 min	1 h 20 min
4. 4 cells	2 h 50 min	1 h 30 min
5. 8 cells	3 h 35 min	2 h 30 min
6. 16 cells	4 h 5 min	3 h 30 min
7. 32 cells	4 h 45 min	4 h 45 min
8. 64 cells	5 h 55 min	5 h 30 min
9. 128 cells	6 h 50 min	6 h 10 min
10. Cell cleavage with little increase in size of blastoderm	9.5-10.5 h	8.5-10 h
11. Central area of blastoderm becomes elevated. Fine droplets at vegetal pole	16 h	16 h
12. Gastrulation has commenced and gastrula covers half the yolk	23 h	22 h
13. Extra embryonic ectoderm covers 75% of yolk surface	27 h	26.5 h
14. Groove clearly visible in middle of shield, indicating the beginning of formation of the central nervous system	28 h	29.5 h
15. Rudimentary optic vesicles visible	29 h	32 h
16. Kupffer's vesicle present	29.5 h	34.5 h
17. Several caudal somites present	34 h	43.5 h
18. Pericardial cavity formed	48.5 h	50.5 h
19. Otic vesicles clearly visible and the brain is defined	48.5 h	50.5 h
20. Melanophores on body of embryo	50 h	54 h
21. Heart begins to beat	53.5 h	59 h
22. Melanophores on yolk sac vessels	54 h	67 h
23. Circulation begins	59.5 h	69 h
24. Tip of tail free and twitching	72 h	75 h
25. Rudiments of pectoral fins visible	72 h	77.5 h
26. Earliest sign of retinal pigmenation. Outline of swim bladder	72 h	90.5 h
27. Blood showing signs of pigmentation	84.5 h	110 h
28. Gut and liver visible	187 h	200 h
29. Caudal rays well defined	187 h	233.5 h
30. Mouth opens and shuts frequently	220 h	290 h
31. Rapid pectoral fin movement accompanied by mouth opening and closing, signs which indicate that hatching is imminent		
Hatching	243-295 h (10-12 days)	316-322 h (13-13.5 days)

The tanks were maintained at 26 ± 1°C and the young hatched from about 10-13.5 days later, depending on the species. From the first day of hatching, the larvae were fed 2-3 drops of Liquifry No. 1 and TetraMin E five times a day. From day 8, live brine shrimp larvae were also introduced to the fish. Finely crushed TetraMin Staple Food replaced TetraMin E when the fish reached about 16-20 mm TL, or about two months after hatching.

The tanks were cleaned at least once every three weeks to remove pollutants and avoid infection. A water exchanger (The Automatic Aquarium Water Changer), which continuously removes and replaces a small amount of water, was tried for 12 hours at a time so as to exchange about 50% of the water during that period. The procedure was discontinued after loss of some blue-eyes and normal cleaning procedures were resumed. The water exchanger, however, was found to work well with *Melanotaenia* subspecies and with hardyheads.

Whenever possible, up to 20 fish were measured at various intervals to obtain a growth rate for the first 5 months of life.

Results and discussion. At hatching, the fish are active and have a well-developed mouth and digestive system. Pectoral fins and dorsal and ventral fin folds which develop during incubation are present and the fry swim strongly. Food is readily taken from the surface of the water and the young appear to thrive on commercial preparations.

The fry initially swim at the surface of the water and aggregate near floating weed. Subsequently, by about 8 weeks, they move throughout the water column and school with the adults. By the 10 mm TL stage, the young feed together with the adults.

In larvae of *P. gertrudae*, the dorsal and ventral fin folds (present at hatching) are still continuous at 15 days (8 mm TL) (Fig. 7), with the ventral fold reaching anteriorly as far as the anus. The ventral fins are yet to appear. The caudal fin, which starts to develop during incubation, is still only moderately developed. The eyes are shiny, mottled black. The larvae are heavily spotted on the head and body as far as the anus. The pectoral fins have two distinctive spots at the base. A single row of dots extends on either side of the dorsal surface of the body. The ventral fin fold is also slightly pigmented.

By 20 days, 9 mm TL larvae of *P. gertrudae* (Fig. 8) show well-developed and rayed dorsal and anal fins. The ventral fin fold still persists between the anal fin and the anus, and between the anal fin and the caudal fin. The first dorsal fin is just visible and the ventrals have just appeared. The entire body is peppered with fine melanophores. The dorsal surface of the head is very heavily pigmented.

Twelve hours after hatching, 4 mm TL larvae of *P. tenellus* (Fig. 9) have a well developed caudal fin. The buds of the ventral fins are just visible. The dorsal and ventral fin folds are very prominent, extending from the vertical line drawn through the anus to the caudal fin. The top of the head is heavily pigmented, with melanophores extending in rows along the dorsal aspect of the body. The ventral surface is similarly pigmented.

At 7.4 mm TL (8 days), *P. tenellus* (Fig. 10) had well-rayed anal and second dorsal fins. The development of the first dorsal fin had begun. The ventral fin fold still persisted at this stage, as did part of the fin fold posterior to the second dorsal fin. The ventrals were still undeveloped. The pigmentation was essentially the same as at the 12 hour stage. The growth rates of the larvae studied show a wide variation between individuals as do the *Melanotaenia* spp. (Tables 10 and 11). At 26 ± 1°C both species roughly double their length in the first 20 days of life, and double their length again in two months (*P. gertrudae*) or three months (*P. tenellus*) and proceed then to grow less rapidly to almost adult size by the end of the 5th month. At this stage, *P. gertrudae* is sexually mature and spawning. Likewise,

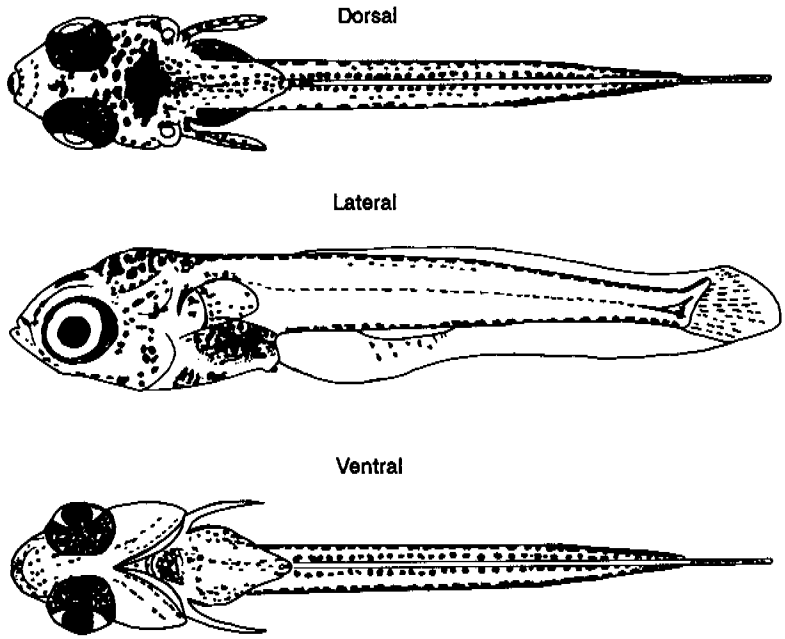


Figure 7. Dorsal, lateral and ventral views of *P. gertrudae* larva at 8 mm TL (15 days after hatching)

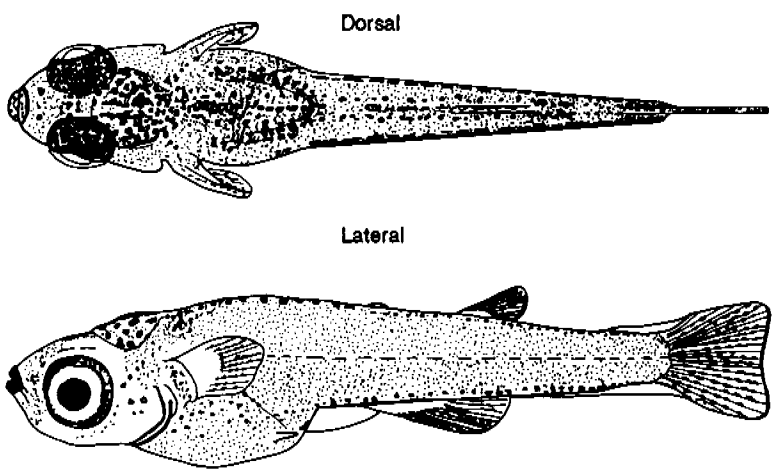


Figure 8. Dorsal and lateral views of *P. gertrudae* larva at 9 mm TL (20 days after hatching)

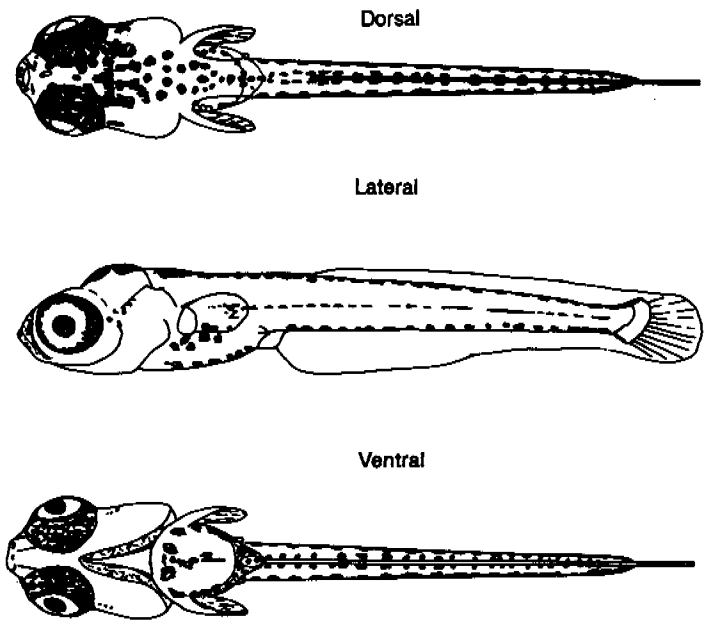


Figure 9. Dorsal, lateral and ventral views of *P. tenellus* larva at 4 mm TL (12 hours after hatching)

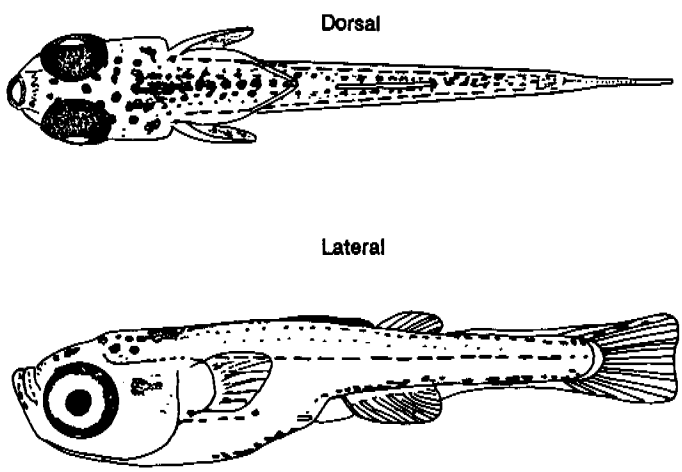


Figure 10. Dorsal and lateral views of *P. tenellus* larva at 7 mm TL (8 days after hatching)

P. tenellus is observed to enter weeds and exhibit mating behaviour at 20 mm SL (less than 5 months of age) although no spawning was ever observed. The length of *P. tenellus* at sexual maturity was found by Bishop et al. (in press) to be at about 18-23 mm TL, which is in close agreement with the results of this study. The growth rates for *P. tenellus* reported here are significantly faster than those calculated by Bishop et al. (in press), possibly because of an abundant food supply and lack of competition in aquarium conditions. The assumption of these authors that *P. tenellus* attains sexual maturity within its first year of life is, however, substantiated.

Smaller fry tend to stay at the surface whilst the larger individuals utilise the whole water column. At this stage of development, when size disparity had arisen, some chasing of the smaller fish by the larger took place, a situation which can be overcome by sorting the fish and separating them into similar sizes.

No significant difference was found between the lengths of males and females in *P. tenellus* up to 148 days (Table 12). Bishop et al. (in press), working on the same species, explained the bimodal frequency distribution observed by differential growth of sexes but stated that this prediction warranted verification. The present study is also inconclusive and requires further investigation.

From about 15 mm SL, the fish could be sexed on colour pattern and the size of the dorsal fin in *P. tenellus*. Bishop et al. (in press) indicated that these fish were sexually distinguishable at 14 mm; they also noted that the females of *P. tenellus* showed a significant peak in gonad development in the early Wet season. This finding is consistent with the mating behaviour observed in the laboratory. Spawning was more frequent during the summer (corresponding to the early Wet) and the viability of eggs was greater than at any other time of the year. The fecundity values quoted by Bishop et al. (in press) are confirmed by the present study. The number of eggs laid in any one spawning is small.

Table 10. Lengths (sexes combined) of *P. gertrudae* from hatching to 152 days

Lengths are given in mm TL; n = 10

Days after hatching	Min. TL	Max. TL
0	3.8	4.0
15	4.3	6.0
20	6.0	9.0
32	7.1	11.2
42	12.5	15.5
50	15.3	17.8
62	15.1	17.9
95	16.7	20.7
152	18.0 males*	22.2
	17.0 females*	21.5

* 10 of each sex

Table 11. Lengths (sexes combined) of *P. tenellus* from hatching to 13 months of age

Lengths are given in mm TL; n = 20-30 except where shown otherwise.

Days after hatching	Min. TL	Max. TL
0	4.1	4.3
9	5.6	6.0
17	8.0	8.5
30	6.1	12.4
50	8.5	19.3
63	10.5	21.2
85	10.5	25.5
90	10.5	25.6
107	14.4	27.5
122	15.0	27.5
144	18.5	27.5
13 months, 3 males left	39.5	42.5

Table 12. Maximum and minimum lengths of males and females of *P. tenellus* between 90 and 148 days

Lengths are given in mm TL; n = 20

Age in days	Males		Females	
	Min. TL	Max. TL	Min. TL	Max. TL
90	17.8	21.5	10.5	25.6
107	18.0	26.1	14.4	27.5
122	18.1	26.7	15.0	27.5
148	18.7	27.5	18.5	27.5

Recommendations

Because of the small size of the mouth of the blue-eyes studied, food particle size appears to be important. Finely crushed food is accepted a month after hatching; large flakes should never be used for feeding, frozen adult brine shrimp are also too large and should be avoided. Live brine shrimp larvae, on the other hand, are eagerly accepted by the young (from about 10 days after hatching) and adult blue-eyes and should be used to supplement the flake diet at least two or three times a week. The frequency of feeding can be reduced to 3 times a day once the fish are over 8 weeks old.

Because of the moderately low number of eggs per clutch, an increase in egg production can be achieved by mass spawning rather than by individual pairings. Up to 100 young have been produced by 9 pairs of *P. gertrudae* in two days of spawning. The tanks should be well planted with weeds such as Java moss for *P. gertrudae* and plants such as *Ceratopteris* spp. or *Nitella* spp. for *P. tenellus*. After spawning, the adults should be removed to avoid egg predation.

As spawning is sporadic, and as the stimulus for mating behaviour is unknown, the number of individuals produced per annum cannot be determined with any accuracy but, with 10 breeding tanks each with nine pairs and a number of holding tanks, it would be possible to produce a total of about 500 fish a year or double that number with 20 tanks. There would be no certainty of continuity of production but as the fish will survive for two years (and perhaps even three) without any difficulty, the numbers could be built up in the first year in the summer months and then maintained at the required level.

3.3 Northern Territory hardyheads

Ivantsoff (1980) summarised the anecdotal information about the reproductive biology of hardyheads known to that time. The first detailed description on development was made by Llewellyn in 1979 on *Craterocephalus fluviatilis* and a recent study by Milton & Arthington (1983*a*) has examined the reproductive cycle of two species of hardyheads, *C. marjoriae* and *C. stercusmuscarum*. Work on embryonic development of *Craterocephalus* is yet to be described.

Hardyheads are ubiquitous and relatively abundant in Australian waters (Ivantsoff 1978). They are well known both to the amateur and scientist and have stimulated interest in both groups because of the ease with which they can be caught. The specific status of many species, the population variability, and their distribution and range, however, are poorly known. Ivantsoff (1978) has examined the taxonomic position of the known species but much of that work is yet to be published. Observations, both in the field and in the laboratory, indicate that the species of hardyheads in the Alligator Rivers Region are *C. stercusmuscarum* and an undescribed species of *Craterocephalus* closely related to, and regarded by Ivantsoff (1978) as conspecific with, *C. marjoriae* (a species from Queensland and northern N.S.W.).

Although both the undescribed species and *C. stercusmuscarum* exhibit great variability in colour pattern, they are quite distinct and readily recognisable from one another.

C. stercusmuscarum

General description

Colour. *Body:* Pigmentation is variable between individuals within a population and in any one individual from one time to another. The juveniles are translucent, dusky or fawn coloured, with little or no spotting on the side of the body. The adults are translucent, varying from light yellow to rich gold, with spots of various intensity and size along the side of the body, with pigment concentrated in the middle of each scale so as to form rows of black stripes along the side of the body. However, these stripes are frequently absent in the hardyheads from the Magela Creek system. A black band commencing on the snout and extending through the eye, the opercle and continuing as an interrupted stripe as far as the hypural joint, on the other hand, is always present. This band is usually skirted with a thinner line of gold below and above it. The abdomen may be butter yellow or cream coloured. The dorsal part of the head is heavily pigmented.

Fins: All fins are transparent, yellowish and slightly dusky, showing little or no variation from specimen to specimen.

Sexual dimorphism. Not marked although the abdomen may become distended in a gravid female. Milton & Arthington (1983*a*) found that male specimens in Queensland waters became more intensely golden below the mid-lateral stripe. Llewellyn (1979), in his study of a subspecies of the spotted hardyhead stated that, at breeding time, the females were easily sexed by observing the black mesovarium through the semitransparent abdominal wall, by the swelling of the lips around the vent and the silvery white membranes around the vent, in contrast to the golden colour of the males. In the present study, the specimens under observation changed colour frequently and rapidly, making evaluation of colour change at breeding time difficult. Invariably, however, the colours intensified before spawning, with the black mid-lateral stripe becoming somewhat darker in the female. The abdominal region in the male became more yellow. This concurs with the observations made by Bishop et al. (in press) that at the onset of the breeding season, the abdomen and the throat regions become canary yellow to bright golden. It appears also that colouration is related to dominance in the males, with the less dominant males being much paler than the aggressive individuals.

Behaviour. Aspects of the mating behaviour of the southern subspecies of *C. stercus-muscarum* have been described by Whitley (1958), who stated that gravid females rubbed against stones which apparently assisted with the extrusion of the ova. The males, lying immediately behind, presumably fertilised the eggs which were apparently deposited in crevices of rocks or on the rocky bed of the river.

The present study is at variance with the description of mating behaviour described above. At spawning, the female enters the weed and is closely followed by the male. If the male does not follow, the female will come out of the weed and dart in again. The male may then butt the female in the anal fin or the posterior region of the abdomen. The male will then also enter the weed; the female releases her eggs whilst quivering and spreading the eggs widely in the vegetation. The male remains beside or behind its mate and fertilises the eggs immediately after spawning. The spawning medium may be Java moss, *Nitella* sp. or *Ceratopteris* sp. and spawning may occur at any depth in the water column. Spawning was never observed to take place on bare sand at the bottom of the tank.

Whenever more than one pair were present in a tank, and spawning was imminent, all the fish would aggregate at the spawning site and spawning would be simultaneous. However, immediately afterwards the fish would eat the eggs.

Egg numbers. Spawning in *C. stercusmuscarum* is intermittent and unpredictable in aquarium conditions but more common during the early spring and summer months. Breeding pairs observed from September to the end of November spawned at least four times a month, almost invariably between 7.30 and 10.00 a.m., although occasionally spawning would also take place in the early afternoon. Spawning could also take place almost daily for several weeks, especially if more than one pair were present in the tank. The more common pattern, however, was for the fish to spawn for several days, followed by variable periods of rest. The number of eggs shed appears to be small, with about 20 eggs released by each female at spawning.

Spawning becomes infrequent after spring and summer and totally unpredictable in aquarium conditions.

Stimuli to spawning. Spawning in *C. stercusmuscarum* was a sporadic phenomenon which did not appear to be triggered by external stimuli. However, it was noted that a disturbance such as cleaning of the tank, a change of water or a drop in water level, would often be followed by spawning the day after. No controlled experiment to ascertain the validity of this observation could be made, as only a limited number of fish and aquaria were available at the time *C. stercusmuscarum* was spawning.

Eggs and embryonic development

Whitley (1958) stated that the eggs of *C. fluviatilis* (a subspecies of *C. stercusmuscarum*, Ivantsoff 1980) are spherical, transparent and demersal, with a cluster of oil globules at the edge of the yolk mass. Llewellyn (1979) described the embryonic development of the southern subspecies in considerable detail and noted the size and the changes in morphology as development proceeded. He assumed that the larvae would be well developed and at least 3.4 mm long at hatching.

The eggs tend to be scattered in the lower part of the water column, often failing to adhere to the coarser weeds. Egg size varied from 1.3 to 1.7 mm in diameter at spawning and the eggs are sometimes not quite spherical in shape. The surface of the chorion is covered by many adhesive filamentous strands (0.5-0.9 mm long) regularly distributed over the entire chorion.

Results and discussion. It is assumed that the embryonic development of the Northern Territory *C. stercusmuscarum* is indistinct from that observed by Llewellyn (1979) for *C. fluviatilis*. At spawning, morphology of the chorion and the diameter of the eggs is the same. Early division is rapid, with the 32 cell stage reached (at 26 ± 1°C) 3 hours after spawning. Division is slightly more rapid than that observed for *Pseudomugil* spp.. The eggs have about 7 oil globules which are clustered at the vegetal pole.

At 26 ± 1°C, completion of hatching appears to take place about 312 hours after spawning. In a series of observations, it was noted that a batch of eggs spawned over a period of four days at 26 ± 1°C began hatching at the end of the 13th day. This is comparable to *Pseudomugil* spp., where the time from spawning to completion of hatching was between 10 and 13.5 days. As the eggs Llewellyn (1979) studied died about 138 hours after fertilisation, it is not possible to compare the embryogeny of the two subspecies studied.

Craterocephalus sp. nov.

General description

Colour. *Body*: sandy-yellow, translucent and with a black mid-lateral stripe which becomes more prominent in the posterior half of the body. Colouration is slightly darker above the mid-lateral stripe, the lower half lighter and with the silvery peritoneum clearly outlining the abdominal cavity. Usually, there are two rows of spots below the mid-lateral band. The top and sides of the head are darker than the rest of the body and the opercular region may be opalescent in some of the specimens.

Fins: hyaline and colourless.

Sexual dimorphism. *C. marjoriae*, a closely related species, is considered to be sexually dimorphic by Milton & Arthington (1983*a*) at breeding time. *Craterocephalus* sp. nov., on the other hand, is extremely difficult to sex and only minor differences can be observed between the sexes. The females tend to have a more curved dorsal surface and distended abdomens when gravid and appear to be slightly deeper bodied; the males tend to be more slender, with the dorsal profile almost straight. Sexual dichromatism was not observed at any stage.

Behaviour. Nothing is known about the mating behaviour of *Craterocephalus* sp. nov. The species was first collected and recorded as *C. marjoriae* by Pollard (1974) on the advice of Ivantsoff. Bishop et al. (in press) noted that these fish are commonly found in sandy-bottomed pools in creeks in the Alligator Rivers Region. They have also examined the gonads and population structure of these fish in different seasons.

In the laboratory, the onset of mating behaviour may appear to be expressed in two ways: the male may pursue the female and butt her in the anterior part of the anal fin or in the posterior part of the abdomen; alternatively, the male may repeatedly swim beneath the female, charging from one side and grazing her abdomen with his dorsal fins. Either form of approach eventually causes the female to go into the weeds and release her eggs. The male follows closely and fertilises the eggs immediately. At this stage the eggs are eaten, unless the parents are removed from the tank.

Charging from the side and under the female appears to be the standard form of mating behaviour when more than one pair are present in the tank. Spawning is generally quite high up in the weed, never on the gravel or sand and never just below the water surface either. Group spawning appears to be a common phenomenon, with all the adults entering the weed (observations were restricted to 3 fish most of the time). The spawning medium may be Java moss or any other fine weed such as *Nitella* sp.

Egg numbers. Spawning in *Craterocephalus* sp. nov. is intermittent and unpredictable in aquarium conditions; it is most frequent during September-November, when spawning occurs at least 4 times each month, usually in the morning. Spawning may occur on 3-5 consecutive days, followed by a rest period of 6-9 days. Estimation of egg numbers is extremely difficult as the fish were the shyest of all the species studied. Over a 4 day period, as many as 100 or as few as 30 eggs may appear in the tank. In all probability, the number of eggs would not exceed 20 at one spawning. Spawning becomes less frequent after spring and summer. Viability of eggs is good in the spring-early summer months.

Stimuli to spawning. Mating behaviour of *Craterocephalus* sp. nov. appears to be similar to *C. stercusmuscarum*. Stimuli to induce spawning could not be ascertained but, as in *C. stercusmuscarum*, disturbances such as water change could be followed by spawning on the following day.

Eggs and embryonic development

Nothing is known about the morphology of eggs other than their size whilst still in ovary (Bishop et al., in press). No detailed study on embryonic development appears to have been done either for this species or its close congener, *C. marjoriae*. A detailed study was done at Macquarie University by Mr Greg Semple and Mrs Lucy Crowley confirmed the described development of the eggs.

Materials and methods. Three adult specimens of *Craterocephalus* sp. nov. were held in a large tank (1 m x 30.5 cm x 38 cm) from the time they arrived from the Northern Territory. The tank was placed in a well lit position (natural light as well as fluorescent). The temperature was maintained at about 26 ± 1°C when the eggs were laid. The eggs were transferred into water in 90 mm diameter petri dishes and floated in a tank in which the water temperature was maintained at 26 ± 1°C. A small quantity of methylene blue was added to prevent fungal infection.

Results. The eggs of *Craterocephalus* sp. nov. are spherical with a tuft of 10-12 filaments (Fig. 11a) restricted to the chorion above the animal pole. Elsewhere, the chorion is somewhat rough but not sculptured. The chorion does not appear to be adhesive whilst the filaments often stick to each other or to the adjacent chorion.

On contact with a plant, the filaments adhere well to the fine weed, allowing the egg to be suspended freely from it. There are 27 to 30 oil droplets (0.38-0.50 mm in diameter) as well as numerous small droplets, initially lying below the tuft, then spreading and moving down to the vegetal half of the egg along the periphery of the yolk (Fig. 11a-g).

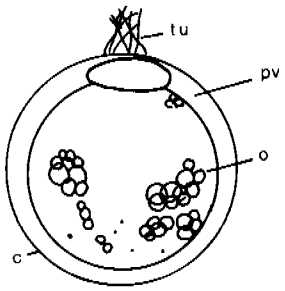
The eggs are telolecithal and the yolk almost fills the entire egg. The egg is creamish to colourless and non-granular. If fertilised, the egg develops the perivitelline space within the first two minutes of spawning (0.10-0.18 mm deep). Division is meroblastic. The egg size varies from 0.87 to 0.96 mm in diameter and there appears to be a positive correlation between the size of the mother and the size of the egg, the larger females producing larger eggs.

For the sake of convenience, the process of embryogenesis is divided into stages. Table 13 gives comparative times of the stages to hatching. Figure 11 is a diagrammatic representation of some of these stages.

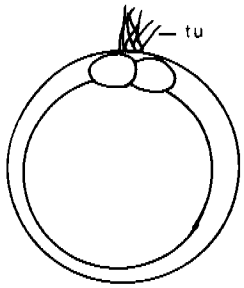
Discussion. Pre-spawning behaviour of the two species of hardyhead only differed in minor detail. In both cases, the fish were quite unpredictable in their spawning and both were most active in the spring and summer months. This result concurs with Bishop's et al. (in press) finding that the peak of gonad development was in the early Wet season although the gonads were well developed throughout the year for both species. In *Craterocephalus* sp. nov., mature and ripe females were more abundant in the late Dry and early Wet season. Bishop's et al. (in press) conclusion that these species can spawn sporadically throughout the year is confirmed by the present study.

The maximum size of ova measured by Bishop et al. (in press) coincides quite accurately with the actual egg size at the time of spawning. Their measurements for the spotted hardyhead were 1.52 mm and for the second species of hardyhead 0.75 mm compared with 1.3-1.7 mm and 0.87-0.96 mm respectively in this study.

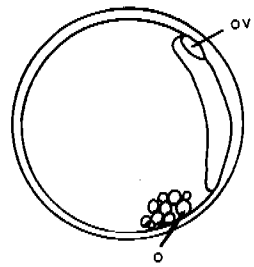
The discrepancy between the two species in the duration of embryogenesis is substantial. The eggs of *C. stercusmuscarum* take as long as 13 days to hatch whilst those of the new species take only 6 days. The difference in size of the eggs may possibly dictate the rate of the development. The trend is similar in *P. tenellus* and *P. gertrudae*, with the former having smaller eggs and a faster hatching time. *M. s. inornata* had marginally smaller



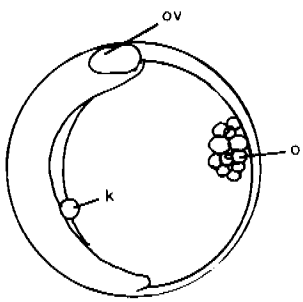
One-cell stage



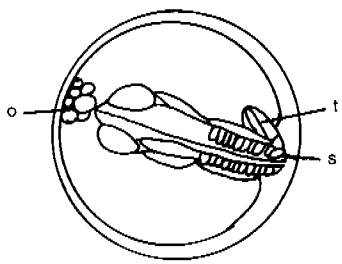
Two-cell stage



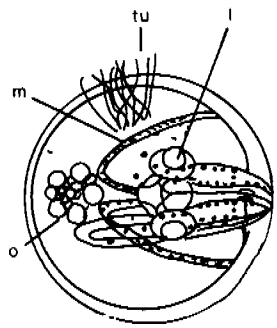
Optic vesicles present with oil droplets near tail end
21 h 20 min



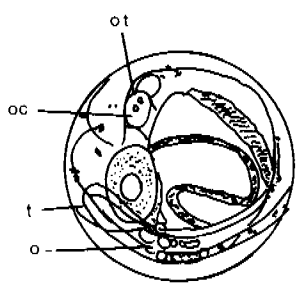
Kupffer's vesicle visible
25 h



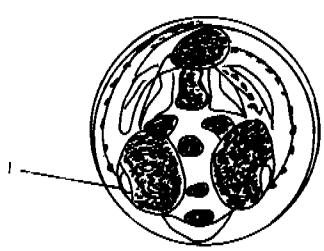
12-14 somites present
30 h



The tail is coiled; melanophores are well defined
About 54 h



The tail grows past anterior end of embryo. Otolith visible:
About 78 h



Just before hatching.
Pigmentation very strong;
oil droplets almost gone:

Key
c: chorion; k: Kupffer's vesicle;
l: lens; m: melanophores;
o: oil droplets; oc: otic capsule;
ot: otolith; ov: optic vesicle;
pv: perivitelline cavity; s: somite;
t: tail; tu: tuft.

Figure 11. Diagrammatic representation of the embryonic development of *Craterocephalus* sp. nov. at 26 ± 1°C.

Table 13. Embryogeny of *Craterocephalus* sp. nov.

Temperature = 26 ± 1°C

Stage of development		Time
1.	Fertilization	5-10 s
2.	1 cell	54 min
3.	2 cells	1 h 10 min
4.	4 cells: oil moving down to vegetal pole	1 h 40 min
5.	8 cells	2 h
6.	16 cells: 2 layers of cells, most of the globules at vegetal pole	2 h 30 min
7.	32 cells	3 h
8.	Epiboly begins. Blastoderm flattens and begins to spread down over the yolk surface. Blastopore visible.	4 h
9.	Embryonic shield and germ ring develop	15 h
10.	Neural plate and groove well formed	17 h 10 min
11.	Optic vesicles and pericardial cavity apparent. Oil globules coalescing (Fig. 11c)	21 h 20 min
12.	Kupffer's vesicle apparent. (Fig. 11d)	25 h
13.	Eight caudal somites visible. Kuppfer's vesicle still present. Brain vesicles enlarged	28 h 40 min
14.	Twelve to fourteen somites. First pigmentation apparent as cream and pink stellate spots appear. (Fig. 11e)	33 h
15.	Rhythmic pulsations of the pericardial cavity. Plasma apparent, coursing through the yolk sac vessels. Heart still a straight tube	40 h 30 min
16.	Melanophores appear in eyes and yolk sac. Tail extends two thirds of the way around the yolk sac. Pigment spots are enlarged, some on the head becoming quite black. The tail bud moves free off the yolk sac. Pectoral fin buds just apparent.	48-49 h
17.	Red blood cells in plasma flow. Otic vesicles apparent. Tail moves frequently (Fig. 11f)	54-57 h
18.	Pectoral fin buds distinctly visible behind the otic vesicles. Tail tends to coil to equatorial plane. Melanophores are more obvious on the yolk sac.	59 h
19.	Pectoral buds well developed. Blood flow is quite red. The tail is almost coiled to equatorial plane.	70 h
20.	Chromatophores become more apparent on eyes, in peritoneal cavity and on the dorsum of the head.	73 h
21.	Break in flow of blood indicates development of heart chambers	75 h
22.	Tail grows past the anterior end of the embryo. Caudal vein is visible. Liver at this stage is a yellow vesicle visible behind the left pectoral fin. Otoliths are distinct (Fig. 11g).	85 h 30 min

Table 13. (Continued)

Stage of development		Time
23.	Mouth forming. Upper jaw is apparent. Branchial arches are visible.	103 h
24.	The heart is now S shaped. Mouth is fully formed and moving frequently. Meckel's cartilage is quite discernible. Eyes appear golden. The tail coils around 1.5 times.	114 h
25.	Yolk sac now greatly reduced. Oil droplets are less numerous and quite small. The heart chambers are very distinct. The chorion is flaccid. The mouth often moves and the embryo turns frequently.	120-123 h
26.	The embryo is again coiled in the polar plane with the tail beside the body and the yolk sac. The oil droplets are still visible in front of the heart. The eyes are copper coloured, with some silver tinge.	132 h
27.	The tail position changes again so as to lie over the head as far back as the rear of the swim bladder. Oil droplet number is very small. Pigmentation darkens progressively (Fig. 11h).	149 h
28.	Movement within the chorion frequent. Hatching occurs.	150-158 h (6.25-6.6 days)

eggs (Table 2) and marginally shorter development time. Without a test of significance, however, it would not be possible to imply that smaller eggs develop faster.

Larval development of the Northern Territory hardyheads

There are no known descriptions of the larval development of the genus *Craterocephalus*. Until the present study, only amateur breeders had reported success in maintaining and breeding hardyheads in captivity. Llewellyn (1979) was unable to maintain the eggs of *C. fluviatilis* (= *C. stercusmuscarum*) alive beyond 138 hour stage whilst Midgley (in Bishop et. al., in press) had stated that *C. marjoriae* (= *Craterocephalus* sp.) would not survive in still-water ponds. Although the mortality rate of larval hardyheads was much higher than those of the rainbow-fishes and blue-eyes, a sufficient number were brought through at each spawning to continue with the study of this stage of development.

Materials and methods. Adult fish of *C. stercusmuscarum*, one male and three females, were placed in one breeding tank and three adult specimens of *Craterocephalus* sp. in another. Tanks were well supplied with weed, including an abundant supply of Java moss. Tanks were examined daily for eggs (a x4 magnifying glass is very useful in locating eggs). If eggs were found, the adults were removed and the eggs allowed to develop at temperatures of about 26 ± 1°C. Methylene blue was added to the water to minimise any fungal infection. From the first day of hatching, the fish were fed 2-3 drops of Liquifry No. 1 and TetraMin E at least 5 times a day. From day 12, live brine shrimp nauplii were added and the frequency of feeding was reduced to 3-4 times a day. Minced liver, chopped lettuce and pond water were occasionally added to the diet. Eventually, the baby food was replaced by finely crushed TetraMin Staple Food and a supplement of frozen brine shrimp was also given at least once every 3 days. Small quantities of the latter were tried first, to ascertain whether the food would be accepted. Tanks were given a partial water change once every three weeks, usually by siphoning off a third of the water to avoid losing the larvae

or the larvae were removed to a clean tank in which the water had been warmed to 26 ± 1°C to avoid chilling, and allowed to stand for at least 24 hours. Whenever possible, as many fish as available (20-60) were measured at various intervals to obtain a growth rate for the first four months of life. Some representative stages were recorded by drawing (Figs 12-17).

Results and discussion. *Craterocephalus* sp.: The newly hatched larvae are active, swimming strongly from the beginning. The yolk sac is still present with several oil droplets discernible anteriorly on the ventral surface of the yolk. Feeding begins after the yolk is absorbed which may be several hours after hatching. At this stage, the liver and kidneys are visible in the anterior part of the body cavity. The heart is displaced forward on the yolk sac which is in contrast to the typical position under the neck (Rosen 1964). This position is also typical for *Melanotaenia* spp. (Crowley 1984).

The dorsal and ventral fin folds are continuous with the dorsal fin fold, originating posterior to a vertical through the anus (Fig. 12). The ventral fin buds are yet to appear. The eyes are copper brown, as is the swim bladder at this stage. The pigment spots on the body are now black, although some of these were yellow just before hatching. The mid-lateral band appears as a spotted line, extending from the pectoral fin buds to the caudal end of the body.

The following observations were made on larvae maintained at 26 ± 1°C throughout their growth and development. By day 12, the larvae feed actively from the bottom of the tank, a behaviour pattern that they maintain throughout life. Excursions into the lowermost 10 cm of the water column occur continuously as the fish chase brine shrimp or any other food added to the water.

At 16 days post hatching (Fig. 13), the dorsal and anal fins are discernible in the dorsal and anal fin folds. The bottom half of the caudal fin is well rayed at this stage. A fine row of spots borders the ventral fin fold anteriorly. The caudal fin and the notochord are also spotted.

At 25 days (Fig. 14), the second dorsal fin and the anal and caudal fins are rayed. The ventral fin buds are large and the first dorsal fin has just begun its development. The ventral fin fold persists only anterior to the anal fin and the dorsal fin fold is only present at the first dorsal fin. The abdomen and opercle at this stage become silvery. The notochord is no longer visible and the mid-lateral stripe extends to the caudal peduncle.

At 36 days the fins are all developed and the fish are essentially adult in form and colour pattern.

Craterocephalus stercusmuscarum: the following observations were made on larvae maintained at 26 ± 1°C throughout their growth and development.

The spotted hardyheads are also active swimmers, swimming independently of one another for about the first 12 hours and schooling thereafter in the top half of the water.

On hatching (Fig. 15), the dorsal and the ventral fin folds are continuous, with the dorsal fin fold originating behind a perpendicular through the anus. The ventral fin buds are not apparent at this stage. The eyes are heavily pigmented, as is the dorsal surface of the swim bladder. The top of the head is spotted, forming a circular patch of pigment in the nuchal area. The abdomen, opercle and the mid-lateral stripes are also pigmented.

At 22 days (Fig. 16), the second dorsal and anal fins buds are visible and the ventral fin buds are just beginning to emerge. The dorsal fin fold at this stage has almost disappeared whilst the ventral fin fold persists (in a reduced state) and is continuous with

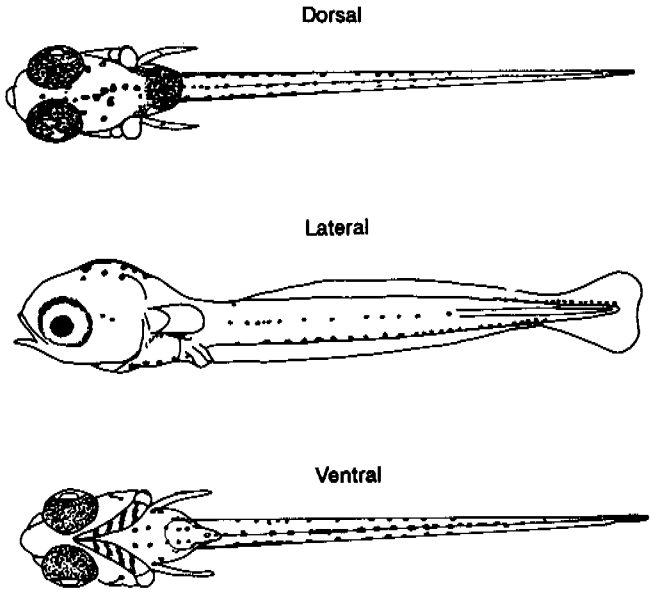


Figure 12. Dorsal, lateral and ventral views of *Craterocephalus* sp., at hatching and 3.8 mm TL *(see Fig. 3 for details of structures)*

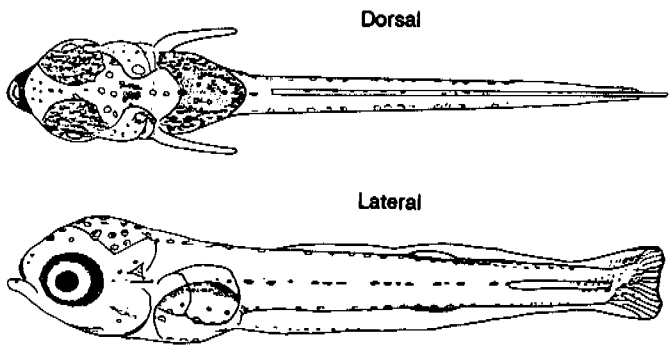


Figure 13. Dorsal and lateral views of *Craterocephalus* sp. nov., 16 days after hatching and 6.7 mm TL

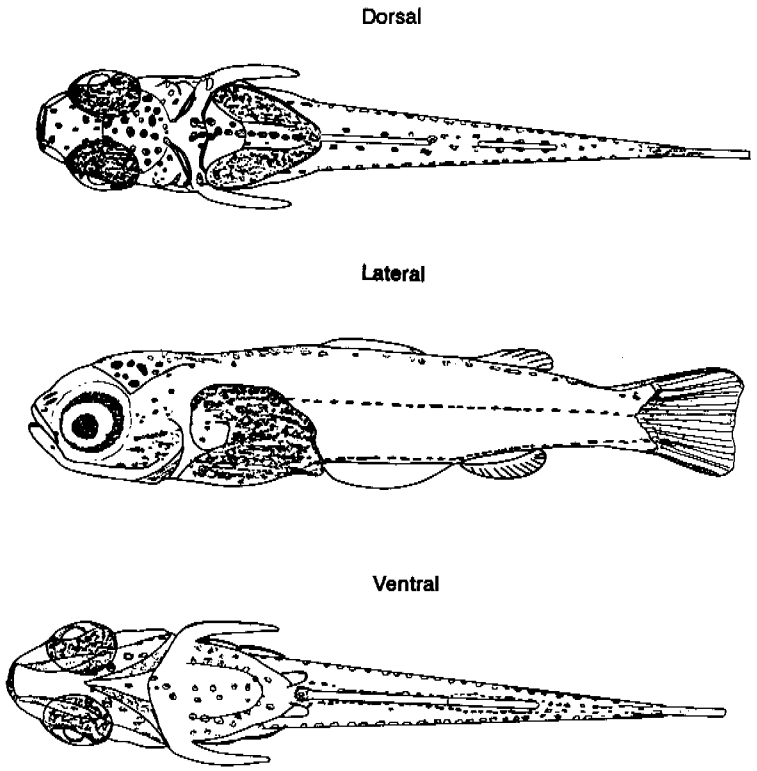


Figure 14. Dorsal, lateral and ventral views of *Craterocephalus* sp. nov., 25 days after hatching and 9 mm TL

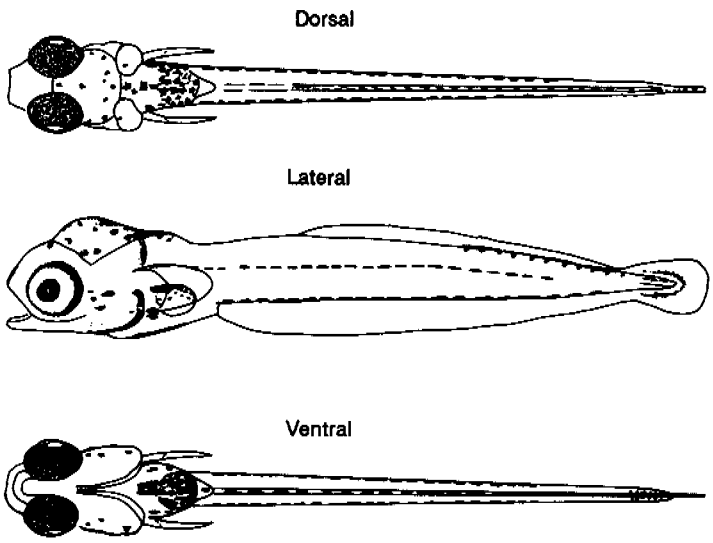


Figure 15. Dorsal, lateral and ventral views of *C. stercusmuscarum* at hatching and 4.2 mm TL

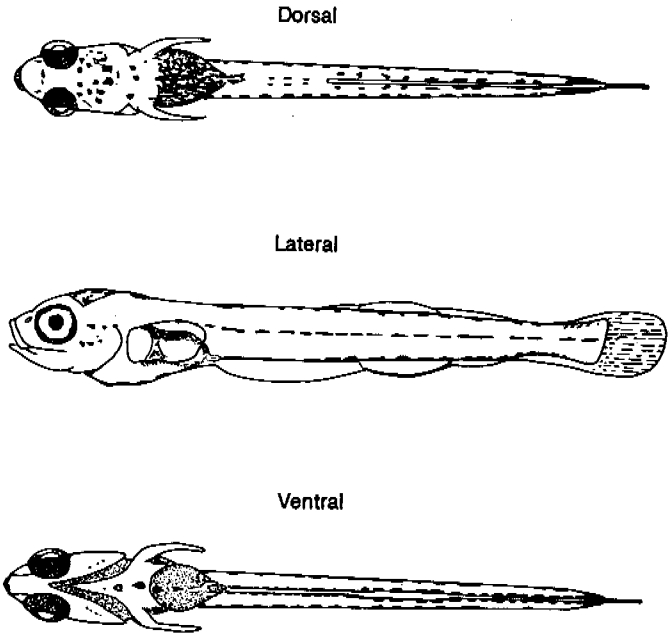


Figure 16. Dorsal, lateral and ventral views of *C. stercusmuscarum* 22 days after hatching and 8.5 mm TL

the caudal fin which is well formed and rayed. The eyes and the dorsal half of the swim bladder are black. The other markings remain the same as those of the post-hatch larvae.

At 30 days (Fig. 17), the dorsal and anal fins are well developed and the first dorsal fin is already present as a bud. The ventral fin fold persists between the anus and the anal fin and the remnant of the dorsal fin fold persists at the origin of the first dorsal fin.

The anal fin at this stage becomes pigmented and there may also be some spotting on the second dorsal fin. By this stage the fish are feeding and schooling in the bottom half of the tank but never feed off the bottom. When disturbed or frightened, younger fish will disperse to avoid the source of disturbance, slightly older fish, on the other hand, maintain themselves in a tight school formation even when disturbed.

Rates of development and growth

The growth rates of the hardyheads studied varied considerably as did the growth rates of the rainbow-fishes and blue-eyes (see Tables 14 and 15). On the average, *C. stercusmuscarum* roughly trebled its size (from 4.5 mm to 14.3 mm) in the first 40 days and then doubled its size by the time the fish were 3.5 months old. Thereafter the rate of growth slowed, with the size increasing by a factor of about 1.8 (calculated for the 4 males) in the following 10 months. There was no observable difference in size between sexes until the fish reached 50 mm SL. Since only 4 specimens of each sex were left in the population under study, no significance can be attached to this difference until other studies are undertaken.

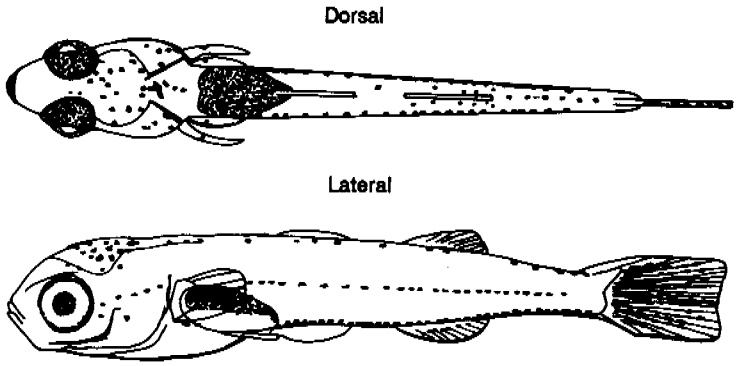


Figure 17. Dorsal and lateral views of *C. stercusmuscarum* 30 days after hatching and 9.8 mm TL

Table 14. Lengths of *Craterocephalus stercusmuscarum* from hatching to 14 months of age

Temperature = 26 ± 1°C

Period after hatching	Sex	Number of fish measured	Min. TL (mm)	Max. TL (mm)
1-3 d	combined	60	4.0	4.5
22 d	"	50	6.5	8.5
30 d	"	50	8.4	9.8
41 d	"	40	11.0	14.3
62 d	"	60	18.7	18.8
108 d	females	10	20.0	27.3
108 d	males	10	21.5	26.8
118 d	females	9	20.1	32.3
118 d	males	9	22.3	29.8
127 d	females	4	29.0	32.3
127 d	males	4	28.5	32.2
14 months	females	4	52.5	57.0
14 months	males	4	46.2	47.6

At hatching, *Craterocephalus* sp. was slightly smaller (3.4-3.8 mm) than *C. stercusmuscarum*. The larvae took approximately 60 days to treble their size and then doubled their size again in just under 4 months. By 8 months the size had doubled again, indicating that the rate of growth did not decline as rapidly for this species as for the spotted hardyhead. As *Craterocephalus* sp. could not be sexed, the size difference between sexes could not be determined. By 18 months, *Craterocephalus* sp. reached 64.4 mm in length. This is smaller than the maximum size recorded by Ivantsoff (1980) and would suggest that the species lives well into its second year and possibly longer. Observations on growth were not made on *C. stercusmuscarum* beyond the 14th month. In Queensland, *Craterocephalus stercusmuscarum* may attain a length of 90 mm SL (Ivantsoff 1980) whilst Bishop et al. (in press) report fish of 65 mm SL from the Magela Creek system.

Table 15. Lengths (sexes combined) of *Craterocephalus* sp. nov., from hatching to 17 months of age

Temperature = 26 ± 1°C

Period after hatching	Number of fish	Min. TL (mm)	Max. TL (mm)
1-3 d	60	3.4	3.8
16 d	60	4.5	6.7
25 d	60	7.6	9.0
50 d	60	10.0	11.8
60 d	60	11.1	11.4
68 d	60	8.3	13.5
78 d	50	9.2	18.1
100 d	26	10.7	21.5
117 d	24	12.5	21.2
124 d	26	12.7	23.7
127 d	16	21.0	27.5
245 d	9	43.0	43.8
17 months	3	58.8	63.8

This suggests that fish may attain an age of 2+ years, a finding corroborated by Milton & Arthington's (1983*a*) study on *C. stercusmuscarum* and a sister species of *Craterocephalus* sp. nov. (*C. marjoriae*).

At 8 weeks the second generation of the spotted hardyheads exhibited sexual display and at 3.5 months (about 32 mm SL), the abdomen of the fish became yellow, indicating sexual maturity at this stage. Bishop et al. (in press) record the length at first maturity as 27-29 mm, that is a slightly earlier time than found by the present workers. In this study, however, displaying was first observed at about 18 mm and it is possible that reproductive maturity had preceded the colouration of the abdomen which is often regarded as a sign of breeding condition. Comparison of the growth rate with the southern subspecies of the spotted hardyhead is of little value, since the temperatures of the southern waters are considerably below those found in the Northern Territory. However, it is interesting to observe that Llewellyn (1979) recorded that *C. fluviatilis* reached 25 mm in 4 months under natural conditions with water temperatures 24.6-27.8°C, which is a slower rate of growth than obtained for the northern subspecies in the laboratory at 26 ± 1°C. It is also interesting to note that thereafter, the rate of growth slows down even further in *C. fluviatilis*, which only reaches 35 mm in the first year. The northern subspecies attains this length between 4 and 5 months of age.

In their estimation of growth rates for *Craterocephalus* sp. (= *C. marjoriae*), Bishop et al. (in press) calculated that this species reaches 38-39 mm SL in approximately four months. This result indicates that this species grows somewhat faster in the wild than under the conditions of this study. The fish only reached 27.5 mm SL in 3 months and were still only 30 mm SL at 165 days or approximately 5.5 months. This difference could be explained by the fact that the laboratory temperatures at which the fish were reared were somewhat lower than the ambient temperatures of the waters of the Alligator Rivers Region.

Craterocephalus sp. began exhibiting sexual display at 30-35 mm SL. This sexual display took the form of repeated pursuits, with the male moving beneath the female and grazing her abdomen with its dorsal fin. Butting, as described previously, was also observed. Following such displays there were eggs obviously fertilised, viable and attached to the weeds. As in *C. stercusmuscarum*, sexual maturation appears to be somewhat slower than

recorded in the field (Bishop et al., in press) where the fish were mature at 22-28 mm SL as against 30 mm SL, in the present study.

Recommendations

As spotted hardyheads may be easily stressed when held in community tanks in large numbers (> 30 fish), it is advisable never to keep more than that number in a tank for more than about 5-7 days. Stress often results in fin rot and a decreased survival rate. Larger fish tend to attack younger fish and for this reason the fish should be sorted and similar-sized fish kept together.

High temperatures (> 30°C) result in an increase in mortality through greater incidence of fungal infection. *Craterocephalus* sp. nov. responds to temperature changes and crowding in a similar manner and, in addition, it has been observed that sudden pH changes (> 1.5) can result in higher mortality.

Both species are much harder to rear than either the rainbow-fishes or the blue-eyes. Mortality may occur through predation of parents on eggs, changes of temperature or pH, crowding, or dirty tanks. For these reasons, greatest care must be taken when rearing these fish. Although mortality rates have not been studied, Tables 14 and 15 give some idea of the death rate during this study. Frequently all of the eggs were found to be dead, with death occurring at any stage, possibly due to fungal infection. However, the cause of mortality was never determined.

Because of the difficulties in rearing of hardyheads, it is difficult to predict the numbers of fish that can be reared in any one period of time. Assuming that a pair of either species could lay between 20 and 30 eggs at a time, the number of young raised would essentially depend on the number of tanks set up. The successful breeding period appears to be restricted to the September-November. Therefore, provided that the tanks are kept clean and the fish do not become stressed and thus prone to infection, it might be possible to rear 500-1000 fish of both species per annum.

3.4 Northern Territory reticulated perchlet (*A. macleayi*)

General description

Pollard (1974) stated that little or nothing was known about the biology of the reticulated perchlet except that it occurs most commonly in turbid and heavily vegetated billabongs along the Magela Creek system. The work of Bishop et al. (in press) is the only comprehensive field study of this species. The taxonomic position of the reticulated perchlet is uncertain, both at the species and familial levels despite Allen's (1982) recent placement of *A. macleayi* in the family Ambassidae, distinguishing it from its sympatrically occurring relative the sail-fin perchlet, *A. agrammus*, by a widely divergent gill raker count on the first gill arch. Bishop et al. (in press), however, noted that the two nominal species were not easily distinguished as they appeared to have overlapping or intermediate key characteristics. In the present study, it was found that the colours which were supposedly distinctive in the two species overlapped or were intermediate between the two. It is thought, however, that the fish which were bred, are described here, are indeed *A. macleayi*. The taxonomic and systematic position of the family Ambassidae will be resolved in the near future as Dr G.R. Allen (pers. commm.) is currently revising the group.

Colour. *Body:* the overall colour of a non-breeding adult is olive green to copper or golden, especially when the light falls directly onto the fish. The upper half of the body is darker, with the edges of scales outlined with black pigment giving the fish its 'reticulated' appearance. The curved mid-lateral band is not distinct but can be inferred on close observation, its colouration being slightly lighter and yellower than the rest of the body.

Caudally, the band narrows almost to a line which may appear as a golden flash as the fish flicks its tail. The ventral surface is lighter, more copper or golden in colour, with little or no black pigment to outline the scales. The opercle and the pre-opercle are also light coloured. The upper part of the iris is black, the rest golden. The dorsum, the sides of the head and chin are dusky. The dorsal and ventral contours are outlined with a thin gold line.

Fins: always erect. The spines of ventrals and anal are white and distinct from the rest of the fin. The edges of dorsal and anal fins are reddish. The rays of all of the fins are coppery, standing out clearly from the light yellow transparent fin membranes. There is a distinct black patch at the base of the pectorals. The caudal fin appeared to be relatively clear of pigmentation in the specimens observed.

Sexual dimorphism. There appear to be absolutely no differences between sexes, either in colour or in morphology. Sexing can only be done at spawning time, by observing the behaviour of the fish and devising some form of marking to distinguish each sex.

Behaviour. The reticulated perchlet tends to prefer dense vegetation when spawning, thus making it difficult to observe its mating behaviour. It has been noted that the males are more aggressive and are often seen pursuing the females into the weed. The male swims from underneath and behind the female, nudging her anal fin with the top of his head. Such pursuits occur at regular intervals but this behaviour may not lead to pairing, as the female may swim away and join other fish in normal feeding and schooling activity. If the female is receptive, the pair will move to a sheltered position, close to the bottom. The male, which often appears to be darker than the female, adopts a head down position, whilst the lighter bodied female maintains herself horizontal, just above the gravel or sand. Both fish may stay motionless for some time, remaining close to each other until spawning takes place. After spawning, the pair remain in the same area, presumably either to defend their eggs or eat them.

A change in colour (from dark to light and vice versa) may not necessarily be associated with mating behaviour as the perchlets tend to blend into the background of the tank. Colour change may be rapid (within about 30 seconds), going from very dark, almost purple to a light copper shade. The spines on the ventrals and anals may change from relatively colourless to white as described above. Fish which tend to be more aggressive (presumed to be male) are often much darker during sexual or aggressive displays.

Egg numbers. The breeding program of the reticulated perchlet had initially met with very little success and had almost been abandoned when, in the spring of 1982, Mr G. Semple noticed that the perchlets had spawned.

The perchlets spawned readily in Java moss. The eggs were released high up in the weed, the fish presumably moving up from their position near the bottom to disperse the eggs. Initially, only a few eggs were spawned a day but the number increased, possibly to above two hundred, on the second and third days. Spawning continued for about a week, followed by a period of reduced activity, with only a few eggs being laid. Further observations of egg-laying activity of these fish were reported elsewhere (Semple 1985).

Stimuli to spawning. None was found.

Eggs and embryonic development

Bishop et al. (in press) had reported that the ovaries of the reticulated perchlet could contain between 320 and 2360 eggs with a mean egg diameter of 0.3 mm. This finding, and the statement by the same authors that a large number of small eggs were laid amongst the vegetation, is in agreement with the observation made in this study.

Once the spawning activity has been established, the eggs are scattered high in the weed, in large numbers. The eggs are small, about 0.45 mm in diameter at spawning, but acquire a thick mucilaginous coat around the chorion, making the total diameter of the egg about 0.6 mm. The coat is sticky and the eggs adhere well to the weed in which they are spawned.

There are no filaments on the outer surface. At spawning, the yolk of the eggs examined contained one large and four small oil globules.

No data are available on the embryonic development of the reticulated perchlet.

Larval development

There is no known descriptions of the larval development of the reticulated perchlet, either by a scientist or an amateur breeder. In this study, all early attempts to keep the larvae alive beyond two or three days were unsuccessful. Eventually, 3 larvae survived to maturity and were last measured at 150 days.

Materials and methods. Three adult specimens of the reticulated perchlet were observed to spawn in an aquarium. The parents were immediately removed from the tank allowing the eggs to develop in the tank which was thickly planted with Java moss. The eggs were allowed to develop undisturbed until hatching, in water temperatures of 26 ± 1°C. Once hatched, the larvae were fed on Liquifry and TetraMin Baby Food, ground liver and an infusion of fresh lettuce leaves. Subsequently, this regime was changed to feeding on concentrated pond water extract, until the fish were capable of feeding on brine shrimp larvae. The concentrated pond water extract was obtained by using a small, fine mesh, hand held dip net to remove cyclopoids and other microscopic organisms from a pond within the university grounds. These were then rinsed in tap water and placed in 500 mL clean tap water, a small amount (about 20 mL) of the resulting 'soup' was then given to the larvae. At 25 mm SL, the fish were given adult diet of TetraMin Staple Food and frozen brine shrimp.

The breeding tank was left undisturbed as long as possible to avoid unnecessary mortality. About one third of the water was carefully siphoned off several weeks after hatching and replaced to prevent accumulation of waste products.

The fish were measured whilst restraining them with a fine mesh net held against the glass. This procedure was an attempt to minimise the loss of fish due to handling at the early stages of development.

Results and discussion. When first hatched, the fry attach themselves to stationary objects until the yolk is fully absorbed. The larvae remain in the weed or clinging to filaments of algae or other plant material. By two days, the yolk is absorbed and the fish appear to become neutrally buoyant. Feeding begins and the larvae pick up small items of the given food from then on. The fish actively search for food and congregate in areas where food is more abundant, usually in the corners of the tank or at the bottom. The capture of food is achieved by rapid darting movements if the food is live. By day 6, the fry spend most of their time near the bottom of the tank where the food given may be more plentiful. By day 9, the fish are dispersed throughout the tank, although the tendency to aggregate near the bottom is still strong. Schooling behaviour begins on about day 12 and the fry appear to be well developed and feeding vigorously at day 18.

First attempts to rear the larvae with Liquifry, TetraMin E Baby food and ground liver failed and all larvae died. Addition of pond water to the tanks immediately after hatching allowed the larvae to survive. Frequently, it was possible to collect water with large populations of cladocerans and other small crustaceans, on which the larval perchlets thrived. It was found that once the larvae were of sufficient size (about a month old or about 18 mm TL), the diet could be changed to live brine shrimp nauplii. Small insect

larvae would also be taken at this stage. At about 6 weeks of age, the fish were found to eat adult food, such as dry flake food and adult brine shrimp.

Despite the eventual success in breeding and bringing a number of perchlets to maturity, the perchlet breeding program was not successful. The study of the larval development could not be completed in the time available under the contract. The thirty fish initially observed and measured were reduced to 3 by the 40th day of study.

Rates of development and growth. At hatching the larvae are very small, about 2 mm TL. At 12 hours after hatching (Fig. 18) the larva has continuous dorsal and ventral fin folds, with the dorsal fin fold terminating at the vertical through the anus. The pectoral fins are well developed, whilst the ventrals are still absent. The eyes are heavily pigmented. The nuchal region may have sparse black marking, whilst the abdominal region is more heavily pigmented. There are lines of spots on either side of the ventral fin fold. By the seventh day (Table 16), the larvae double their size and then triple their initial length by day 12, provided the diet is adequate and the fish are feeding. By day 18, the fish grow to 10 mm TL and at this stage are identical with adults in their external morphology.

By about day 40, the fish showed first signs of aggression by engaging in chasing activity. This behaviour appears to be a prelude to sexual display, as at about the same time (43 days and 21 mm TL), the fish began butting and pursuing each other, in much the same way as the adults do before spawning. New colour markings appeared at the same time: the iris darkened as did the anterior edge of the dorsal fin, these possibly being signs of sexual maturation.

Bishop et al. (in press) report that the length at first maturity of the reticulated perchlet was between 29 and 33 mm TL. It is possible that display commences well before the fish is sexually mature or, alternatively, the behaviour observed was not behaviour prior to spawning but aggressive display.

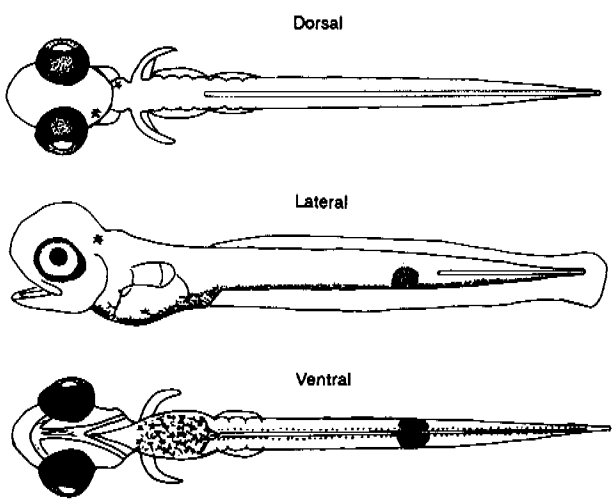


Figure 18. Dorsal, lateral and ventral views of *Ambassis macleayi* two days after hatching and 2.25 mm TL

Table 16. Lengths of *Ambassis macleayi* from hatching to 150 days

Period after hatching	Number of fish	Min. TL (mm)	Max. TL (mm)
0	30	2.00	2.25
2	30	2.25	2.50
7	*	*	4.00
12	*	*	6.00
18	*	*	10.00
41	3	18.5	20.90
43	3	18.5	21.00
54	3	25.0	29.20
80	3	30.00	34.20
150	3	35.60	39.80
Fish captured at 35.0-40.0mm TL and held in the laboratory for 248 days, grew to 50-52mm TL. Therefore-			
398 (approx.)	*-	50.00	52.00

* values not available

Recommendations

Early stages of larval development require live food consisting of crustaceans in order to survive. Cladocerans, copepods and ostracods appear to be the right size for the developing fry to eat. It is suggested, therefore, that these crustaceans would have to be either regularly collected or cultured. Numerous techniques on how to culture pond water organisms can be found in the literature (e.g. Galtsoff et al. 1937).

As the fish grow, they can be introduced to commercial flake food and frozen brine shrimp. Adult fish can therefore be fed in the same way as the rainbows-fishes, blue-eyes and hardyheads.

Temperature tolerance of larvae appears to be high (28-29°C) but it is suggested that the fish should not be subjected to temperatures lower than 23°C and higher than 30°C as this may introduce other problems, such as higher susceptibility to fungal infection and possible abnormalities, if the perchlets respond to temperatures in the same way as the other species that were studied.

The tanks holding perchlet fry should not be cleaned during the early stages of development. For this reason, larger tanks (70 x 38 x 30 cm) would be better for their development. Adults should always be removed from the tanks where they have spawned.

Young fry appear to be quite weak in the very early stages of their development. For this reason, only subsand filters should be used, even with larger tanks, in order to avoid the larvae being sucked into the filter.

Vegetation should be abundantly planted, as for the other fishes studied. Java moss appears to be most acceptable but other plants such as *Ceratopteris* and *Elodea* could also be of use. Perchlets apparently prefer fine leafed or filamentous plants for spawning.

The tanks should always be covered with lids as perchlets, like the rainbow-fish and hardyheads, tend to jump out of tanks.

At this stage, there can be no guarantee that the reticulated perchlet could be bred in sufficient numbers for toxicological studies. More work has to be done on its food preferences, although it is already known that microcrustaceans are an important component of their diet in the wild (Bishop et al., in press). It is also necessary, if possible, to determine if there are external factors which can induce the fish to breed. Effects of light intensity, day length, water content, change of water levels, current strength, population composition and size, substrate and vegetation should all be examined to ascertain whether fish will breed only under a certain set of conditions.

Present indications suggest that all the fish examined in this study maintain the same biological pattern as has been established in the wild.

Until more is known about the perchlet's breeding behaviour, it is suggested that the species should be collected as it occurs in large numbers in the wild. With careful maintenance, the reticulated perchlet can be transported and kept in captivity with relative ease. In contrast, breeding these fish at this time may only produce small numbers of fish at great expense.

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