REARING EXPERIMENTS WITH FIVE SPECIES OF AUSTRALIAN FRESHWATER FISHES

I. INDUCEMENT TO SPAWNING

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Summary

Experimental work on the reproduction of five species of fish endemic to Australia indicates the importance of specific water temperatures and floods as triggering mechanisms for spawning and for subsequent survival of young. Evidence has been obtained which suggests that the factor which stimulates the fish to spawn is produced when water comes into contact with dry soil.

The species studied were *Tandanus tandanus*, *Plectroplites ambiguus*, *Maccullochella macquariensis*, *Bidyanus bidyanus*, and *Carassiops klunzingeri*. The spawning of these species is compared with some observations made on the heterochthonous *Perca fluviatilis*.

Plectroplites ambiguus and *Bidyanus bidyanus* spawn at water temperatures above 23°C provided there is an accompanying rise in water level; both species produce pelagic eggs. *Tandanus tandanus* spawns at a temperature of 24°C and demersal eggs are laid in a gravel nest; a rise in water level is not essential. *Maccullochella macquariensis* spawns at 20°C provided there is a slight "run off" of water into the pond; eggs are laid in hollow logs or in similar situations. *Carassiops klunzingeri* spawns at 22·5°C and the eggs adhere to grass and twigs at the water's edge. *Perca fluviatilis* spawns at 11·5°C and all fish spawn in ponds over a short period if additional water is added, this ensures a more uniform water temperature throughout the pond.

I. INTRODUCTION

A total of 27 species of fish are known to inhabit rivers of the Murray–Darling river system within New South Wales; eight of these are introduced fishes. The species mentioned in this paper are listed in the following tabulation.

Family	Genus and species	Common name
Retropinnidae	*Retropinna semoni* (Weber)	Australian smelt
Plotosidae	*Tandanus tandanus* Mitchell	Freshwater catfish†
Serranidae	*Macquaria australasica* Cuvier	Macquarie perch
	Plectroplites ambiguus (Richardson)	Golden perch†
	Maccullochella macquariensis (Cuvier)	Murray cod†
Theraponidae	*Bidyanus bidyanus* (Mitchell)	Silver perch†
Percidae	*Perca fluviatilis* L.	European perch†‡
Eleotridae	*Carassiops klunzingeri* Ogilby	Western carp gudgeon†

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† Details of reproduction of these species are given in this paper.

‡ Introduced to Murray–Darling river system in 1888.

The classification of Berg (1947) has been followed. Only four native species have a wide distribution within the system and are of commercial importance; these are *Tandanus tandanus, Plectroplites ambiguus, Maccullochella macquariensis,* and *Bidyanus bidyanus.* All four species have been kept in farm dams where *T. tandanus* is known to breed regularly, the others rarely.

However, apart from essentially taxonomic studies and a few anecdotal notes, little has been published concerning the biology of Australian native fishes. McKeown (1934) dealt briefly with the food of *Macquaria australasica*; Dakin and Kesteven (1938) with the development of *M. macquariensis*; Butcher (1945 and 1946) with the food of several species; Stephenson and Grant (1957) with the artificial feeding of *P. ambiguus*; Lake (1959) with various aspects of the biology of all fishes known to inhabit the rivers of New South Wales; and Whitley (1960) published a guide to freshwater fishes of Australia which contained brief notes on their biology and distribution.

In 1949 and 1950, J. O. Langtry accumulated a large quantity of data on relative abundance of fish captured, length–weight relationship, gonad development, selectivity of fishing gear, etc. These data were collected in the course of an unfinished general ecological survey of the Murray–Darling river system and were issued as a report by the Victorian Fisheries and Wildlife Department in 1960.

The establishment in 1960 of the Inland Fisheries Research Station, near Narrandera, N.S.W., provided facilities for intensive research on fishes of eastern Australia. An account of the morphogenesis and ontogeny of one species, *Retropinna semoni,* was subsequently published (Milward 1966). In addition a summary of our present knowledge of all fish of the Murray–Darling river system was published recently (Lake 1967*a*).

Experienced fishermen have maintained for many years that the larger fishes of the Murray–Darling system spawn during floods, and it has been claimed that a delay in the annual flooding from spring to autumn led to a corresponding delay in spawning. It was known that *T. tandanus* laid their eggs in nests of a metre or so in diameter. Nests have also been reported for *M. macquariensis*; such reports possibly arose as the result of seeing old nests of *T. tandanus.* There seemed to be no generally held beliefs about the spawning of *P. ambiguus* and *B. bidyanus,* although Ogilby (1920) recorded that the eggs of *P. ambiguus* were planktonic.

Most of the experimental work reported in this paper is based upon fish contained in ponds. However, it is pertinent to emphasize that the Murray–Darling river system presents a habitat where enormous fluctuations in water level and other factors occur. This is caused by the uncertainty and irregularity of rainfall on the watershed and the low gradient of the rivers throughout the greater part of their lengths. It is possible for hundreds of miles of rivers to be reduced to chains of waterholes and even most of these dry out during prolonged droughts. On the other hand, heavy snowfalls and rain in the eastern highlands and monsoonal rains in Queensland cause floods which virtually produce inland seas which cover hundreds of miles of flood plain.

Frith (1959) made a study of the breeding and other habits of wild ducks in parts of this system and found that environmental factors, particularly changes in water level, were of prime importance in regulating sexual activity in these animals. He also showed that increasing water levels are followed by an increase in plankton and insects which largely determine the survival of young.

In Indonesia, species of *Puntius* spawn in the rivers during floods but fish farmers in that country have found that if a pond is dried out, then filled with water, and adult fish introduced to the pond while it is filling, the fish usually spawn. Some of the species of major carps of India and Pakistan have also been induced to spawn in this way (Hora and Pillay 1955). Swingle (1953) showed that optimal spawning with several species of fish was only attained when fresh water entered a pond at times when other conditions — mainly temperature — were satisfactory. John (1963) studied the reproductive cycle of the speckled dace (*Rhinicthys osculus*) in Arizona and found it to be bimodal, with discrete peaks in early spring and late summer, if the precipitation were normal. In periods of drought, overcrowded and under-nourished populations failed to reproduce.

Gonads and advanced larval stages of fishes were studied from material collected in the Darling River between Bourke and Brewarrina during the period 1957–59. At that time the larvae could not be specifically identified although juvenile and very small *P. ambiguus* were collected from flooded backwaters. From these initial observations it was assumed that *P. ambiguus* would only spawn after substantial rises in the water level. Other earlier conclusions pointed to the fact that *P. ambiguus*, *B. bidyanus*, and *T. tandanus* would not spawn below 24°C, whereas the optimal temperature for spawning of *M. macquariensis* was 20°C.

Controlled experiments were carried out at Narrandera, in artificial ponds, from September 1960 to May 1966. Each species, and some of the same individuals, were induced to spawn for at least 2 and for up to 4 consecutive years. Since the main object of these experiments was to induce spawning in the ponds and to record the stimuli which must operate in order to achieve this, no attempt was made to obtain maximum numbers of small fish; this paper deals with the induced reproduction of the species listed. Other results from this research are given in Part II of this series (Lake 1967*b*).

II. Materials and Methods

During the earlier field studies in the late 1950's, some hydrological data were obtained from many areas throughout this river system including pH, temperature, turbidity, total dissolved solids, and total alkalinity. These were recorded under varying conditions ranging from floods to very low river levels when flow had ceased. Some of these data have been published (Lake 1967*c*). At the same times, fish were collected, gonads examined and weighed, and length–weight data recorded for fish captured. Larval and juvenile fishes were also collected by the use of rotenone. The information obtained during this period helped to suggest the design of experiments in ponds where the factors which stimulate spawning may be determined.

(*a*) *The Ponds*

The ponds were excavated in earth and clay during 1960. Although it was found possible to induce breeding in small ponds (6 by 6 by 1 m depth) with all species except *M. macquariensis*, larger ponds, designated as flood ponds, were generally used. The flood ponds had a surface area of about 0·1 ha being a little over 30 m square. The larger portion of these ponds was 1·2 m in depth while the section along one side, of about 30 by 7 m, was 2·4 m in depth. This deeper section allowed lengthy retention of fish in the pond while the shallower "flood plain" could be drained and dried. All ponds contained an outlet tower enabling the water level to be set at any desired height. The tower also provided facilities for holding screens of various mesh. There was a screened underwater inlet, at the opposite end of the pond to the tower. Between this inlet and the outlet there were raceways set into the bottom of the ponds. At the inlet of larger ponds there was an extension of the raceway into a concrete sump. Since all ponds were of clay construction these sumps and raceways allowed ready collection of all fish, which congregated there, when the ponds were drained.

The water supply for the ponds was pumped from the Murrumbidgee River into an earthen reservoir from which it was reticulated by gravity to the ponds. Water could be drawn from the reservoir at any desired depth. This gave considerable selection of temperature of the water reticulated to a pond. It was possible to fill or empty a pond at a rate of up to 130,000 l. per hour which meant that a flood pond could be completely filled or emptied within 5 hr. Ponds were filled at rates varying from 5 to 48 hr.

Field studies had suggested that turbid water may be necessary for gonad development or for final maturation and spawning. When in flood the inland rivers are always very turbid, the Darling River produced a Secchi disk reading as low as 10 cm on a number of occasions. Provisions were available to vary the turbidity in the ponds.

Lengths of fibro-cement pipe of diameters from about 8 to 30 cm were placed in ponds to provide some cover and protection for the fish. In the ponds used for *Maccullochella macquariensis*, sand, gravel, and sunken logs were also introduced. Gravel was added to some of the catfish ponds.

(*b*) *Stocking Rate in Ponds*

The ponds were stocked at various rates ranging from 1 pair to 20 pairs of a particular species of fish. Different sex ratios were also used.

(*c*) *Experimental Fish*

Most of the adult *Plectroplites ambiguus, Bidyanus bidyanus*, and *Maccullochella macquariensis* were netted from the Murrumbidgee River and flooded anabranches near Narrandera. During 1965 additional experiments were carried out with *P. ambiguus* and the adult specimens were obtained from the Lachlan River. This river was in drought condition at that time and there had been no significant rise in water level for several years.

Tandanus tandanus were netted from Barren Box Swamp near Griffith. Most *P. fluviatilis* were netted from an irrigation canal a little upstream from Narrandera. *Carassiops klunzingeri* entered the ponds, when very small, with the water pumped from the river to the station.

Tandanus tandanus can be readily sexed, after the fish reach 1 year of age by the shape of the urogenital papilla. *Carassiops klunzingeri* also shows marked sexual dimorphism (Lake 1967*a*). The remaining species show no sexual dimorphism although the sexes can be separated at advanced stages of gonad development. For at least 1 month before water temperatures are sufficiently high for breeding to take place, milt can be forced from the male fish. Female *P. ambiguus* and *M. macquariensis* become considerably distended and show a swelling and inflammation around the cloacal region. It is rare to find mature *P. ambiguus* females less than 1·3 kg in weight (total length (T.L.) 43 cm) although the male fish may be mature at 0·5 kg. (T.L. 33 cm). *B. bidyanus* and *P. fluviatilis* may be slightly distended but show little change in the cloacal region.

The size range of fishes used for breeding work was 0·9–2·6 kg (T.L. 45–60 cm) for *T. tandanus*, 0·7–6·4 kg (T.L. 34–65 cm) for *P. ambiguus*, 2·3–6·8 kg (T.L. 57–74 cm) for *M. macquariensis*, 0·7–3·2 kg (length to caudal fork (L.C.F.) 34–55 cm) for *B. bidyanus*, and 0·4–2·3 kg (L.C.F. 32–45 cm) for *P. fluviatilis*. *Carassiops klunzingeri* is a small fish and those observed spawning ranged in total length from 3 to 5 cm.

The fish were sexed and sorted into ponds at various times ranging from 2 days to 10 months before breeding was expected to take place. In order to reduce damage to the fish and allow careful examination they were anaesthetized with 2-methyl-quinoline at a concentration of 10–20 p.p.m.

Laparotomies were performed on a number of fish after spawning in order to see if all ova were shed. An incision of 5–6 cm was sufficient to examine the ovaries of fish of 1·5–4·5 kg in weight. Before making the incision, scales were removed with a scalpel. Michel clips were used to close the incision and a concentrated solution of potassium permanganate was applied to the wound. These fish were held singly in 400-l. fibro-cement tanks through which a flow of water was maintained. After 2–3 weeks the wound was clean and had healed.

(*d*) *Confirmation of Spawning*

The courtship of one species and actual spawning of two other species were observed. Plankton nets were hauled through the ponds and the presence of eggs or larvae confirmed that a spawning had taken place. A very slight oil film on the water surface also suggested a spawning had occurred. The use of lights during the night enabled the pelagic eggs of *Plectroplites ambiguus* and *Bidyanus bidyanus* to be seen.

To collect eggs of *Tandanus tandanus* it was necessary to lower ponds and with *Maccullochella macquariensis* removal of fibro-cement pipes proved to be quite satisfactory since eggs were laid inside these pipes. The eggs of *P. fluviatilis* could be seen from the edge of the ponds.

(*e*) *Chemical and Physical Tests*

As a routine procedure water temperatures were taken in the early morning and late afternoon near the surface and bottom of the ponds. During flooding of ponds water temperatures were taken frequently using a thermistor at all depths until spawning occurred or the experiment was terminated. Conductivity and pH were recorded with electrical meters, turbidity was determined with a Secchi disk, and total alkalinity was measured by titrating with hydrochloric acid using methyl orange as indicator.

III. Results

The numbers of fish used in a pond, the sex ratio, or the rate of adding water to a pond, had no apparent influence on whether spawning occurred. Ponds with flood-plain areas were convenient to use but they were not essential. Where no specific flood plain was provided the sloping walls of a partly filled pond acted in the same way in that dry ground was inundated when water was added to the pond.

Within the pH ranges encountered (7·1–7·8), total alkalinity (63–90 p.p.m. as $CaCO_3$), conductivity (220–400 μmhos at 20°C), turbidity (Secchi disk readings from 12 to 240 cm), there was no noticeable effect on gonad development or spawning. Spawning has occurred in the Darling River when the conductivity was 3000 μmhos at 20°C. Williams (1966) has shown that there is a fairly constant relationship between conductivity and total dissolved salts in Australian inland waters and that Na^+ and Cl^- are the predominant ions.

(*a*) *Plectroplites ambiguus*

The number of spawned eggs was very much greater when several pairs of fish were employed during the tests. After 5 months, however, the resulting juveniles were larger, in better condition, and more numerous in those ponds which contained only one or two pairs of parental fish. In view of the fact that artificial food was not supplied during these experiments, it can be assumed that this phenomenon must have been the result of the scarcity of the natural food supply (plankton) in the heavily populated ponds. Plankton tows in these ponds yielded only negligible quantities of cladocerans and copepods, while these crustaceans were quite abundant in other ponds which originally contained a smaller number of young fish. By the time that most of the fish in heavily populated ponds had apparently starved to death, the plankton became affected by cooler weather conditions, and no further increase in its density could be noted. The remaining few fish remained very weak and emaciated and many died when collected. The larger and stronger fish, however, were quite hardy.

Counts of ova, both by gravimetric and volumetric methods, using three fish of 2·2, 2·3, and 2·4 kg (T.L. about 50 cm), demonstrated a fecundity of about 500,000 per individual. The weights were measured before the removal of ovaries.

Experiments were carried out with this species from November 1960 to November 1965. During the period from early November 1960 to mid-February 1961 seven ponds were used. Six spawnings were induced in November and December 1961. Three in November 1962, and four in October and November 1965. In November and early December 1960, three flood ponds were filled at different times, a few days apart. The males in these ponds yielded milt on slight pressure, and all females possessed ovaries at an advanced stage of development, causing a perceptible distention of the body wall. However, even considerable pressure failed to produce any ova.

After the ponds had been filled, water temperatures did not exceed 22·2°C and cool weather followed in each instance causing water temperatures to drop to about 20°C. These ponds were emptied, the flood plain dried, and then refilled on a number of occasions. No spawning occurred as the result of these floodings and a pair of fish were removed and an attempt was made at artificial fertilization. Ova were obtained by cutting open the fish since none was obtained by applying pressure to the body wall. No fertilized eggs resulted. On December 9, 1960, surface temperatures rose to 28·4°C but it was not possible at that time to produce temperatures above 23°C at depths below 70 cm. Again no spawning occurred. Between December 20 and December 31, 1960, air temperatures during the day rose to over 38°C and at night did not fall below 27°C.

The filling of one flood pond was commenced on December 20, 1960, and when full on December 21, the surface temperature was 30°C with a gradual fall to 24·5°C at the bottom at a depth of 2·4 m. Water temperatures did not fall below 24°C for the next 10 days and surface temperatures went as high as 33·4°C on December 27, 1960. At 4 p.m. on December 22, 1960, 45 hr after the filling of the pond commenced, courtship behaviour was observed. Three male fish of about 1 kg and one large female of 4·4 kg were seen near the surface at the edge of the flooded area of the pond. The males repeatedly nosed the cloacal region of the female and used their snouts to "bump" the female in this area and along the ventral sides of the body. The female was placid with her head pointing downwards at an angle of about 60°. Occasionally her tail would break the water surface. From time to time a male would swim off some little distance and move around the female; no fighting was evident between the males. This behaviour was observed for 4 hr until dusk. During this period the fish were not easily disturbed although they would move away when approached very closely. They would return after several minutes, usually to the same spot. This may suggest some evidence of territorality but sufficient data are not available to confirm this. No actual spawning was observed during this period but subsequent collection of eggs showed that spawning occurred soon after dark. This pond was reasonably clear having a Secchi disk reading of 63 cm.

During the next few days, two more flood ponds were filled and spawning occurred in each about 30 hr after they were filled. In each instance the water temperature did not drop below 24°C anywhere in the pond. One pond was filled with clear water where it was possible to see a Secchi disk at the bottom at a depth of 240 cm. The other pond was one that had been turbid before flooding and muddy water was used to fill it. In this instance the Secchi disk reading was only 12 cm.

Some adult *P. ambiguus* were retained in a deep pond where water temperatures, at the bottom, did not rise above 23°C. Others were kept in water of only 90 cm depth where temperatures exceeded 24°C. No water was added to these ponds and no breeding occurred.

On February 8, 1961, the fish from the deeper and cooler pond, were placed in a pond *after* it had been filled with water over 24°C and spawning took place on February 10, 1961, and fertilized eggs were collected. This was repeated, at the same time, in another pond, with fish from the shallow pond. Again the fish spawned but all eggs were dead and not fertilized. It appeared that once the fish experienced temperatures above those at which they could spawn the eggs started to break down unless an accompanying rise in water level induced spawning.

The induction of spawning of *P. ambiguus* was repeated in 1961 and 1962 in order to confirm and extend the information collected during these earlier experiments. The spawning experiments in 1965 were conducted principally to obtain young *P. ambiguus* for other experimental work. These were carried out in the light of previous results and spawnings were achieved, in each instance, the first time ponds were filled.

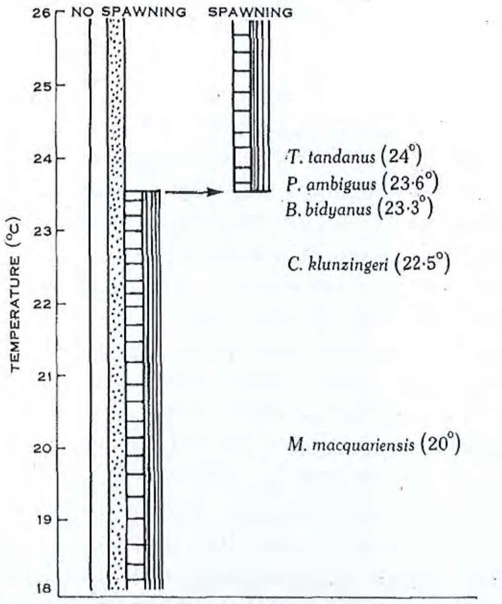

Fig. 1.—Conditions for spawning of *Plectroplites ambiguus*, and minimum temperatures for spawning of all species studied. Unshaded column, no additional water added to pond; stippled column, water added to pond and released with no rise in level; horizontally shaded columns, water added to pond; vertically shaded columns, pond filled, fish then introduced.

The ovaries of several fish were examined after spawning and it was found that some fish did not shed all their ova. It was essential to know if a fully spent fish would mature in a pond without flowing water. Laparotomies were conducted on three females and one of these was found to be fully spent. This was the large female of 4·4 kg which was observed during courtship behaviour. This fish matured in a pond and spawned in December 1961. It was induced to spawn again in November 1962 and November 1965. No attempt to induce spawning was carried out in 1963 or 1964 with this species. Without rising water levels the oocytes were presumably resorbed.

In many cases only some ova (as little as 25% of gonad weight) were shed from a fish. The residual mature ova were not free in the lumen of the ovary and so ovulation had not been complete. This fact suggested that this species may be a batch spawner producing batches of ova at short intervals during the spawning season. However, observations of wild fish do not confirm this possibility except that when floods are of a minor nature incomplete ovulation is common. During major floods ovulation is complete and all ova are shed from the ovary at the one time.

The factors associated with the spawning of *P. ambiguus* are summarized below and are shown diagrammatically in Figure 1.

(1) Sexual development took place in ponds, flowing water was not necessary.

(2) Development of gonads and spawning took place in clear and turbid waters (Secchi disk readings of 12–240 cm). Total dissolved solids in pond water always exceeded 140 p.p.m.

(3) Spawning was induced by adding water to a pond so that the minimum temperature of the water at the bottom exceeded 23·6°C. This was the lowest temperature at which spawning occurred. In several instances temperatures reached 23·5°C but spawning did not take place.

(4) Spawning only occurred if water temperatures remained above 23·6°C for the whole period between commencement of filling of the pond and time of spawning. This period was usually less than 48 hr.

(5) Spawning did not occur unless water was added to the pond, even if temperatures exceeded 23·6°C.

(6) Provided temperatures were 23·6°C or greater, spawning occurred if the adult fish were placed in the pond after filling was completed.

(7) If fish were held at temperatures below 23·6°C spawning could be delayed for several months and then induced.

(8) If fish were held at temperatures above 23·6°C for several weeks breakdown of oocytes took place gradually. If the stimulus of "flooding" was then given ova were sometimes shed but these were dead.

(9) Spawning could be induced with water from the river even during drought times provided dry ground was inundated in the pond and water temperatures exceeded 23·6°C throughout the pond.

(10) Inundation of dry ground gave blooms of plankton which were used as food by the larvae and young fish.

(11) Many fish which spawned in ponds shed only a portion of their ova. A few shed all ova and these were invariably large females greater than 3 kg in weight.

(12) Fish collected from the river during drought periods could be induced to spawn.

(13) Spawning always occurred at night, most frequently within 3–5 hr after sunset, irrespective of the time the pond was filled.

(14) If water was added to a pond and the water level kept constant, or reduced, by allowing water to flow out of the pond at the same time, spawning did not occur even if temperatures were suitable. However, on raising the water level and inundating dry ground, spawning occurred.

(b) Bidyanus bidyanus

This species spawned unexpectedly in two ponds in December 1960. The first that was known of this was when larval stages were collected in a plankton net. It was suspected that spawning occurred during hot weather when the ponds were "topped" with water to replenish losses due to evaporation and seepage. Other spawnings took place in 1961. However, no controlled experiments were conducted with this species until November and December 1962 and November 1965.

The experiments with *B. bidyanus* were carried out in a similar way to those on *P. ambiguus*. Unlike *P. ambiguus*, *B. bidyanus* often schooled and spawned late in the afternoon; there was a high fecundity similar to that of *P. ambiguus*.

Actual spawning was observed at 4.30 p.m. on November 18, 1962, following the filling of a flood pond on November 16 and 17.

Shortly after 4 p.m. one male was seen swimming swiftly around the shallow edge of a pond pursuing a large female fish. Occasionally a second and third male would join the chase and then disappear for a while. At 4.30 p.m. when only one male and the female could be seen they broke the surface of the water in vigorous activity and an immediate short haul of a plankton net at this point resulted in the collection of about 200 eggs all of which were fertilized. The first blastomere had not yet formed.

Factors which stimulated the spawning of *B. bidyanus* closely paralleled those required by *P. ambiguus* (Fig. 1) but with the following important differences.

(1) Water had to be added to a pond to induce spawning but increasing the water level as little as 15 cm was sufficient.

(2) Spawning took place provided the water temperature exceeded 23·3°C to a depth of 90 cm. Temperatures below this level could be cooler.

(3) Spawning usually occurred late in the afternoon and before sunset.

(4) After spawning some individuals died.

(5) All fish shed most ova at the one spawning.

(c) Tandanus tandanus

This species was placed in three ponds in 1962. One pond was 12 by 4 by 0·5 m, one was 30 by 15 by 1·2 m, and the third was 30 by 30 by 1·2 m. Gravel was added to the smallest and largest ponds; the other had a mud bottom. Five specimens of *T. tandanus* which weighed from 1½ to 2 kg prior to removal of ovaries (T.L. *c*. 47–55 cm), each contained between 18,000 and 26,000 ova.

During the latter part of November 1962 water temperatures reached 25°C and nest-building commenced. This could be seen in the shallow pond without lowering the water level although the activities of these fish caused the water to become fairly turbid. Water levels in all three ponds were lowered and a search was made for eggs amongst the gravel. In the pond lacking gravel, nests were made in the mud and these contained a few stones and hard pieces of earth which must have been collected by the fish from amongst the mud. The nests were circular, saucer-shaped, depressions and ranged in size from 0·7 to 1·2 m in diameter in the larger ponds. In the small pond the nest was oval in shape and had a length of 2 m and a width of nearly 1 m.

The larger fish made the larger nests. In the ponds which contained gravel the nests were built at the positions where the gravel was placed. The circumference of the nests was built up higher than the surrounding pond bottom.

It was soon evident that once water levels were lowered to expose the nests the catfish abandoned these and commenced building new nests after the pond was refilled. In one pond lowering and raising of the water level was continued at least once a week throughout the summer and spawning did not take place. In the remaining two ponds, including the one with mud bottom, spawning occurred but only the young fish were collected and eggs were not obtained. Very few young fish resulted from the pond devoid of gravel. This work was extended in 1963 and eggs at an early stage of development were obtained from nests.

The factors associated with the spawning of *T. tandanus* were:

(1) Water temperature reached about 24°C before nest-building commenced. Spawning did not occur at a water temperature of less than 24°C.

(2) Gravel was preferred to mud for nest-building although nests were built and spawning took place in mud, coarse sand, and gravel. Various sizes of gravel were used for nest building up to a size of 5 cm in diameter.

(3) One pair of fish used one nest for spawning.

(4) One parent (sometimes both) remained in attendance at the nest until after hatching.

(5) The eggs passed down between the gravel and did not adhere to it.

(6) Spawning occurred at about sunrise in the two instances when this could be determined.

(7) Addition of water to a pond hastened spawning but spawning took place even when no additional water was added.

(8) Nest building commenced 1–2 weeks before spawning.

(9) Lowering of water levels to expose a nest caused it to be abandoned and another nest was built. If nests were continually exposed by lowering water levels the oocytes were eventually resorbed and spawning did not take place.

(*d*) *Maccullochella macquariensis*

In 1961 only a few adult fish were available. These were subjected to rises in water level in "flood ponds" similar to the ones used for *P. ambiguus* and *B. bidyanus*. This was attempted when water temperatures approached 18°C and repeated at temperatures up to 25°C. Spawning did not occur.

In May 1962, 35 adult *M. macquariensis* were obtained from the river. These fish ranged in size from 2·3 to 6·8 kg (T.L. *c.* 57–74 cm). At this stage sexing was difficult and the fish were sorted and sexed in October when the males readily yielded milt and the females were distended by the ovaries. Although several attempts were made to induce spawning in a number of ponds, under various conditions, spawning did not occur.

Since the sexes of these fish were now known it was possible to sort some of them into a pond in early 1963 so that there would be no need to handle them just before spawning was expected to take place. It was thought that handling them prior to spawning may have some inhibiting effect, although this had not been the case with the three species bred previously.

Seven fish, four males and three females, were placed in a new and larger flood pond in April 1963. This pond covered an area of about 0·6 ha, although an area of 0·4 ha was very shallow flood plain with a maximum depth of 1 m. The remaining 0·2 ha was nearly 3 m deep. This allowed the fish in this pond a depth of water of 2 m even when the flood plain was dry. The pond was lowered late in June 1963 to expose the flood plain.

On October 17, 1963, filling of the pond and subsequent inundation of the flood plain was commenced at 10 a.m. At this time the surface temperature of the pond was 18·4°C and at the bottom the temperature was 16·7°C. Warm weather was forecast and the river temperature, from which water would have to be pumped to fill this large pond, was 19°C. The pond was filled slowly and cold water from the bottom was let out through the pentstock. Because of these two factors the pond was not full until the early afternoon of October 21. At this time the temperature was fairly uniform throughout the pond at 20·5°C. The following morning a very slight oil film was seem on the pond surface and when temperatures were being recorded later that morning a fish broke the surface of the water. This was a rare occurrence with this species particularly during daylight. It was thought that spawning was taking place.

No further indications were available as to whether spawning had occurred and it was known that the eggs, if present, would take over a week to hatch (Dakin and Kesteven 1938). At 3 p.m. on October 25 a length of fibro-cement pipe 120 cm in length and 20 cm in diameter was removed from the pond and adhered to the inside of the pipe were the eggs of *M. macquariensis*. This pipe had been situated in the deeper section of the pond along one of the banks and was partially submerged prior to inundation of the flood plain. It was located near to where the cod had been seen to splash. Examination of the eggs indicated that spawning almost certainly occurred on the morning of October 22.

Spawning of this species was again achieved in this pond in 1964. The fish were not handled or disturbed after July 7, 1964. Spawning did not take place until mid-November 1964 because water temperatures did not reach 20°C until that time. On that occasion water was kept much clearer before and during flooding. In 1963 Secchi disk values were maintained between 20 and 30 cm before flooding and rose to about 40 cm after flooding. In 1964 Secchi disk readings were never less than 100 cm.

It has been confirmed that spawning of *M. macquariensis* has occurred in three farm dams, close to Narrandera, N.S.W. In each instance it rained when water temperatures were about 20°C although in one case there was only a very slight rise in water level in the dam, probably no more than a few centimetres. Each of these dams contained hard objects, an old galvanized water tank in one, hollow logs in another, and a concrete trough in the third. Eggs could have been attached inside or underneath these objects. These dams however, were not as large as the pond in which spawning occurred at the Narrandera station, in fact one of them was of similar size to the smaller flood ponds in which spawning did not occur. The fish in the larger flood pond were not handled for 4 months before spawning. Whether the effects of handling acts as an inhibitor to spawning was not determined. However, one reason why spawning did not occur in some of the smaller ponds was ascertained. During October and November wide fluctuations in weather and air temperatures were common and this caused wide fluctuations in water temperatures particularly where the water volume was small and the depth shallow. Subjecting *M. macquariensis* to temperatures over 21°C resulted in the death of the oocytes and they were resorbed within a month. Macroscopically the ovaries in which resorbtion of oocytes had taken place were easily mistaken for spent ovaries. It was very probable that the lowering of ponds and the handling of fish close to spawning time affected the fish by subjecting them to temperatures above 21°C since the fish would have been in the very shallow water collected from the surface of the pond. Counts of ova in five fish 3–3½ kg in weight (T.L. *c.* 60 cm) showed that there were 20,000–30,000 ova per fish, and two fish of 20 and 22 kg (T.L. 100 and 110 cm) contained about 200,000 ova each. The weights were measured before the removal of ovaries from the fish.

The major factors associated with the spawning of *M. macquariensis* were as follows.

(1) Addition of water to a pond when water temperatures first reached 20°C induced spawning provided there were suitable objects present for attachment of eggs.

(2) Neither flowing water nor high turbidity were required for maturation of gonads or spawning.

(3) A short period of exposure of this species to temperatures over 20°C caused damage to the oocytes which were then rapidly resorbed.

(4) Eggs were attached to the inside of fibro-cement pipes. It is assumed that hollow logs are commonly used for this purpose, under river conditions. Rivers in inland Australia are well supplied with branches and whole trees of *Eucalyptus camaldulensis* (river red gums) which are continually falling into the rivers as banks erode.

(5) All the eggs from one female were laid in one pipe.

(*e*) *Carassiops klunzingeri*

This species was observed spawning on November 5, 1963. Eggs were laid around the shallow edges of the pond in which *M. macquariensis* had spawned 14 days previously. A male fish "nosed" a female from time to time as they moved rapidly between the grass around the edges of the pond, very close to the water surface. The fish paid little attention to observers and remained in a fairly restricted area, rarely moving more than 30 cm distant. Eggs were so small (0·40–0·51 mm in diameter when water-hardened) that it was impossible to see them being deposited. However, the eggs adhered to grass and twigs and they could be readily collected and examined. Eggs were found from 5 cm below the surface to a maximum of 25 cm depth.

This spawning took place at 3 p.m. when water temperature in the vicinity of the eggs was 22·5°C. Temperature alone rather than flooding at a specific temperature would therefore seem to be the main factor stimulating spawning in this species. Adult fish of this species were from 3 to 5 cm in length and the ovaries contained from 1000 to 2000 ova depending on the size of the female. Observations suggested that spawning was spread out over several days and even weeks. When spawning ceased the male fish assumed a vertical position, with the head either up or down, and forced a flow of water over the eggs. While caring for the eggs, the males became quite aggressive and chased away other small fish and shrimps (*Macrobrachium* sp.) some of which were larger than *C. klunzingeri*. This species has been known to spawn in aquaria.

(*f*) *Perca fluviatilis*

Although some information was available on the spawning of this species in the northern hemisphere, and apparently has been for a considerable time (Jenkins 1961), no detailed accounts of the reproduction of this species have been published from studies in Australia.

Perca fluviatilis spawned in ponds at Narrandera when water temperatures reached 11·5°C in early spring. This temperature is fairly consistently reached during the last week in August in that locality. However, it was noted that some specimens in the same pond did not spawn until additional water was added to the pond. If a flood pond was used all fish spawned on inundation of the flood plain provided water temperature reached 11·5°C. If water was not added to a pond then even those fish which did spawn were several days to a week later in spawning than those in a pond which had water added. This effect could be due to some stimulus which was provided by adding water, since it was found to be essential with several endemic fish species. However, it could also be explained by assuming that the adult fish in a deep pond were at the bottom in colder water.

Spawning occurred at night in each of the eight ponds used. In 1961 when the ponds were devoid of aquatic plants and the banks were not grassed, eggs were laid on bundles of twigs. In subsequent years eggs were laid at depths of 120–180 cm on rooted aquatic plants on the bottom of the pond. In flood ponds there were no aquatic plants and eggs were laid on the grassed edges of the flood plain at shallow depths of 10–50 cm.

Although the spawning and survival of native fish species was controlled largely by environmental factors, particularly rises in water level, it would appear that sexual activity in *P. fluviatilis* was influenced more by fixed annual factors, particularly temperature. Photoperiodism may also be significant with this species. However, the increased space and food provided by floods must also increase the survival of *P. fluviatilis* to a considerable extent.

IV. Discussion

The reproduction of these fishes, which inhabit the extensive Murray–Darling river system, show some interesting adaptations to this extreme environment where conditions fluctuate on a very large scale. Extensive flooding, with the subsequent plankton blooms and other food development, accompanied by the huge increase in the area of fish habitat, has resulted in the best survival of fish at these times. The fish have become largely dependent on these floods for the initiation and induction of spawning when water temperatures are rising and relatively high.

Plectroplites ambiguus and *Bidyanus bidyanus* have high fecundity and fast-hatching pelagic eggs which they have retained from their marine precursors. *Tandanus tandanus* is more typical of an inland fish with its demersal eggs placed in a gravel nest. The same can be said of *Maccullochella macquariensis.*

One significant result was the dependence of *M. macquariensis* on small fish and shrimps following the very short plankton-feeding stage. The wide distribution and abundance of *C. klunzingeri* together with its time of spawning, make this species very important and a contributing factor which could control cod abundance. This bionomic relationship between these two species is supported by the fact that *C. klunzingeri* always spawns after *M. macquariensis* because of the higher temperature requirement for the spawning of the former species. Unfortunately, this relationship cannot as yet be supported from observations in natural waters because no one has been able to collect *M. macquariensis* of the required size in spite of many concentrated efforts to do so. The habit of *C. klunzingeri* of spawning in very shallow water suggested the deleterious effects of any sudden reductions in water level, now quite common due to irrigation demands.

Although it is now certain that floods, or even freshets, in the river, at specific temperatures, induce the final maturation of gonads and subsequent spawning, no satisfactory answer has been given as to what specific factor is "detected" by the fish. Swingle (1953) postulated a repressive factor, secreted or excreted by the fish, which prevented spawning and that dilution by rising water levels was followed by spawning. However, several species of fish spawned at Narrandera by adding water from a river in drought condition. The river contained these same fish species. It would appear to be more acceptable to postulate that the fish is stimulated by some factor resulting from the inundation of dry ground or from water entering a pond or river after flowing over dry ground. If water was added to a pond and allowed to flow out again so that the level did not rise, spawning did not occur even when temperatures were suitable. Yet filling a pond and then adding the fish after the flow had stopped did induce spawning. Ample evidence is available to show that fish have remarkable powers of odour perception and it is pertinent to note that particularly in hot arid regions a characteristic odour is associated with rain falling on dry soil. An oil (petrichor) which possesses the odour in highly concentrated form has been obtained by steam distillation of most silicate minerals and rocks, the yield being proportional to the exposed surface area, increasing with the time of exposure, and being about 30 mg/m^2 (Bear and Thomas 1964). This oil has been shown to be a complex mixture of organic compounds containing basic, neutral, and acidic fractions (Bear and Thomas 1966). The neutral oil, which is the largest fraction and has the dominant petrichor odour, contains aliphatic hydrocarbons, aldehydes, ketones, esters, lactones, and probably alcohols. Bear and Thomas (1965) and Bear and Kranz (1965) have given other results of analyses, particularly of the acid fraction and the effect of this oil on seed germination and plant growth. They have also mentioned that there is evidence that drought-stricken cattle respond in a restless manner to this "smell of rain" which may drift with the wind for considerable distances.

Observations of fish gonads, particularly those of *P. ambiguus*, show that during years when floods and high rivers are common throughout the winter and early spring the gonads develop earlier and more uniformly in all fish whereas during dry winters when rivers are low gonads are far less developed by early spring. However, fish kept in ponds where water levels were raised regularly throughout the winter, to replace losses by evaporation and leakage, gonad development was similar to that observed in the rivers subjected to flooding. All the evidence, therefore, suggests that the fish are stimulated by some factor resulting from the inundation of dry ground; it is known that there is an oil released when this happens. It is also known that constituents in the oil affect plant growth, that some animals can readily detect the odour of petrichor which indicates to them the presence of water, and fish are known to have remarkable senses of odour perception. Further work is contemplated to test this hypothesis. There is certainly little, if anything, in Swingle's results which contradicts this possibility.

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