Native Fish Australia Technical Report #1

METHODS FOR THE HATCHERY PRODUCTION OF THE FRESHWATER JEWFISH OR EEL TAILED CATFISH

Tandanus tandanus

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Cover Photo: Adult catfish used in fingerling production.
(Photo courtesy of Bruce Sambell, Ausyfish Pty. Ltd., Childers, Queensland)

Preface

Native Fish Australia is a non-profit organisation founded in 1981 and dedicated to the conservation of Australian native freshwater fish. It has state branches in New South Wales, South Australia and Victoria with a number of regional branches in those states. Objectives of the organisation include:

(1) The promotion of the scientific study, conservation, propagation and management of native fish species, their environment and ecology;

(2) The dissemination of information and knowledge of scientific work on native fish and related matters to members, governments, scientific bodies and the general public;

(3) The participation and assistance in the collection, preparation and analysis of any information relevant to native fish;

(4) The encouragement of members to instigate and participate in, where appropriate, programs or scientific projects related to native fish.

The Victorian branch is the largest and most active and one of its activities is the operation of a native fish hatchery using facilities provided by Latrobe University. At this facility members have successfully undertaken the artificial propagation of trout cod, Macquarie perch and Australian bass to assist government departments in the conservation of these species and to provide recreational angling.

Native Fish Australia (Victoria) has published this report in order to make more widely available information on work completed to date on the artificial propagation of a native fish of conservation significance, the freshwater catfish. The author reviews the historic development of methods for producing catfish by hatcheries up to the present time. Although not actively involved in the development of the procedures discussed in the report, Native Fish Australia acknowledges its value and the importance of making the author's work freely available. The opinions and views expressed in this document are those of the author and not necessarily those of Native Fish Australia.

Native Fish Australia (Victoria) Incorporated

September 2006

Introduction

The freshwater catfish or jewfish, *Tandanus tandanus* (Mitchell 1838, Plotosidae) is a warm water fish found in the Murray-Darling drainage system and rivers of the east coast of Australia from about Sydney to Cooktown. Recently it has been suggested that some of the populations of catfish found in east coast drainages may be undescribed new species or subspecies and this is an area of active research (Clunie and Koehn, 2001).

Concern has been expressed for the conservation of the catfish, which has undergone a decline in its range and abundance, at least in the Murray-Darling basin (Clunie and Koehn, 2001). This decline has been occurring since at least the early twentieth century with catfish becoming extinct in the Murray River upstream of Lake Hume around 1935 (Bill Hollis, personal communication 2006). The catfish is considered endangered in Victoria and was listed under the *Flora and Fauna Guarantee Act 1988* in 2003, as an endangered taxon.

The catfish exhibits a high potential for aquaculture, being hardy, having excellent table qualities and accepting prepared diets (Smith 1993). As a consequence, there is interest in the development of methods for the mass production of catfish fingerlings. Export of catfish larvae to Asian fish farmers is believed to have taken place from Queensland.

This report reviews methods that have been utilised for the production of juvenile catfish and presents previously unpublished data and protocols. It should not be viewed as an exhaustive study of methods for the production of catfish fingerlings. Rather, the intention of this report is to review the work done to date, making the information freely available as a stimulus for further work. It is hoped that ultimately the information contained within will provide the genesis for further development of techniques for the hatchery production of the catfish to aid in recovery efforts.

History

Lake (1967) described the factors associated with the natural spawning of catfish in ponds at the Inland Fisheries Research Station near Narrandera NSW. He reported that spawning took place when water temperatures exceeded 24ºC. Davis (1977 a, b) described the ovarian and testicular development of catfish in the Gwydir River NSW and inferred that spawning took place from October to early January when water temperatures exceeded 24°C. Merrick & Midgley (1981) described the spawning behaviour of catfish in a coastal stream in south east Queensland and reported that particular spawning event to have taken place at a temperature of 20°C. The author has observed nest construction and found nests containing eggs in both natural streams and farm dams in Queensland when temperatures have ranged from 20 to 25°C. A number of female catfish close to ovulation have been captured from the Billabong Creek NSW by the author at a temperature of 22°C.

Since the publication of Lake's work, catfish fingerlings have been produced as a result of the uncontrolled reproduction of spawners in ponds by a number of commercial fish hatcheries. Production of fingerlings using this method has proved to be a limited source, probably due to poor recruitment of larvae. The method is unreliable and total production from individual hatcheries utilising this approach has generally been in the order of hundreds to perhaps tens of thousands. Natural reproduction also precludes the management of genetics for conservation purposes, development of improved genetic lines, and extension of reproduction outside the natural breeding season for aquaculture.

With an interest in the culture of catfish, work was undertaken in the 1980s to induce ovulation of females and to produce high quality eggs and larvae. Initial trials were conducted at a commercial hatchery in Victoria during January 1980. This work was based largely on the methodology used by Rowland (1983) for spawning induction of golden perch. Both carp pituitary extract (CPE) and human chorionic gonadotrophin (HCG) had been used successfully by Rowland to induce ovulation and spawning in a number of species of Murray-Darling fish.

These early trials involved a small number of females, which provided a guide for the development of methods for hormone-induced ovulation of female catfish. Subsequent to the early Victorian work, production of catfish fingerlings has taken place in south east Queensland at a number of commercial hatcheries from the mid 1980s to the present. A commercial hatchery near Wartook, Victoria, commenced mass production of catfish fingerlings in the mid 1990s for farm dam stocking and similarly produced large numbers of fish.

In both Queensland and Victoria, production of catfish fingerlings occurs on an 'as required' basis. In individual years many tens of thousands of juveniles have been produced which usually has met market demands for a number of seasons. It is understood that one hatchery on at least one occasion has hatched in the order of 100 000 catfish eggs. Subsequently, little production may take place for a number of years until the market recovers. The existing technology provides these hatcheries with the potential to reliably produce hundreds of thousands of fingerlings per annum if there is the market demand and if sufficient broodstock are available.

Artificial Reproduction

Methodologies for Spawning Induction

Endocrine intervention is used to induce final oocyte maturation, ovulation and spawning in those species of fish that will not reproduce spontaneously in captivity, or fail to respond to environmental manipulation to induce spawning (Zohar 1989, Crim 1991). It is also used to provide control over the timing of reproduction and to permit genetic selection and management (Lam 1982). In most cases, females are the focus of these techniques as males generally undergo development and extended spermiation in captivity though there are exceptions (Zohar 1989).

Various options for endocrine intervention exist at different physiological 'command' levels. The choice of which level in these pathways intervention takes place is largely determined by where in the reproductive cycle disruptions occur due to the impacts of captivity. Such disruptions may range from a simple inhibition of spawning to complete lack of gonadal development (Shelton 1989, Crim 1991).

The oldest form of endocrine intervention is the technique of hypophysation, its use dating back to the 1930's in South America (Shehadeh 1975, Lam 1982). Hypophysation in its strictest sense is the injection of pituitary material into fish to induce final oocyte development and/or spawning. The rationale behind hypophysation is to mimic the transient surge of maturational gonadotropin, GtH II, that initiates the onset of final oocyte maturation, ovulation and spawning (Peter 1981, Lam 1982).

While hypophysation was of enormous benefit to aquaculture it suffers from a number of limitations. Crude pituitary extracts vary in their content of gonadotropin(s) depending on the state of maturity of the donor fish and contain a range of other hormones which may have synergistic or antagonistic effects on reproductive processes. It is therefore difficult to quantify the gonadotropin content of such preparations and standardise dosage (Shehadeh 1975, Zohar 1989). There is also the potential for transferring disease to recipient fish through the use of such material of direct biological origin.

Mammalian gonadotropins of placental origin have been widely used as substitutes for pituitary extracts since the 1960s for the controlled reproduction of finfish. The most widely used is human chorionic gonadotropin (HCG). Being relatively pure, sterile preparations of known potency, they are admirably suited for induced spawning of finfish. Unfortunately due to problems of species specificity their use is not universally successful in fish (Zohar 1989, Shelton 1989) and in some cases use of HCG has lead to death through ovarian swelling (Forteath 1993). Being high molecular weight glycoproteins they have considerable immunogenic potential and there have been reports of finfish becoming refractory to HCG with repeated usage (Lam 1982). Some Australian native fish treated with HCG have the following season failed to show significant ovarian development and can be in poor physical condition or over a succession of seasons have proved less responsive to HCG treatment (W. Trueman, personal observations).

In the 1980s increasing use was made of hypothalamic gonadotropin releasing factors (GnRH) which regulate pituitary production of gonadotropins. They are easily synthesised permitting use of pure preparations of known potency and have the advantage of stimulating the secretion of endogenous gonadotropin (Zohar 1989, Yarron 1995). Being low molecular weight decapeptides they have low immunogenic potential and very limited opportunity for species specificity. Modification of GnRH molecules by substitution of amino acids has resulted in the production of molecules that in vivo are degraded far slower than native

forms. The development of these super-active analogues has permitted the use of doses measured in micrograms (Peter et al 1993).

In some cases GnRH has failed to produce surges of GtH II or very high doses have been required. In some (but not all) teleosts the gonadotrophs are innervated by nerve fibres producing the neurotransmitter dopamine which inhibits GtH II production by the gonadotrophs (Peter et al 1986). Treatment of fish with dopamine antagonists such as pimozide and domperidone negates the inhibition on GtH II secretion by these dopaminergic neurones. A combination therapy involving treatment with GnRH analogues and dopamine antagonists, termed the 'linpe technique' has been found to be a successful technique for inducing final oocyte maturation and ovulation in fish that do not respond well to GnRH alone (Peter et al 1988, Yarron 1995). A combined commercial preparation under the trade name of '*Ovaprim*' utilises such a combination in a propylene glycol medium to provide extended release of analogue and antagonist (Peter et al 1993) and is widely used overseas for the induced spawning of some fish species.

The successful use of endocrine intervention to induce ovulation is largely dependant upon the stage of maturity of the female. The timing of endocrine intervention with pituitary extracts, HCG or GnRH is critical in many species. Applied too early in the maturational cycle the surge of GtH II will fail to induce final oocyte maturation and ovulation due to the oocytes lacking maturational competence (Lam 1982). In general fish whose ovaries contain oocytes which have completed the process of vitellogenesis are receptive to induced ovulation/spawning. This is indicated by the nucleus or germinal vesicle having commenced /completed its migration to the periphery of the oocyte. Such oocytes have developed the receptors and pathways responsive to surges of GtH II and traditional endocrine intervention.

Generally, detailed histological observation of oocyte maturity is not used for identification of females likely to be receptive to spawning induction as it is time consuming and may increase handling stress upon the fish. Rather, it has usually been found more convenient to identify a convenient 'yardstick' for assessing maturity and through trial and error relate this 'yardstick' to the state of maturity of female fish and spawning success. Criteria utilised for assessing the maturity of female fish have included the degree of distension of the abdomen, the appearance of the vent, and more reliably the size and gross appearance of the oocytes. For example Rowland (1983) classified the oocytes of the golden perch into a series of stages based on the transparency of the oocytes and the size and position of oil and yolk droplets. However some fish contain oocytes which are largely opaque and little internal structure can be discerned under the light microscope.

Davis (1977a, b) provided a detailed description of the gross and histological appearance of catfish oocytes through the annual reproductive cycle. He classified the appearance of the oocytes into nine stages utilised by Japanese workers. Although not describing the migration and breakdown of the germinal vesicle in catfish oocytes he attributed the Japanese workers as stating that the migration of the nucleus takes place at the completion of the tertiary yolk globule stage and prior to the mature yolk stage. He described oocytes of the catfish in the former stage as having diameters up to 2.2 mm while those of the latter were up to 2.8 mm in diameter. From this information it was postulated that female catfish containing oocytes in the range 2.2 – 2.8 mm may be responsive to traditional methods of hormone-induced ovulation and spawning.

Early Work

In the early Victorian trials broodstock were obtained by angling from the Billabong creek near Jerilderie N.S.W. nine months prior to the trials and were held in earthen ponds. They were removed from the ponds in early January when bottom temperatures had reached 24°C.

Broodstock were anaesthetised with quinaldine and sexed according to the shape of the urino-genital papilla which is cylindrical in males and pyramidal in females (Lake 1967). Male fish were darker than female fish at this time. Males expressed seminal fluid only when considerable abdominal pressure was applied and in general the volume was very low, sometimes a few individual drops. From some fish the fluid appeared to be a slightly grey colour and examination revealed the presence of spermatozoa. In other males the fluid was clear but examination in these cases also revealed the presence of active spermatozoa. In male catfish the spermatozoa appeared to be present in much lower densities in the seminal fluid relative to other Murray-Darling fish species.

A sample of the oocytes was obtained from females by inserting the broad end of a 4 mm diameter disposable plastic serological pipette into the oviduct. Smaller females (minimum weight = 350 g) had the membrane of the papilla stretched with the tapered end of the pipette followed by sampling with the broad end. Oocytes were examined microscopically, their diameters measured and general appearance recorded. The observation of Davis (1977b) that larger females produced larger ova was confirmed. During the breeding season most females had distended abdomens and the ovaries contained oocytes that were orange, packed with numerous tiny droplets, almost totally opaque and ranged from 2.0-2.8 mm. Small oocytes under 1.0 mm and a few large semi-translucent oocytes over 2.8 mm were present in most samples (Fig. 1).

Some females had ovaries containing oocytes the majority of which were large (about 2.5 – 3.2 mm), semi-opaque and which appeared to contain numerous very fine yolk droplets. In some fish these oocytes were uniform in appearance (Figure 2) while in others also present were large, flaccid oocytes and shells which were presumed to be produced by the atresia of oocytes (Figure 3).

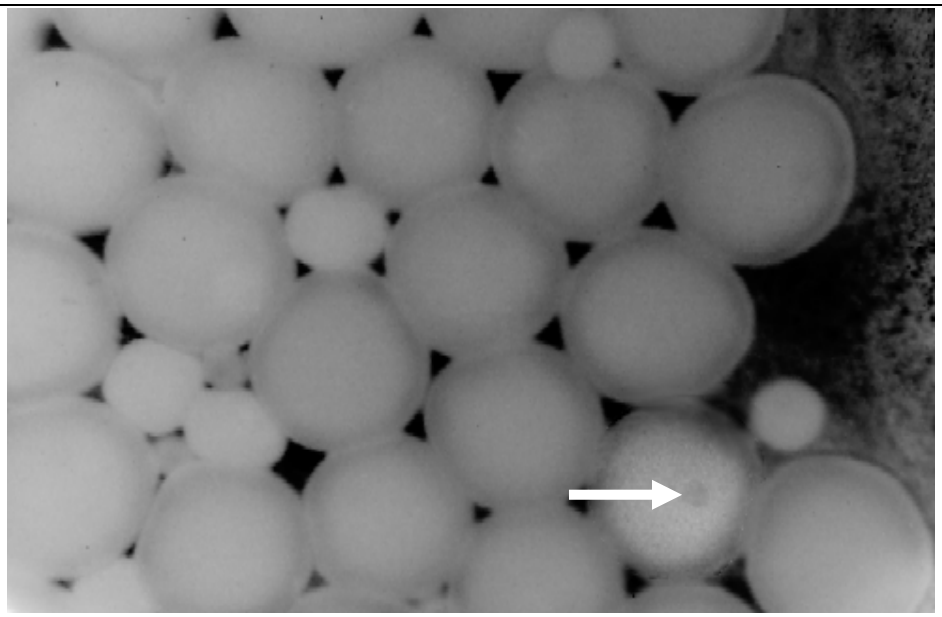

Figure 1.

Typical appearance of oocytes from most female catfish sampled during the spawning season. The majority of the oocytes are large and opaque and also present are a few smaller oocytes. They match the description of Davis (1977a) for completion of the tertiary yolk globule stage to an early mature yolk stage. At one pole of the larger oocytes the germinal vesicle is sometimes just discernible (arrowed).

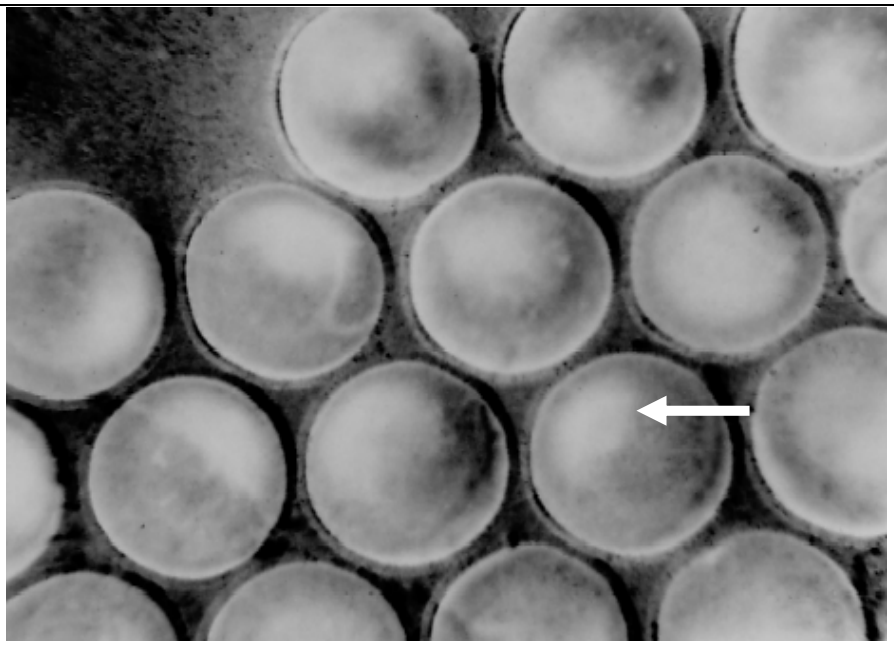

Figure 2.

Oocyte sample from a female catfish at an advanced stage of maturity. The majority of the oocytes are large and semi translucent. They match the description of Davis (1977a) for completion of the mature yolk stage with progression towards the ripe egg stage. The germinal vesicle appears to be undergoing breakdown, creating a discoloured area at one pole (arrowed).

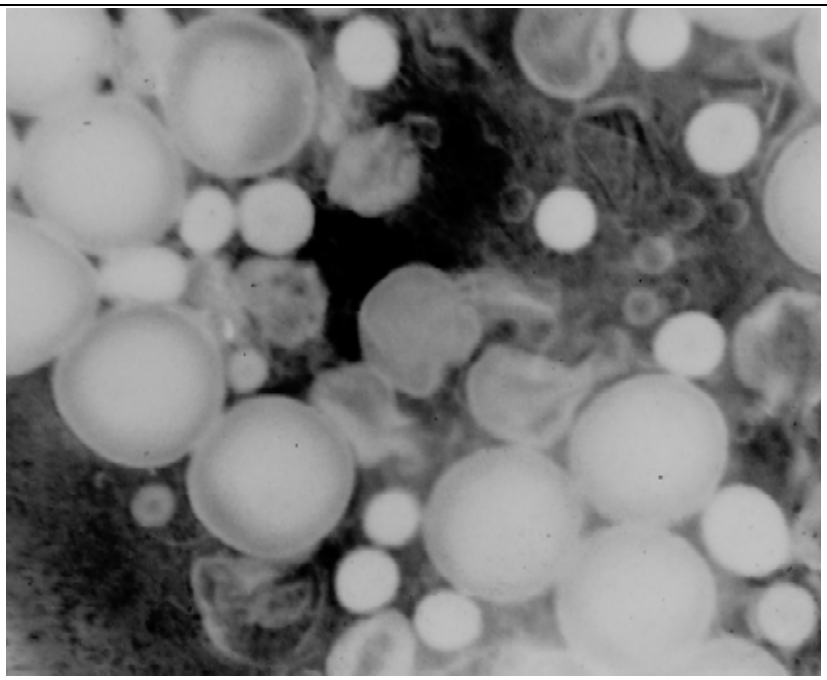

Figure 3.

Oocyte sample from a female catfish containing oocytes at a variety of stages. Visible are the shells of oocytes which have become atretic and are undergoing resorption.

Initial trials assumed that germinal vesicle migration occurred in catfish oocytes ranging from 2.2 to 2.8 mm in diameter (Davis 1977a) and that females containing such oocytes would be suitable for hormone induced ovulation/spawning. Females were selected on the basis of the majority of their oocytes being within or exceeding this size range. Female catfish (weight range 350 - 700 g) were weighed and injected via the intra-peritoneal route at the base of a ventral fin with either CPE at a dose rate of 5 mg/kg or HCG (*Chorulon*, Intervet) at a rate of 1000 i.u./kg. The male fish routinely received a single dose of CPE at 5 mg/kg to promote spermiation.

The CPE was prepared by collecting pituitary glands from wild carp at various times of the year, excluding the spawning season of September-October. The glands were desiccated in acetone and stored in this fluid in a refrigerator for several months until required. Prior to use the glands were removed from the acetone, allowed to dry in the air for 10-15 minutes, weighed and then ground in a mortar and pestle. It was found as a rough rule of thumb that the weight of the individual pituitary glands in mg approximated the body weight of the donor fish in pounds, though in very large fish the glands were much heavier. Prior to use the ground glands were mixed with iso-osmotic saline or distilled water and the supernatant collected in a hypodermic with care taken to avoid blockage with large particles.

After injection the fish were held in100 L aquaria at a temperature of 25°C. Twenty four hours after being injected the females were anaesthetised, the oocytes re-sampled and most received additional hormone treatment. Females were subsequently re-examined 40 hr after the first injection and thereafter at intervals as required dependant upon the appearance of the oocytes.

Females containing opaque oocytes injected with CPE showed no obvious change in the appearance of the oocytes after 24 hours and were reinjected. Females responding to CPE

treatment exhibited oocyte clearing about 40 hr after the first injection and shortly before ovulation the chorion became visible as did an area of disrupted cytoplasmic yolk droplets probably caused by previous germinal vesicle migration. Fish that had completely ovulated could be identified by the stream of ova appearing at the papilla under abdominal pressure. In most trials ovulation occurred from 48 to 60 hr after the first injection. Ova were clear, amber to green in colour, sometimes irregular in shape and often exhibited a considerable degree of oblateness. The thick chorion was clearly visible (Figure 4).

Figure 4.

Recently ovulated catfish ovum. Note path of disrupted yolk droplets from the centre to the animal pole probably created by the migration of the germinal vesicle. The thick chorion is clearly visible as is the space above the yolk mass to accommodate the eventual blastodisc.

Male fish darkened noticeably in colour after injection with CPE. Despite the treatment of male fish with CPE very little seminal fluid could be expressed from them. Considerable abdominal force was required to express any fluid and it was difficult to distinguish between urine and seminal fluid. However all batches of eggs into which this small quantity of fluid was mixed subsequently underwent division and ultimately hatching suggesting that at the very least spermatozoa were present in reasonable quantities. This observation did suggest that male fish could be a limiting factor in mass production of juvenile catfish.

After ovulation the ova were stripped into an enamel bowl and fertilised by the dry method, the contents being swirled for a period of a few minutes before water was added to flush away ovarian tissue. After fertilisation and the addition of fresh water the eggs became slightly adhesive sticking to the bowl and each other but could be readily separated through the swirling action of water in moderate motion. The eggs were not as adhesive as those of Murray cod, *Maccullochella peelii peelii*, but exhibited a degree of adhesiveness similar to recently fertilised eggs of Macquarie perch (*Macquaria australasica*). The adhesive properties disappeared within half an hour presumably upon water hardening. This

characteristic of catfish eggs has not been previously reported and may facilitate the eggs initially embedding in the interstices of nest material.

About thirty minutes post fertilisation the first blastomere appeared on the surface of the yolk mass adjacent to the region of disrupted droplets (Figure 5). Twenty to thirty minutes later the first division took place (Figure 6) leading to the formation of a blastodisc within 24 hours. Gastrulation occurred 48 hours post fertilisation and it was around this time that mortality occurred in some eggs showing developmental abnormalities leading to death and the eggs subsequently turning white.

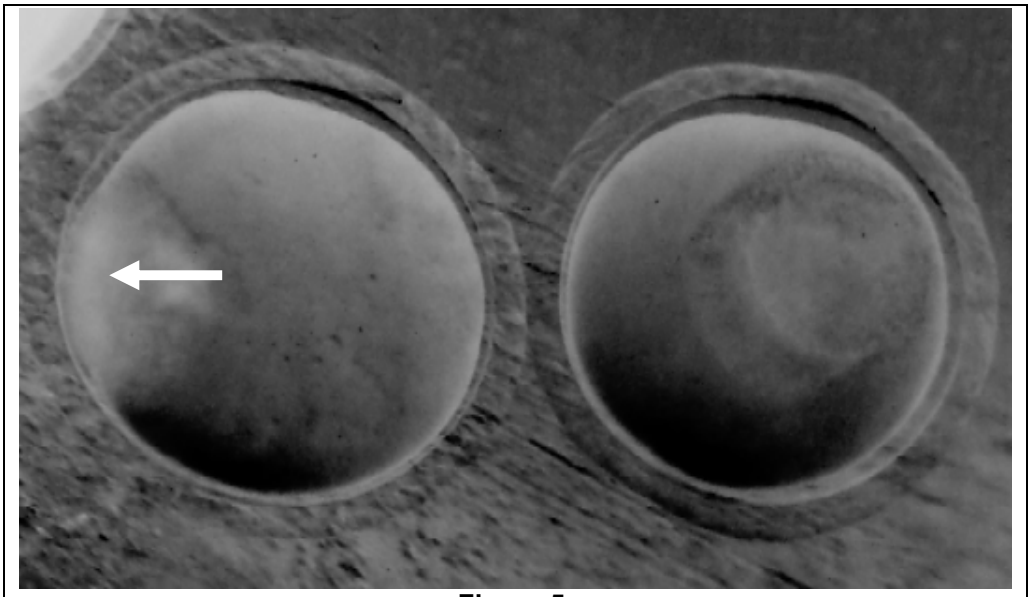

Figure 5.

Catfish eggs about forty minutes post fertilisation showing the first blastomere (arrowed).

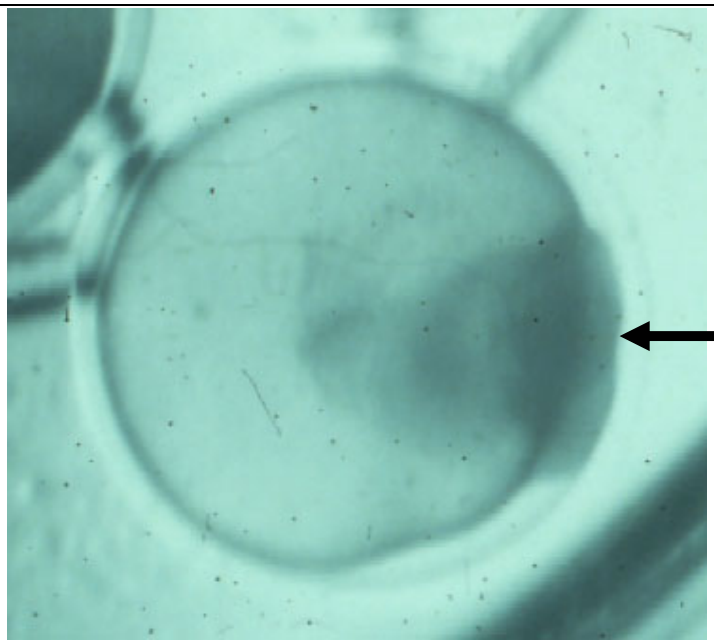

Figure 6.

Catfish egg about ninety minutes post fertilisation showing the first cleavage (arrowed).

A female catfish containing opaque oocytes injected with HCG on consecutive days showed no obvious changes in the appearance of the oocytes after 72 hours. This fish was then treated with two injections 24 hours apart of CPE at 5 mg/Kg and subsequently ovulated. Another female containing semitranslucent oocytes that was treated with HCG was close to ovulation after 24 hours and subsequently ovulated at 27 hours post injection.

Fertilised eggs were placed onto screens suspended in aquaria. To retard the growth of *Saprolegnia* on dead eggs 24 hr post-fertilisation the screens were dipped in a malachite green bath (1 mg/l) for 1 minute and thereafter daily until hatching commenced. In general these early trials produced eggs of high quality with hatch rates being >70%. Approximately 4,000 ova were stripped per kg body weight of the female.

After hatching catfish larvae were kept in aquaria containing under gravel filters and once yolk sac adsorption was completed were fed initially on collected copepods and cladocerans for approximately four weeks. Some were then on-grown in outdoor plankton ponds and harvested after six weeks and at a length of about 5 cm.

The results of this early work suggested that female catfish were not responsive to treatment with HCG unless their ovaries contained semi-translucent oocytes. However females readily responded to CPE even if the oocytes were large and opaque.

Later Trials

The results and protocols developed in the initial trials were subsequently utilised by a number of commercial hatcheries for the production of catfish fingerlings in south east Queensland and Victoria. In Queensland induced ovulation took place from late September to early November when maximum water temperatures were in the range 20-26°C which corresponded to the spawning season of wild fish. In Queensland broodstock were held in earthen ponds or sourced from streams, irrigation channels, farm dams and large

impoundments. In Victoria the breeding season of pond held fish has extended from late October to January. From this work additional data was collected.

Hormones used included CPE (range 1-10 mg/kg) collected from wild carp or a commercial preparation (Argent Chemical Laboratories), or HCG (*Chorulon*, Intervet) (range 1,000-5,000 i.u./kg). It is understood that on a number of occasions pituitary material from other species including eel tailed catfish and 'freshwater mullet' (species not identified) have been utilised to induce ovulation of female catfish. Fish were anaesthetised with quinaldine, benzocaine or clove oil. Only females containing oocytes of largely uniform appearance were utilised in trials for collecting data. Male fish routinely received a single dose of either CPE at 5 mg/kg or HCG at 2000 i.u./kg to promote spermiation. Fish were held in either 100 litre aquaria or 2000 litre circular fibreglass tanks at temperatures from 22 – 26°C.

When ovulation occurred the latency period, (the time interval from the first injection to ovulation) was recorded. Fertilised eggs were placed into perforated trays supplied with a flow through of filtered water at a rate of 20 l/min. To retard the growth of *Saprolegnia* fungus on dead eggs, commencing 24 hr post-fertilisation the eggs were treated in a formalin bath (1 ml/l) for 15 min and thereafter daily until hatching commenced. Samples of eggs in batches of approximately 100 were used for determining hatching rate 72 hr post-fertilisation.

When large numbers of fish were available it was found convenient to sacrifice a fertile male and express milt from the testis in order to fertilise ova collected from females. Sufficient milt could be obtained in this way to fertilise the ova of up to four females. Whole testis were in some cases stored dry in sealed plastic containers in a refrigerator for up to six hours without any apparent loss of sperm motility.

Results with CPE

The results of the trials using CPE treatment are presented in Table 1. All females containing large opaque oocytes that received two equal injections of CPE at rates greater than 5 mg/kg ovulated and generally produced ova of high viability. Lower doses sometimes resulted in ovulation but fertilised eggs showed little development. In these cases the ova appeared to be partially covered in a white, semi opaque membrane, possibly part of the follicle wall. Females receiving single treatments of CPE at 7 mg/kg showed no gross change in oocyte appearance and none had ovulated 72 hr after injection whereas all females receiving two injections at 5 mg/kg or higher ovulated within 57 hr. Control fish treated with distilled water failed to ovulate and showed no changes in oocyte appearance when examine 72 hr after treatment.

Table 1. Response of female catfish containing opaque oocytes to CPE treatment. Treatments administered 24 hr apart.

Dose (mg/kg)	**# of Fish**	**# Ovulated**	**Mean Latency hr** (range)	**% Hatch** mean (range)
Control (distilled water)	3	0	-	-
7/0	3	0	-	-
1/1	2	1	60.6	0
2/2	2	1	50.0	0
4/4	3	2	52.9 (48.8-57.0)	21.0 (0 - 42)
5/5	1	1	55.4	71.0
6/6	3	3	51.3 (49.0-55.6)	39.3 (0 - 76)
7/7	3	3	50.0 (48.1-52.8)	79.6 (72 - 86)
10/10	3	3	50.9 (48.9-52.1)	77.6 (69 - 91)

Results with HCG

In general, treatment of females containing large opaque oocytes with HCG either failed to induce ovulation or where ovulation occurred the ova were semi-opaque and subsequently failed to undergo embryonic development (Table 2). However three females containing large (>2.8 mm), semi-opaque oocytes prior to injection treated with HCG ovulated and produced viable ova within 27 hr of injection (Table 3). While in general HCG alone proved unsuitable for inducing ovulation in female catfish containing opaque oocytes it was found that HCG could be substituted successfully for the second injection of CPE at 2000 i.u./Kg in fish receiving an initial treatment of CPE (Table 4).

Table 2. Response of female catfish containing opaque oocytes to HCG treatment.

Treatments administered 24 hr apart

Dose (i.u./Kg)	**# of Fish**	**# Ovulated**	**Mean Latency hr** (range)	**% Hatch** mean (range)
Control (distilled water)	1	0	-	-
1000/1000	1	0	-	-
1000/1000/1000	1	0	-	-
2000/2000/2000	1	0	-	-
5000/5000/5000	1	1	122	0

Table 3. Response of female catfish containing semi-translucent oocytes to HCG treatment.

Treatments administered 24 hr apart

Dose (i.u./Kg)	**# of Fish**	**# Ovulated**	**Mean Latency hr** (range)	**% Hatch** mean (range)
Control (distilled water)	1	0	-	-
2500	3	3	13.6 (7.1-26.5)	88.0 (76 - 94)

Table 4. Response of female catfish containing opaque oocytes to CPE followed by HCG treatment.

Treatments administered 24 hr apart

Dose **CPE** (mg/Kg) / **HCG** (i.u./Kg)	**# of Fish**	**# Ovulated**	**Mean Latency hr** (range)	**% Hatch** mean (range)
Control (distilled water)	1	0	-	-
5/1000/1000	1	1	71.9	71
7/2000	4	4	49.7 (49.1-50.0)	79.0 (72 - 89)

Attempts at Natural Spawning

Despite the fact that on many occasions injected pairs of catfish were held together, no natural spawning or signs of courtship behaviour have been observed by the author. In some cases nest building material in the form of pebbles and sticks was provided in large tanks containing pairs of injected catfish but this failed to induce courtship behaviour or spawning. In instances when multiple pairs were held together male catfish proved aggressive towards each other with fighting and biting occurring.

It is known that on one occasion courtship and spawning has taken place after treatment with CPE without any nesting material being provided (Bruce McInnes, Wartook Native Fish Culture, personal communication). Apart from this singular occurrence it has proved necessary to strip the fish by hand and artificially fertilise the ova. Wether the lack of spawning is the result of a lack of synchronisation of endocrine and behavioural cues or the result of an inappropriate environment is purely speculative.

Viability of Ova

Rowland (1988) reported a narrow window of viability for Murray cod ova produced by hormone treatment. Although detailed trials were not conducted it appears that ovulated catfish ova remain viable inside the ovary for many hours post ovulation. During routine production broodstock were sometimes collected by netting at night and received hormone injections at around 7 – 10 p.m. In these circumstances it was found convenient from the perspective of hatchery staff to examine females and strip eggs at around 8 a.m., rather than at the anticipated time of ovulation some hours earlier. It was found that the ova flowed very freely at this time and the delay had no apparent effect on viability. At this point the period for which ovulated ova remain viable remains unknown but appears to be at least 3-4 hours post ovulation.

Batch/Multiple Spawning

The ovaries of the majority of female catfish examined during the spawning season contained large opaque oocytes of relatively uniform appearance. A few smaller oocytes were often present (Figure 1). Females examined that were in poor general condition or captured late in the season often contained oocytes which varied greatly in size and appearance. Oocyte samples obtained from these fish usually contained some atretic oocytes and shells. In general, larger fish had more advanced oocytes and matured earlier than smaller fish.

However a proportion of females contained oocytes that were not uniform but were in distinctive size classes. On at least one occasion one such female which was induced to ovulate in early October was subsequently induced again in late January. This demonstrates that at least some female catfish can behave as batch spawners and perhaps take part in multiple spawning events under captive conditions. Whether this occurrence was a product of the captive environment or is a natural phenomenon that takes place in the wild remains unknown. But it does suggest the potential for female catfish to act as batch spawners under natural conditions.

Fingerling Production

Figure 7.

Catfish fingerlings produced through hormone-induced ovulation at harvest and ready for dispatch to customers.
(Photo courtesy of Bruce Sambell, Ausyfish Pty. Ltd., Childers, Queensland)

In general hatcheries producing catfish fingerlings have released larvae into plankton ponds towards the end of yolk sac absorption about 7-10 days post hatching. A stocking rate of around 50-80 000 per hectare has been generally adopted. Protocols used for the management of the ponds are similar to those described by Rowland et al (1983) for production of other species of native fish. Provided good water quality is maintained recovery rates have been high and few problems encountered. The fry grow rapidly from a length of release of about 1 cm to 4-7 cm in six to eight weeks.

The only significant problem peculiar to catfish encountered to date has been severe injuries caused through fingerlings spiking each other when confined in situations creating high densities, such as in seine nets or scoops. It has been found necessary to modify harvest techniques to reduce the risk of these injuries to the fingerlings. Staff have to exercise care in handling both fingerlings and broodstock, both of which can inflict painful wounds.

At times, larvae have been reared in aquaria and tanks on supplied plankton harvested from ponds. This has proved useful when plankton densities have been relatively low in ponds allowing maintenance of fry until food sources have built up. At high fish densities outbreaks of *Trichodina* have occurred. These have been successfully treated using a long term bath of malachite green added to aquaria at 0.08 mg/l.

Recent Developments

Use of Clearing Solutions

A common problem encountered with endocrine intervention to induce ovulation and spawning in fish is accurately assessing the state of maturity of the female. Ideally spawning induction should take place when the germinal vesicle has commenced or preferably completed its migration from the centre of the oocyte to a peripheral position at the animal pole of the yolk. It is at this time that the oocyte develops maturational competence incorporating the development of receptors permitting the induction of final spawning and the necessary physiological pathways supporting this process.

In the case of the catfish, selecting females with ovaries containing opaque oocytes exceeding 2.2 mm in diameter and of uniform appearance has been a successful protocol. Females containing oocytes which have commenced to clear have usually been suitable for spawning though in some cases the clearing is associated with the commencement of the resorption of the oocytes. Hormonal treatment in the case of the latter has resulted in an acceleration of the atretic process. In the case of clearing oocytes, it has proved difficult to judge the precise time of ovulation after hormone treatment, necessitating regular handling of the female and the associated stress.

The use of a more accurate process to assess oocyte maturity, particularly with respect to the germinal vesicle, would overcome some of these problems. Overseas workers investigating the reproductive physiology of fish and controlled reproduction for aquaculture have reported the use of the serra clearing solution to facilitate their investigations (Goetz & Bergman 1978; Kestemont 1991; Dasgupta et al 2001). This solution contains ethanol/formalin/acetic acid in the ratio of 6:3:1 and acts to dissolve/bleach fine yolk particles present in the follicle and oocyte, making the latter appear translucent. It is particularly useful for discerning internal structure in fish whose oocytes are opaque, such as the catfish, which do not lend themselves to the use of other protocols such as the size and distribution of oil droplets.

Since the late 1980's the author has utilised a modified serra solution to assess the maturity of oocytes of a number of species of Australian native fish. It has proved to be particularly useful for those species producing large, relatively opaque oocytes and those with a thick chorion such as the various *Maccullochella* species and the Macquarie perch. The solution was modified to a new ratio consisting of formalin : alcohol : glacial acetic acid at 3:2:1 or 3:3:1 diluted with twice the volume of the mixture with water to reduce distortion of the oocytes through osmotic shock.

When applied to the oocytes of these species, clearing took place within a few minutes revealing oil and yolk droplets. Eventually, the germinal vesicle became visible as an oval brown structure which ultimately became bleached by the solution but remained visible. To accurately assess germinal vesicle position it was necessary to roll oocytes individually so as to remove the effect of perspective. The solution has also proved useful in highlighting the position of the germinal vesicle in species with delicate or relatively clear oocytes though some distortion takes place. It acts rapidly enough to clear the oocytes so that fish can remain in anaesthetic baths, while the process takes place, without harm.

Recent spawning work with catfish has utilised the clearing solution to assess germinal vesicle position. It has revealed that the large opaque oocytes present in females induced to spawn with CPE have germinal vesicles which are commonly partially polarised and some nearly/fully polarised (Figure 9). Smaller oocytes have oocytes with germinal vesicles in a slightly eccentric, central position. The semi-translucent oocytes found in some mature

female catfish have germinal vesicles that are either fully polarised or have undergone breakdown to produce a brown, diffuse mass at the animal pole.

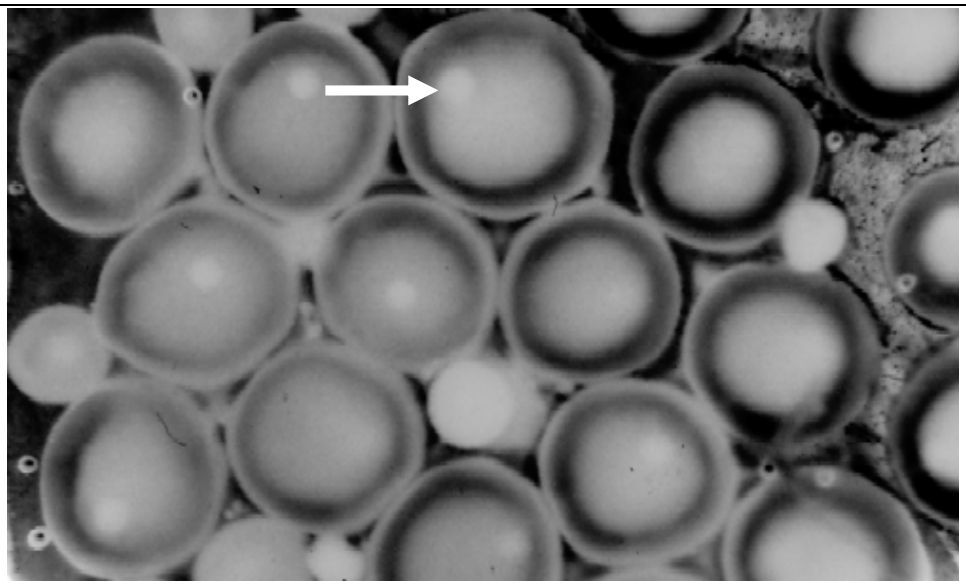

Figure 8.

Oocyte sample from the same female catfish as in Figure 1 after treatment with the modified serra clearing solution. Clearly visible in most oocytes are the germinal vesicles which have started their migration and are partially to fully polarised (arrowed).

It is suggested future work should adopt a classification system assessing germinal vesicle position similar to those used for overseas species of fish. Some systems are largely descriptive (eg. Dasgupta et al 2001), while others divide the process into a series of discrete stages (eg. Wen-Shiun Yueh and Ching-Fong Chang 2000).

Use of Ovaprim

While CPE has proved to be a highly successful tool for inducing spawning in cultured fish, it has a number of problems. Pituitary glands are highly variable in their gonadotrophin content, are potentially inconvenient to collect and provide a means of communicating pathogens. While such problems have not been encountered to date with its use on catfish, it would be preferable to utilise an alternative hormone without these potential problems. Commercial bulk preparations of CPE previously sourced from overseas may no longer be available due to quarantine restrictions.

Despite the practical advantages of using HCG, it has only proved effective on females with very late stage oocytes and due to the brevity of this phase, the proportion of responsive fish likely to be encountered during a season is low. Where significant catfish populations exist, such as in large dams or impoundments, hatcheries are known to have netted large numbers of individuals and selected females with clearing oocytes for HCG treatment. In most circumstances however this approach is not practical.

Like HCG, *Ovaprim* on face value avoids some of the potential disadvantages involved with the use of CPE. It is understood that some preliminary use of *Ovaprim* on female catfish has taken place, with limited success. The use of two injections spaced twenty four hours apart at a dose of 1 ml/kg, has been unsuccessful with females containing opaque oocytes (Bruce McInnes, Wartook Native Fish Culture, personal communication). Female catfish containing semi-translucent oocytes have been induced to ovulate with a single dose of *Ovaprim* at rates from ½ - 1 ml/kg.

Some success has been achieved using *Ovaprim* on female catfish in Queensland (Bruce Sambell, Ausyfish, personal communication) though the state of maturity of the fish has not been identified. Further investigation as to the effectiveness of *Ovaprim* in correlation with oocyte maturity involving germinal vesicle position needs to be undertaken.

Discussion of Existing Methodologies and Future Work

The results presented can be considered preliminary but it is apparent that crude carp pituitary extract can be utilised to reliably induce ovulation in catfish. A number of hatcheries in Victoria and Queensland are now producing catfish fingerlings through the induced ovulation of females and artificial fertilisation of the ova. They have adopted the procedure of administering two injections of CPE at 5-7 mg/Kg spaced 24 hours apart and monitoring fish 48 hr post first injection. Use of HCG has only been successful in females containing late stage oocytes which have commenced germinal vesicle breakdown. Preliminary results with *Ovaprim* suggests that it too may only be suitable in these females though more detailed investigation relating its effectiveness to oocyte maturity is required to clarify its usefulness in catfish.

The failure of female catfish to respond to HCG may be due to a number of possible causes. One possibility is that HCG may not closely resemble endogenous catfish GtH II. However the positive response of females containing oocytes which have commenced germinal vesicle breakdown suggests that the problem may not be one of species specificity. Another possibility is that catfish oocytes are not receptive to GtH II stimulation (and consequently HCG) until germinal vesicle migration is completed or once germinal vesicle breakdown has commenced. Current endocrine models describing oocyte development, maturation and ovulation involve a variety of pathways with pituitary control via GtH I controlling vitellogenesis and GtH II controlling final maturation and ovulation.

The effectiveness of CPE in female catfish could be explained in terms of the CPE containing GtH I, stimulating the completion of vitellogenesis, completion of germinal vesicle migration and the development of maturational competence. This contrasts with reports that the oocytes of some other species of fish acquire responsiveness to GtH II once germinal vesicle migration has commenced but prior to full polarisation (e.g., Goetz and Bergman1978, Wen-Shiun and Ching-Fong, 2000).

One possibility could be that other factors present in the pituitary extract promote the development of maturational competence in female catfish. Substances such as piscine growth hormone and piscine prolactin have been reported to play a role in the reproductive process in teleosts (Singh et al 1998). In fact the robust nature of catfish together with its large oocytes and readily observed germinal vesicle makes the species an attractive species for investigations of the reproductive endocrinology of fish. Nonetheless answers to these questions will only be obtained with further, more detailed investigations.

A potential limiting factor in producing catfish fry is the small quantity of seminal fluid that can be extracted from males. To date treatment with CPE and HCG has not produced a significant improvement in spermiation. The practice of sacrificing males to extract sufficient milt to fertilise multiple batches of eggs is not attractive where broodstock are a scarce resource, particularly to those involved in conservation recovery programs. One possible option is to conduct trials involving the use of steroids which have been demonstrated to promote spermiation in other fish species (Fostier et al 1984). Another option may be to catheterise males so that a greater volume of seminal fluid can be extracted without risk of injury through the use of excessive abdominal pressure.

While there is no doubt that further investigation, development and refinement is required to improve production practices for catfish fingerlings, the existing procedures and protocols provide an immediate potential to mass produce fingerlings for recovery programs. Government agencies contemplating such activities can be assured of early success. The principle uncertainty for any given facility will be determining the yearly window in which

female catfish will be responsive to endocrine intervention for that particular location. It is considered that the seasonal and temperature data reported in this document will provide a useful yardstick for many localities.

With the importance of creating a broad genetic base for stocked populations, collection of wild broodstock from remnant populations, spawning them, and returning them to the wild may prove feasible if they can be reliably caught. Wether wild caught or captive, provided sufficient broodstock are initially available there is a high probability of success during the first season. Hopefully the dissemination of the techniques outlined in this report will improve the availability of fingerlings for culture trials and for restocking natural waters for recreational and conservation purposes.

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References

Clunie, P. and Koehn, J.D. (2001). *Freshwater Catfish: A Resource Document* Final Report for Natural Resource Management Strategy Project R7002 to the Murray Darling Basin Commission. Department of Natural Resources and Environment, Heidelberg, Vic.

Crim, L.W. (1991). Hormonal manipulation of fish seasonal reproductive cycles. In: *Proceedings of the fourth international symposium on the reproductive physiology of fish*. (Eds: Scott, A., Sumpter, J.P., Kine, D.E. & Rolfe, M.S.). pp 43-47, FishSymp 91, Sheffield 1991.

Dasgupta, S., Basu, D., Ravi Kumar, L. and Bhattacharya, S. (2001). Insulin alone can lead to a withdrawal of meiotic arrest in the carp oocyte. *J. Biosci.* 26 (3):341-347.

Davis, T.L.O. (1977a). Reproductive biology of the freshwater catfish *Tandanus tandanus* Mitchell in the Gwydir river, Australia. I. Structure of the gonads. *Aust. J. Mar. Freshwater Res.* 28: 139-158.

Davis, T.L.O. (1977b). Reproductive biology of the freshwater catfish *Tandanus tandanus* Mitchell in the Gwydir river, Australia. II. Gonadal cycle and fecundity. *Aust.. Mar. Freshwater Res.* 28: 159-169.

Forteath, N. (1993). Reproductive biology of new finfish species. *Austasia Aquaculture*, 7 (6), pp54-55.

Fostier, A., Jalabert B., Billatd., Brenton, B. and Zohar, Y. (1984). The gonadal steroids. In: *Fish Physiology*, Vol IX. B. (Eds: Hoar, W.S., Randall, D.J. and Donaldson, E.M.). pp 277-372. Academic Press, New York.

Goetz, F.W. and Bergman, H.L. (1978). The effects of steroids on final maturation and ovulation of oocytes from brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). *Biol. Reprod.* 18, 293-298.

Kestemont, P. (1991). Attempts to assess ovarian maturity in a multi-spawning fish, the gudgeon *Gobio gobio* L., before induced ovulation. In: *Proceedings of the fourth international symposium on the reproductive physiology of fish.* (Eds: Scott, A., Sumpter, J.P., Kine, D.E. & Rolfe, M.S.). pp 278, FishSymp 91, Sheffield 1991.

Lake, J.S. (1967). Rearing experiments with five species of Australian freshwater fishes. I. Inducement to spawning. *Aust. J. Mar. freshwater Res.* 18: 137-153.

Lam, T.J. (1982). Applications of endocrinology to fish culture. *Canad. J. Fisheries & Aquatic Science*, 39; 11-137.

Merrick, J.R. and Midgley, S.H. (1981) Spawning behaviour of the freshwater catfish *Tandanus tandanus* (Plotosidae). *Aust. J. Mar. freshwater Res*. 32: 1002-1006.

Peter, R.E. (1981). Gonadotropin secretion during reproductive cycles in teleosts: influences of environmental factors. *Gen. Comp.Endocrinol.*, 45 (3), 294-305.

Peter, R.E., Lin, H. & van der Kraak, G. (1988). Induced ovulation and spawning of cultured feshwater fish in China: Advances in the application of GnRH analogues and dopamine antagonists. *Aquaculture*, 74; 1-10.

Rowland, S.J. (1983). The hormone-induced ovulation and spawning of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson) (Percichthyidae). *Aquaculture* 35: 221-238.

Rowland, S.J., Dirou, J.F. and Selosse, P.M. (1983) Production and stocking of golden perch and silver perch in New South Wales.
Australian Fisheries 42(9): 24-28.

Rowland, S.J. (1988). Hormone-induced ovulation and spawning of the Australian freshwater fish Murray cod, *Maccullochella peeli* (Mitchell) (Percichthyidae). *Aquaculture* 70: 371-389.

Saddlier, S. (2005) Freshwater Catfish, *Tandanus tandanus*.
Flora and Fauna Guarantee Action Statement # 201

Shehadeh, Z.H. (1975). *Induced breeding techniques - a review of progress and problems.* EIFAC Tech. Pap. #25, pp72-89.

Singh, H., Griffith, R.W., Takahashi,A., Kawauchi, H. Thomas, P. and Stegeman, J.J. (1988). Regulation of Gonadal Steroidogenesis in *Fundulus heteroclitus* by Recombinant Salmon Growth Hormone and Purified Salmon Prolactin. *Gen. Comp. Endocrinol.* 72, 144-153

Smith, E.A. (1993). Candidates for freshwater aquaculture in New South Wales (and anywhere else for that matter). *Austasia Aquaculture* 7(6), 38-40.

Wen-Shiun Yueh and Ching-Fong Chang (2000) Morphological changes and competence of maturing oocytes in the protandrous black porgy, *Acanthopagrus schlegeli.*
Zoological Studies 39(2): 114-122.

Zohar, Y. (1989). Fish reproduction: Its physiology and artificial manipulation. In: *Fish culture in warm water systems, problems and trends*. (Eds:Shilo, M & Sarig, S.), pp 65-119. CRC Press, Boca Raton, Florida.