Native Fish Australia Technical Report #2

A REVIEW OF ATTEMPTS AT THE ARTIFICIAL PROPAGATION OF THE MACQUARIE PERCH *Macquaria australasica*

WITH RECOMMENDATIONS FOR FUTURE ACTION

W.T. Trueman

August 2007

NATIVE FISH AUSTRALIA (Victoria) INCORPORATED

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Cover Photo: Adult Macquarie perch. (Photo courtesy Stephen Kerris.)

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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 Macquarie perch. The author reviews historical attempts at the development of reliable methods for producing Macquarie perch up to the present time.

Native Fish Australia has supported the preparation of this report to stimulate and support government agencies in re-establishing programs aimed at producing juvenile Macquarie perch for recovery efforts and to provide recreational angling. It is intended that this report highlight past progress and problems and make available previously unpublished data to provide a foundation for future efforts. In addition it is hoped that it may be used as a resource by people in government or tertiary institutions in the preparation of proposals and funding applications for research on the controlled reproduction of Macquarie perch.

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

August 2007

Introduction

The Macquarie perch, *Macquaria australasica* (Cuvier) (Percichthyidae), also known as black bream, white eye, goggle eyes or freshwater butterfish, is an Australian freshwater fish found in the Murray-Darling drainage system and a number of rivers of the east coast including the Nepean and Shoalhaven systems. Recent work suggests that the populations of Macquarie perch found in east coast drainages may be undescribed new species or subspecies (Dufty 1986).

Macquarie perch were once widely distributed throughout the southern half of the Murray-Darling river system with confirmed specimens being taken as far downstream as South Australia and regular captures in the Swan Hill area. However, it was most common in the Murray River from the Barmah Lakes lowland region upstream through foothill and upland habitats and in some cases penetrated into montane habitat in headwater areas (Cadwallader 1977; Cadwallader 1981; Trueman 2007). Historical accounts indicate that, together with the bluenose or trout cod (*Maccullochella macquariensis*), river blackfish (*Gadopsis marmoratus/bispinosus*), a number of *Galaxias* species and to a lesser extent the Murray cod (*M. peelii peelii),* the Macquarie perch formed part of a unique assemblage of native fish which typically occupied upland and some montane habitats in north eastern Victoria and southern New South Wales (Trueman 2007).

Lake (1971) considered the Macquarie perch to possibly have been the best Australian freshwater table fish. It once formed a small but important component of the past commercial fishery of the southern Murray-Darling system commanding a high price at market. The Macquarie perch was an esteemed sportfish due to its excellent fighting qualities, willingness to take artificial flies and at times lures, and its outstanding table qualities. It supported important recreational fisheries in Victoria and southern NSW including some in relatively small streams.

Large populations developed historically in impoundments constructed in foothill areas and these were on occasion the target of intense angler activity resulting in weekly harvests from inflowing rivers which at times were measured in tonnes (Cadwallader and Rogan 1977). An indication of the perceived value of the species is demonstrated by the fact that Macquarie perch have been translocated into waters outside of their natural range (Wilson 1857; Cadwallader 1981) and self-supporting populations persist in a number including the Yarra, Wannon and upper Shoalhaven rivers and the upper Seven Creeks. The species was also recommended for translocation to waters in New Zealand (NSW Legislative Assembly 1914).

Since the arrival of Europeans in Australia, the Macquarie perch has undergone a drastic decline in its range and abundance, at least in the Murray-Darling basin (Ingram et al 1990). This decline has been occurring since the nineteenth century with some populations disappearing about the time of the gold rush due to overfishing, cyanide poisoning and general habitat destruction (ACT Gov. 1999). Habitat degradation such as siltation and snag removal has undoubtedly played an important part in the historical decline Macquarie perch. During the late nineteenth and early twentieth centuries exotic salmonid species were introduced into waters containing Macquarie perch. Along with habitat degradation created through land clearing and altered fire regimes their introduction coincides with documented rapid declines taking place in some upland waters around the time of World War 1 (Trueman 2007). However, some populations survive in areas of highly modified habitat such as the Hughes Creek which has been severely silted by a sand slug since the early 1920's and the urban Yarra River.

In the first half of the twentieth century the English perch or redfin (*Perca fluviatilis*) rapidly spread through the lower reaches of the range of the Macquarie perch, their arrival often coinciding with the decline of the latter. This decline may have been the result of predation and/or competition from the redfin. Redfin can carry Epizootic Haematopoietic Necrosis Virus (EHNV) to which Macquarie perch are known to be extremely susceptible (Langdon 1989). However, the virus is not believed to have been present in redfin populations prior to the 1960's and therefore is unlikely to have been responsible for earlier historic declines of Macquarie perch populations. Further evidence of redfin impacting upon Macquarie perch comes from the Yarra River. Macquarie perch were an uncommon catch by anglers from that water during the 1970's, a period coinciding with the presence of large

numbers of redfin. Subsequent to the arrival of the European carp, *Cyprinus carpio,* redfin populations dramatically declined and this coincided with an increase in the number of Macquarie perch taken by anglers which are now commonly captured (W. Trueman, pers. obs.).

The erection of weirs and small dams provided barriers to the migration of Macquarie perch which historically are known to have made major movements upstream for spawning, assisting in the fragmentation of populations. The construction of large, deep impoundments in the 1950's on upland rivers, which drastically altered flow regimes and thermally polluted downstream waters, has been responsible for the decline of some Macquarie perch populations. The primary impact of thermal pollution has been to remove the rise in water temperature in spring or early summer believed to cue the spawning of Macquarie perch though other less obvious affects such as physiological affects and changes to food supplies may also be significant (Koehn et al 2005). While impoundments have for a few decades after filling supported major populations of Macquarie perch, they have typically declined in subsequent decades. Notable examples include Lakes Eildon and Dartmouth.

It has been previously suggested that the decline of Macquarie perch in impoundments has been in part due to changed habitat and interaction with redfin (Cadwallader & Rogan 1977). Also, Macquarie perch generally are considered to have a habitat preference for flowing waters. Despite this, the historical evidence indicates that the species was formerly abundant in billabong environments. This suggests that the static environment created by the impoundment of rivers, while not suitable for their reproduction, cannot directly be responsible for the historical declines observed. Also, historical evidence suggests that in many cases Macquarie perch populations were already in decline in feeder rivers, prior to their impoundment.

Interactions with introduced trout are implicated in the decline of Macquarie perch in at least some habitats which had not undergone significant environmental degradation (Trueman 2007). Adult trout are known to prey upon juvenile Macquarie perch but the extent to which this or other potential mechanisms such as larval predation or competition have impacted on the species are not clear. Reproductive success of Macquarie perch has been linked to body condition (Sheikh-Eldin et al 1995, Gray et al 2000) and it is plausible that competition for food with trout and redfin could have disrupted their reproduction. Detailed studies of the invertebrate fauna in some New Zealand trout streams have indicated that trout can greatly reduce invertebrate food supplies (McDowall 2003).

An alternate interpretation is that the exceptional conditions for recruitment of juvenile Macquarie perch, produced by the filling of impoundments, temporarily arrested the decline of these populations. That decline resumed once filling was completed. Support for this proposition comes in the example of Wyangala Dam constructed on the upper Lachlan River in the mid 1930s. Macquarie perch flourished in both the dam and the river upstream until the mid 1950s when numbers of brown trout arrived in the Lachlan River, this coinciding with a rapid decline in the perch population. This contrasts with the original Eildon (Sugarloaf) Dam completed on the upper Goulburn River in 1927. Macquarie perch flourished in the weir initially after filling but went into a slow decline and had become scarce in feeder streams other than during spawning time by the World War Two. Enlargement of the dam in 1956 produced a resurgence of the perch population which rapidly declined (Trueman 2007). The arrival of redfin in this water may have accelerated the decline along with heavy overfishing.

The conservation status of the Macquarie perch was upgraded from indeterminate to vulnerable by the Australian Society for Fish Biology in 1998. In Victoria it is listed under the *Flora and Fauna Guarantee Act 1988* as a threatened taxon and is classified as endangered. In the Australian Capital Territory it is listed as endangered under the *Nature Conservation Act1980* while in New Wales it is classified as Vulnerable. Currently the taking of Macquarie perch by angling is prohibited in all states except Victoria where they can be retained by anglers from three waters under restrictive bag limits.

Due to its past popularity as a sporting and table fish, and more recently as a result of concern for its conservation, there has been ongoing interest in the development of methods for the artificial propagation of the Macquarie perch for nearly a century. To date, all past attempts have met with limited success and typically have relied upon collecting wild breeding stock. Ultimately as populations have declined breeding programs have met their demise.

The most recent and successful program was operated by the then Victoria Fisheries and Wildlife Division in the 1980's and early 1990's utilizing the wild population in Lake Dartmouth. However with the decline of the population that program too came to an end with the last fish being produced in the mid 1990's (Gray et al 2000). At the present time the only organization attempting the artificial propagation of the Macquarie perch is Native Fish Australia (Victoria) Incorporated, which has on a number of occasions produced juveniles utilising wild broodstock sourced from the translocated population in the Yarra River.

Juvenile Macquarie perch produced from the Victorian Government program, as well as adult fish translocated from Lake Dartmouth, were stocked into a range of waters in an attempt to restore self sustaining populations. Initially those stockings appeared to result in little or no success with no evidence for recruitment from stocked fish. However in recent years it has become apparent that small self-sustaining populations appeared to have become established in Lake Eildon, the Yea River system, the middle Broken River and its tributaries, the upper Buffalo River and possibly the King River upstream of Lake William Hovell. A similar phenomenon has been observed with translocated fish in the Australian Capital Territory taking many years to become recruiting (Lintermans 2003, 2006) perhaps reflecting a form of pulsed recruitment dependent upon variations in environmental conditions in upland rivers. The recently discovered success of these early stockings have stimulated renewed interest in the development of stocking programs for conservation purposes as well as to develop recreational fisheries.

This report reviews methods that have been attempted or utilized for the artificial propagation of Macquarie perch and presents previously unpublished data and protocols. The intention was to review the work done to date by all workers, making the information freely available as a stimulus for further work. It is hoped that ultimately the information contained within will provide renewed interest in research into the development of reliable techniques for the hatchery production of the Macquarie perch by government agencies to aid in recovery efforts and provide recreational angling.

History

Early Attempts

With the considerable activity expended during the early twentieth century into creating translocated populations of Macquarie perch in new waters (Cadwallader 1981) the species was an early target for artificial propagation. In 1914 efforts at the attempted artificial propagation of a number of native fish species were made at the government trout hatchery at Prospect Inlet Ponds near Sydney. Three ponds were set aside '*in case of being able to obtain some golden, silver, and Macquarie perch from Berembed weir a little later on*' though reports in subsequent years fail to mention Macquarie perch being stocked into them (NSW Legislative Assembly 1915).

The NSW State Fisheries Department attempted the artificial propagation of a number of species of native fish captured from the Murray River near Albury in October and November 1928. The rationale behind the operation was to catch running ripe fish of both sexes and artificially fertilise the ova. Although '*Macquarie perch were found to be plentiful ……. No ripe Macquarie perch of either sex was captured*' and the efforts abandoned at the end of November (NSW Legislative Assembly 1929).

It is believed that the government trout hatchery at Studley Park in Melbourne under the direction of Mr Pullen may have attempted the artificial propagation of the species utilizing captive and wild fish sourced from the Yarra River but was unsuccessful. In past decades elderly Melbourne anglers have on a number of occasions recalled a story about a hatchery operating on the outskirts of Melbourne prior to World War two that was breeding Macquarie perch and stocking them into the Yarra River. Recently more information on this operation has come to light.

Former Fisheries and Wildlife Inspector Mr J. O. (Jack) Rhodes in the 1950s was made aware of the operation by Mr. Thomas Kneebone, a well known fishing identity. He reported that his father had successfully spawned captive Macquarie perch in the Heidelberg area which Jack believed occurred in the 1930's. Thomas Kneebone told Jack that '*he was required in all weather conditions to regularly record the water temperature of the breeding ponds'* which Jack believed to be in the backyard of their home. Although Mr Kneebone senior made the then Fisheries and Game Department aware of his success the exact procedure used was never disclosed (J. O. Rhodes, personal communication, October 2006). These efforts probably represent the first successful attempt at the artificial propagation of the Macquarie perch and, significantly, suggest the potential for contemporary efforts to succeed through environmental manipulation.

Cadwallader (1977) reported the work of researcher J. A. Tubb who in 1937 attempted the artificial propagation of a number of native fish species in the Barmah Lakes district. Eggs were collected from 'running ripe' females, fertilised with milt from males, and the eggs incubated in enamel basins or submerged trays. It was reported that a small number of Macquarie perch eggs were fertilised in late September (20-26th) but these subsequently died, the failure being attributed to rapid temperature changes in the enamel basins.

Ripe Macquarie perch eggs were fertilized on October 27 and November 3 with it being reported that '*the first lot failed to develop; the second lot was placed in gauze-bottomed trays to facilitate handling*'. This suggests that development took place in the second batch of eggs though no further reference is made to their fate. Of significance is the presence of 'running ripe' female Macquarie perch in the river for an extended period, from late September to early November encompassing a time span of about five weeks.

In the post war era a number of private individuals attempted the artificial propagation of Macquarie perch. In the 1960's the late Keith Henderson of Harcourt, a passionate angler who for many years had been involved in district trout stocking, held a number of native fish species in ponds in the hope of breeding them. A small number of Macquarie perch sourced from the Broken River were held but the limited number of available fish thwarted any success (Mrs L. Allen, personal communication).

Around this time the late C. C. (Barney) Kipping of Strathbogie operated a volunteer trout hatchery for the Strathbogie-Euroa Angling Club on his farm. For over two decades Macquarie perch sourced from the nearby Seven Creeks were held in small dams in the hope that they would breed. In 1976 a pair of fish was noticed engaged in a flurry of activity in one small pond where a small perennial brook entered and there was present an accumulation of granite sand. The fish were seine netted from the pond and as the female was found to be running ripe the eggs were stripped and artificially fertilized. The ova were apparently viable and developed to the 'eyed' stage where the developing embryos were visible. Unfortunately, due to an accident which cut off the water supply to the eggs, they perished. Although Macquarie perch continued to be held for a number of years afterwards, and were at intervals removed and examined, no 'running ripe' females were encountered again (the late C. C. Kipping, personal communication).

Lake Eildon

In the 1950s the Victorian Fisheries and Game Department established a hatchery for producing trout at Snobs Creek a short distance downstream from the Eildon Dam on the Goulburn River. In the late 1950's to early 1960's attempts were made to produce juvenile Macquarie perch utilizing broodstock sourced from the Goulburn and Jamieson Rivers upstream of Lake Eildon on their annual spawning run. The conditions under which spawning took place in the inflowing rivers to Lake Eildon were investigated and discussed by Wharton (1968). He reported that migration of mature fish into the rivers took place in spring and early summer when water temperatures in the rivers exceeded 16.5 °C, and that temperatures below this suppressed migration out of the lake.

Wharton (1973) also reported attempts at the artificial propagation of Macquarie perch at Snobs Creek between 1962 and 1964. From October to November 1963 a total of 133 adult fish were captured from the Goulburn River, some of which were subsequently utilized in attempts at artificial propagation. A range of trials were conducted utilizing the hormone HCG (Human Chorionic Gonadotrophin) and distilled water to induce ovulation and spawning in females within 7 days of capture. The results were variable with some females ovulating, some shedding eggs and others showing no response. These attempts did not produce viable ova. Two wild females captured on November 21 were found to have a small proportion of ripe eggs (5000 between the two fish) and these were stripped, fertilized and subsequently many hatched. The number of ovulated eggs reported from these females is low compared to the reported fecundity for Macquarie perch of approximately 30 000 eggs per kilogram body weight (Cadwallader and Rogan 1977) suggesting that at the time of capture that the fish had only partially ovulated.

A pair of fish captured on November 21 and held in an aquarium, were injected with the hormone HCG on December 14 and spawned four days later but the eggs proved unviable and the female subsequently died. The experiment was repeated on December 19 and over the following week courtship behaviour was observed and batches of eggs spawned but these too proved unviable and resulted in death of the female. Interpretation of these results require caution, given the considerable period of time that had elapsed between capture and hormonal treatment and the possible influence of stress upon the broodstock. Fish were stocked into a 0.1 Ha pond on November 13 into which a minimal flow of water (15 L/min) was provided but no evidence of spawning was found. The fish were subsequently removed from the pond on December 19 and when examined the females appeared to have commenced resorption.

Wharton's work reported the successful production of viable ova sourced from running ripe females which subsequently hatched and were reared in outdoor plankton ponds. However at the time the work was being undertaken the population of Macquarie perch in Lake Eildon was in decline, which hampered future work, with the species virtually disappearing from the water by 1970 (Cadwallader and Rogan 1977).

Narrandera

The Inland Fisheries Research Station near Narrandera NSW (now the John Lake Centre) commenced operation around 1960 with an early focus being the artificial propagation of native fish. Lake (1967) reported the results of investigations of factors controlling the natural reproduction of five species of warm water native fish. In the mid 1970's efforts were directed towards investigating techniques for the mass production of juvenile native fish. This ultimately led to the development of reliable technology for producing three species, namely Murray cod (*Maccullochella peelii peelii*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*) (Rowland 1983, 1984, 1988).

Ingram et al (1994) reported the results of attempts to induce ovulation/spawning in captive Macquarie perch held in ponds at the IFRS between 1978 and 1990 and utilizing protocols similar to those used successfully for other native fish. Over that period a total of 82 females received a variety hormonal treatments including alone or in combination HCG, LHRH, LHRH analogues, CPE and pimozide. Prior to hormone treatment the ovaries of females were cannulated in order to assess oocyte maturity.

The authors reported that after hormone treatment in a majority of females oocytes increased in diameter and increased in transparency, but ovulation took place in only 13 females. However, of the ova produced by these fish only one female produced any that were viable, a total of 1300 being stripped and fertilized leading to the hatching of 325 larvae. The number of ova produced suggests that on this occasion only partial ovulation had occurred. Other injected females failed to ovulate and the oocytes subsequently swelled and cleared, presumably signalling the onset of atresia and resorption.

The authors speculated that the failure of their attempts to induce spawning and ovulation of pond held Macquarie perch with conventional hormonal intervention was probably due to the fish failing to complete vitellogenesis. They cited similar results being experienced with captive fish held at the Snobs Creek Freshwater Fisheries Station and Hatchery in Victoria. A number of hypotheses were proposed to explain the failure of Macquarie perch to complete vitellogenesis when held in earthen ponds. These included the lack of appropriate environmental cues in the pond environment, such as flowing water and the presence of a suitable substrate, high water temperatures, poor water quality, inadequate nutrition and stress induced by the captive environment. They outlined future areas for urgent research to overcome the problems including improved broodstock nutrition, environmental stimuli for maturation and novel hormonal treatments to promote the completion of vitellogenesis.

Lake Dartmouth

Gooley (1986), and Gooley and McDonald (1988) reported the results of spawning induction trials with Macquarie perch conducted at Lake Dartmouth in Victoria from 1983 to 1985. Females were collected from the wild during their spawning migration out of the lake and into the inflowing Mitta Mitta River. They were selected for use in trials on the basis of external characteristics. Individual fish were sacrificed both before and after hormone treatment to assess ovarian development. They reported little variation in the appearance of oocytes of migrating females, which were generally opaque in appearance and averaged 1.45 mm in diameter. Individual fish used for the production of fertilized ova in the trials did not undergo any form of ovarian assessment.

Captured females were injected with HCG either in single or incremental doses and placed with injected males in holding tanks. They were subsequently monitored for ovulation and spawning. Where spawning failed to occur ovulated ova were stripped from the females and were artificially fertilized utilizing milt from the males. For single dose injections of HCG, ovulation occurred from 33 to 62 hours after injection. Most fish that produced fertilized eggs ovulated from 39 to 43 hours after injection with HCG. At the time of ovulation they noted that the diameter of the ova had increased to 1.60 mm.

In 1983 of a total of 12 females receiving a single dose of HCG, 9 ovulated and produced ova but only one female produced viable eggs. 15 females were injected with incremental doses of HCG of

which 12 produced ova and 2 batches were fertilized. In 1984 only incremental injections of HCG were used. A total of 46 females were injected of which a total of 26 produced ova and 4 produced eggs that were fertilized. In 1985 females received single injections of HCG at 1000 i.u./kg. A total of 51 females were injected of which 31 produced ova and 11 produced eggs that were fertilized. For the total of 124 females injected over the three seasons the percentage which induced to ovulate was 63% while the percentage that produced fertilized eggs was 15%. These are relatively low rates compared to those reported in spawning trials for other native fish (Rowland 1983, 1984).

Gooley and McDonald (1988) considered the correlation between induced spawning success and the minimum and maximum water temperatures of the inflowing Mitta Mitta River. They reported that of the fish that produced fertilized eggs, 78% were captured when minimum temperatures were between 13-16 °C and 89% were captured when maximum water temperatures were between 14-18 °C. The authors concluded that, of the fish that produced fertilized eggs, most were captured on their upstream spawning migration into the Mitta Mitta River and were within a few days of spawning naturally. Appleford et al (1998) described the annual cycle of gonad development for Macquarie perch in Lake Dartmouth utilizing ovarian samples sourced from the population during the filling phase of the impoundment between 1975 and 1982, as well as other waters.

Native Fish Hatcheries, Violet Town

In 1980 a commercial fish hatchery commenced operation near Violet Town in north east Victoria and undertook a small number of spawning trials on Macquarie perch. Broodstock Macquarie Perch were sourced from Lake Dartmouth in April 1980 and held in small earthen ponds. In October 1980 the fish were removed from the ponds when bottom temperatures had reached 16 °C and examined for spawning induction. The protocols used were based on advice from Dr Stuart Rowland at the IFRS at Narrandera as to what were in use for golden perch, which were published some time later (Rowland 1983).

The ovaries of females were cannulated with a plastic pipette and the oocytes examined. Some fish had failed to undergo ovarian development, while others had ovaries containing oocytes varying greatly in size. Three females contained oocytes of fairly uniform size approximately 1.5 mm in diameter and which were almost totally opaque. With intense illumination numerous fine droplets could just be discerned inside the oocytes. To the eye the oocytes had a creamy white appearance. One fish was injected with HCG at 500 i.u./Kg, the second fish with HCG at 1000 i.u./Kg and the third fish was injected with CPE at 5 mg/Kg. The fish were held in aquaria with spermiating males which were injected with HCG at 500 i.u./Kg.

After injection, males showed signs of courtship behaviour with the females which persisted for about 24 hours, after which interest declined. By 48 hours after injection, the only visible change in the females was a slight additional distension of the abdomen. The females were removed from the tank and examined. It was found that in all three females that the oocytes had increased in size slightly and undergone an increase in their transparency though were still fairly opaque. One female had partially ovulated and the ova were stripped and fertilized but proved to be unviable. Both females injected with HCG died within a few days. The female treated with CPE was again examined 5 days after injection. At that time the ovary contained a mass of gelatinous, bloody tissue indicating active resorption of the oocytes. Subsequently the following summer a number of broodstock perished when water temperatures exceeded 27 °C (W. Trueman, pers. obs.).

Recent Work

Lake Dartmouth

Subsequent to the work of Gooley and McDonald (1988) production of juvenile Macquarie perch using broodstock sourced from Lake Dartmouth continued into the 1990s as well as further research into the biology of the species. The Lake Dartmouth hatchery facility was eventually closed with wild broodstock being directly transferred to the Snobs Creek facility. In latter years after stabilization of the water level in Lake Dartmouth, between 1991 and 1997, they reported that the body condition of female fish declined as did artificial spawning success. This decline was attributed to poor nutrition associated with declining food resources in the lake (Gray et al 2000). An additional factor associated with the declining condition of Macquarie perch in Lake Dartmouth may have been competition for food resources with the European carp which appeared in the lake in great numbers during this period. Ultimately the low spawning success led to the cessation of the artificial propagation program for Macquarie perch.

Sheikh-Eldin et al (1995) analysed the composition of oocytes, liver and muscle of wild and tank held females to investigate the role of broodstock nutrition in the reproductive failure of captive fish. They reported unpublished data and protocols from Gooley and Ingram for spawning induction. These included the use of the hormone salmon GnRh Analogue (D-Arg6, Trp7, Leu8, des Gly10-LHRH ethylamide GnRHa) at a dose of 10 g/Kg* and a stripping time of 41-43 hours post injection. (* The dose reported for LHRHa appears to be excessively high and may be an error. Based on reported doseages of this hormone used for other species it may be 10 μg/kg).

The authors reported significant differences in the development of oocytes between the captive and wild fish. In wild females the oocytes fell into two distinct groups, namely small pre-vitellogenic oocytes less than 0.30 mm in diameter and a second group of larger oocytes completing vitellogenesis. In the captive tank-held fish the two groups of oocytes were less distinct with a lower proportion of oocytes approaching maturity. In addition to differences in oocyte development significant differences were reported in the GSI (gonadosomaric index) and HSI (hepatosomatic index) between the wild and captive fish.

The following year the same authors (Sheikh-Eldin et al 1996) provided further detailed information highlighting the differences in the composition of the liver and oocytes of wild and captive fish and their food sources. It was observed that the captive fish had a higher ratio of n – 3 to n – 6 fatty acids than did the wild fish and that wild food sources in general had a higher content of n – 6 fatty acids. Similarly the oocytes of wild fish had a lower ratio of these fatty acids than did the tank-held fish. In particular it was noted that Macquarie perch concentrate n – 6 fatty acids in their tissues generally relative to their dietary supply and that this process appeared to be more efficient in wild fish. Overall the livers of captive fish appeared to be deficient in 20: 4n – 6 and 20: 5n – 3 fatty acids compared to wild fish.

Sheikh-Eldin et al 1996 hypothesized that deficiencies in fatty acids such as the n – 6 series may have been responsible for the ongoing failure experienced in inducing spawning in captive Macquarie perch. In particular they highlighted the importance of 20: 4n – 6 as a precursor for synthesis of eicosanoids such as the prostaglandin series of hormones some of which play important roles in final oocyte maturation, ovulation and spawning behaviour.

Wartook Native Fish Culture

In the mid 1990's Wartook Native Fish Culture investigated the development of techniques for the captive spawning of Macquarie perch. Broodstock were sourced from Lake Dartmouth and its inflowing rivers and held in earthen ponds at the hatchery facility at Wartook. The fish were marked with dart tags, these causing hemorrhagic lesions around the tags. The fish with these legions failed to undergo normal ovarian development. However a number of untagged fish placed in ponds were subsequently utilized in trials which are reported later in this document.

Andrew Tonkin

In recent years the ownership of Wartook Native Fish Culture has changed hands with Mr Chris Vincent becoming the principal. Recently it was reported that some success had been achieved in inducing ovulation and spawning of captive Macquarie perch by Native Fish Australia member Andrew Tonkin in collaboration with the new proprietor (Walker 2006). Although not fully detailing methodology it is reported that some protocols previously developed for assessing maturity of the female fish as described later were utilized.

Native Fish Australia

The following information has been provided by NFA members Ron Lewis, Julian Bowler, Rob Radavicius, Tim Curmi and Nick Thorne who have been actively involved in trials conducted investigating the induced spawning of Macquarie perch.

Native Fish Australia (Victoria) Incorporated has been involved in the artificial propagation of a number of species of native fish since the early 1980s. Initially, work was directed at producing silver and golden perch juveniles at a waste water treatment plant on the Mornington Peninsula. Subsequent to these activities the organisation embarked on a program to breed the trout cod, initially at members' residences and ultimately at a hatchery facility located in premises provided by La Trobe University in 1994. The organization over a number of years produced approximately 44,000 trout cod larvae which were incorporated into the government rearing program at the Snobs Creek Hatchery near Eildon.

Around this time a program to induce spawning of Macquarie perch sourced from the nearby Yarra River was initiated and has continued since. The greatest success was achieved at the first attempt in late 1994 when total of approximately 5000 juveniles were reared up to a size of approximately 25 mm, initially being fed on plankton but then on *Artemia*, *Tubifex* and Cladocerans and subsequently liberated into the Yarra River near Warrandyte. These fish were produced from a number of pairs of Macquarie perch angled from the Yarra River near Warrandyte and transported to the Latrobe University facility. Several successful spawnings were achieved, a number from females injected with HCG, however the greatest success in terms of hatch rate and actual numbers produced came from fish which were found to be running ripe after three or four days in the hatchery when the fish were anaesthetised with the intention of injecting with hormone. These fish were instead stripped and the eggs fertilised. This is the first reported example of Macquarie perch completing FOMO under artificial conditions.

It was estimated at the time that approximately 20,000 eggs hatched, producing live larvae. This level of success was entirely unexpected for this experimental trial and some viable eggs and larvae were lost in the process of developing protocols for handling and rearing these fish. Nevertheless, as mentioned previously, approximately 5,000 juvenile Macquarie perch were raised to a size suitable for stocking, approximately 25 mm.

NFA has attempted to reproduce this approach with non-injected females a number of times since 1994, but without success. It appears that the inability to reliably capture females at the correct stage of oocyte development by rod and line has prevented repetition of this notable achievement.

A display was held at the hatchery in December 1994 where three distinct age classes were displayed (from three separate spawnings), together with eggs in the incubation units. The display was attended by representatives from Victorian Fisheries at all levels including Ministerial representatives.

Since that time efforts at producing juvenile Macquarie perch have proceeded on an intermittent basis, secondary to the production of trout cod. Small numbers were produced in 1995 and 1996, but less effort was put into Macquarie perch due to the emphasis on trout cod which had become acclimated to the new hatchery by that time. In 1997 some 1,500 Macquarie perch fingerlings were produced from two successful spawnings and these were released into the Watts River, a tributary of the Yarra River, approximately 1 km upstream from the confluence of those rivers. Whilst those fish were still in the hatchery, another successful open day was held, displaying both trout cod and

Macquarie perch juveniles, which was attended by representatives of the University amongst other interested parties.

In 1999 further success was achieved. A number of female Macquarie perch were induced to ovulate after treatment with the hormonal preparation *Ovaprim*® and eggs hatched. A small number of juveniles were produced which were liberated into the Diamond Creek a few hundred metres upstream of the Main Road bridge.

Recently NFA has taken the decision to wind back its trout cod breeding activities and concentrate its efforts into the production of juvenile Macquarie perch.

In November 2006 NFA once again successfully bred Macquarie perch in their hatchery at La Trobe University, Melbourne. Broodstock were sourced from the Yarra River around Eltham and Warrandyte from early August through to mid November. Upon relocation back to the NFA hatchery the fish were sexed and the females cannulated and checked for oocyte maturity/development. The females were then given unique identification codes and administered either *Folligon*® or *Ovaprim*® artificial hormones or a combination of both under controlled conditions. The females were then routinely checked for oocyte development, ovulation and signs of spawning, all observations being carefully recorded.. All females caught during the months of August and September experienced no development subsequent to arrival from the river nor did they respond favourably to either artificial hormone.

At the end of October and into early November with the temperature rising in the river some of the newly caught females showed further natural maturation compared to the earlier fish, with others seeming spent or having eggs running freely. The semi-mature fish were moved quickly to the hatchery and immediately administered *Ovaprim*® hormone according to recognised doseages and the oocytes checked 24 hours later. Development continued and the oocytes had reached the stage where they had completed FOMO and were suitable for fertilisation. The females were then anaesthetised and the ova stripped into a large bowl containing spawning solution to prevent water hardening. Two males were then also anaesthetised and milt collected from both using a syringe, added to the bowl containing the ova and gently stirred for a few minutes with a feather. The fertilised eggs were then placed into the dedicated incubators, which keep them rolling and moving to ensure they completed development and hatched. After 72 hours at 20+ ˚C approximately 500 eggs hatched. Feeding of the fry commenced upon yolk sac absorption, 3 to 4 days after hatching, with *Paramecium* and *Artemia* nauplii. This feeding regime continued for 6 weeks and was then replaced with Cladocerans and other zooplankton collected from local ponds. The fry were successfully on-grown to fingerling stage in glass aquaria and a small number still remain in the hatchery (August 2007). A number of the larvae upon hatching from the 2006 trial were collected by Victoria Fisheries scientist Brett Ingram for DNA sampling as part of genetic research being undertaking on the Percichthyidae fishes.

NFA has observed that the oocytes in Macquarie perch do not appear to develop uniformly within the ovary. Generally NFA has observed no more than 50 to 60% of oocytes are at the same stage of maturity. Even in the most successful trial (1994) with the “running ripe” fish that were stripped without endocrine intervention, it is estimated that only about 70-80% of oocytes were harvested. This can be partially explained by the desire not to over stress the fish in the stripping process, but experienced native fish breeders observed at the time that the harvest was less than generally achieved with other species. It is postulated that this asynchronous development may be in part a survival adaption to the highly variable conditions that can exist naturally when these fish breed, allowing females at times to spawn more than once during a breeding season if conditions are suitable.

In recent years, difficulties in procuring broodstock, particularly males at spawning time, has proved to be a limiting factor. NFA has recently adopted the protocol of capturing and holding ripe males in aquaria early in the spawning season to guarantee a supply of milt. Additionally, experience with collecting females has shown that those with the ovarian development responsive to endocrine intervention are usually not receptive to rod and line capture. It is postulated that during this phase, the females stop feeding. NFA has applied for permission to use fyke nets to collect female broodstock as a means of overcoming this bottleneck. Although NFA uses protocols to reduce the

incidence of gut or gill hooked fish through active angling, the use of barbless and "circle" hooks a number of fish do end up with such injuries. Netting will have the extra advantage of reducing the incidence of injury and even mortality caused by these ingested or gill lodged hooks.

NFA's success in producing Macquarie perch in part validates some of the observations reported later in this document on suitable criteria for selecting females for spawning induction. While the number of juveniles produced has been modest this has been in part due to the limited resources available. While the La Trobe University site is well positioned to access the Yarra River perch population and has good facilities for spawning induction, its capacity to rear juveniles to fingerling size is limited.

An Overview and Summary of Work to Date

In the past four decades despite considerable effort, reliable technology for the induced ovulation or spawning of female Macquarie perch has failed to be developed whilst during the same period methods for the production of other Murray-Darling species have been developed and refined. In considering the work done to date the following problems are apparent:

1. Gross oocyte appearance has not been a reliable indicator of the state of maturity of the female fish;
2. The environmental factors controlling vitellogenesis and final maturation remain unclear;
3. Female Macquarie perch have shown a variable response to endocrine intervention;
4. After hormone-induced ovulation/spawning the eggs produced are often unviable;
5. There are differences apparent in the oocytes of fish sourced from the wild, held in earthen ponds or in tanks. It has been proposed that the differences may be caused by a lack of suitable environmental stimuli, stress or by inappropriate nutrition.
6. Variations in body condition of wild fish, linked to nutrition, have been associated with reproductive success in the wild.

A Review of the Reproductive Physiology & Controlled Reproduction of Fish

In order to consider the problems encountered with the controlled reproduction of Macquarie perch it is perhaps useful at this point to briefly review current knowledge on the processes regulating reproduction in teleost fish and how intervention takes place in artificial reproduction. Teleosts represent a diverse group with a long evolutionary history and as a consequence do show differences in their reproductive endocrinology. However a general discussion and overview is considered useful at this point and the information provided has been drawn from a number of reviews (Mommsen and Walsh 1988; Nagahama et al 1994; Goetz and Garczynski 1997; Jalabert 2005). As female Macquarie perch have historically provided the bottleneck in the development of propagation techniques the discussion that follows is largely focused on that sex.

In the past three decades much has been elucidated about the reproductively physiology of fish and with reference to existing knowledge for mammals such as humans. Control of the process takes place across a hierarchy of command levels. The basic model is that the hypothalamic/hypophyseal (hypothalamus/pituitary) axis controls the reproductive process in female fish in response to environmental and internal stimuli. The hypothalamus regulates the function of the hypophysis by means of the hormone Gonadotrophin Releasing Hormone (GnRH) which stimulates the release of two hormones, formerly known as vitellogenic gonadotrophin or GtH I, and maturational gonadotropin or GtH II. GtH I and GtH II are now understood to structurally resemble and to play physiologically similar roles to the mammalian hormones Follicle Stimulating Hormone (FSH) and Leutinising Hormone (LH) and are now so named.

Oocytes are derived from primary germ cells in the ovary and undergo development and growth to ultimately produce ova with capacity to be fertilized. The process of vitellogenesis encompasses the greatest growth phase of oocytes. The protein transport molecule vitellogenin, synthesized in the liver, transports large quantities of lipid, carbohydrate and other substances through the bloodstream to the oocytes for uptake. The precise structure of vitellogenin varies between species, particularly in terms of lipids which vary both in composition and quantity. These molecules are incorporated into the oocyte and integrated into it to form a rage of structures including oil and yolk droplets and the future egg shell or chorion. Ultimately these structures play pivotal roles in the fertilized egg and developing larvae forming the basis of physical structures and acting as sources of energy.

FSH acts on ovarian follicles to stimulate the growth of oocytes including the process of vitellogenesis, the uptake of lipids and proteins from the bloodstream and incorporation into the growing oocytes. Within the oocyte vitellogenin is cleaved into a number of substances and processed. In response to the stimulus from FSH the follicle cells produce the steroid hormone testosterone which is converted to 17β-estradiol, released into the bloodstream and acts upon two principle targets. It stimulates mobilization of materials necessary for vitellogenesis in the liver and secretion of vitellogenin by that organ into the bloodstream, and provides a feedback to the hypothalamus providing modulation of GnRH release. At the completion of vitellogenesis the oocyte has completed the first prophase stage of meiosis and in many species enters a period of meiotic arrest.

Once oocytes have completed vitellogenesis they may enter a phase of maturational competence whereby they have developed the necessary receptors and pathways for final oocyte maturation and ovulation (FOMO). In vitro studies of oocyte development in fish, as well as investigations into induced spawning, have indicated that changes in oocyte structure are associated with the acquisition of maturational competence and the ability to undergo FOMO. Gross changes vary from species to species but commonly involve an increase in the size of the oocytes, increased transparency and coalescence of oil and yolk droplets. Less subjective are changes in the appearance and position of the nucleus or germinal vesicle (GV).

At some stage during oocyte development the GV migrates from a central or slightly eccentric position within the oocyte to a peripheral position against the chorion and adjacent to the micropyle, the small pore in the chorion through which the sperm enters at the time of fertilization. In some species it has been reported that maturational competence is only acquired once the GV is in the

peripheral position while in others competence may be acquired in a sub-peripheral or even the central position. While variations in GV position and maturational competence exist between species it is consistent within a species. Ultimately it is the movement and changes in the GV that signal the cessation of meiotic arrest and the transition to FOMO.

Acquisition of maturational competence involves a switch in steroid production by the follicles from the production of 17β estradiol to maturation inducing steroids (MIS). Concurrently in some species the pituitary gland switches from FSH to LH. In other species both hormones are produced throughout vitellogenesis and rather than their being a switch there are changes in the proportions of these two hormones produced or frequency of release. The mechanism controlling these changes is somewhat unclear in fish although it is known in some species that changes in steroid production by the follicles initiate the variations in pituitary secretions. Some evidence suggests that as in mammals the molecule inhibin produced by the ovary may signal the pituitary that vitellogenesis is complete. At this point if suitable environmental stimuli exist (e.g. photoperiod, temperature, flooding, mates, suitable spawning site) the hypothalamus produces a transient surge of GnRH. This in turn stimulates the pituitary to produce an extended pulse of LH which acts upon the ovarian follicle cells to initiate FOMO.

The processes involved in FOMO are reasonably well understood. The surge of LH stimulates the follicle cells to produce one of two molecules that can act as MIS, depending upon species, namely 17α-hydroxyprogesterone or 17 α,20β,21-trihydroxy-4-pregnen-3-one. These steroids initiate the resumption of meiosis including GV migration and/or breakdown, the second meiotic division and coalescence of oil and yolk droplets. The breakdown of the GV signals the cessation of meiotic arrest and initiation of the final events of maturation. Once these activities have reached completion release of the prostaglandin PGF2α stimulates release of the now mature ova from the follicles and acts as a cue for spawning behaviour.

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). The most common form of endocrine intervention utilized in aquaculture is to mimic or induce the transient surge of LH that initiates the onset of FOMO (Lam 1982). This form of intervention can take place through direct treatment with piscine LH in a crude form as an extract from the pituitary gland of species such as carp (CPE), or purified fish gondotrophin. Alternately mammalian gondadotrophins, such as Human Chorionic Gonadotrophin (HCG), have been used to mimic the effects of endogenous LH. Another approach has been to utilize synthetic hypothalamic gonadotrophin releasing hormone (GnRH) and its superactive analogues (known as GnRHa or LHRHa) to induces surges of LH via the hypothamic/hypophyseal axis.

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. 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). 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 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 LHRHa, is critical in many species. Applied too early in the maturational cycle they 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. Such oocytes have developed the receptors and pathways responsive to surges of LH 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 requires specialist facilities, is time consuming and requires detailed knowledge to identify key indicators of the process. 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. Such approaches were recently reviewed by West (1990) who noted that all suffered from various limitations and, apart from detailed histological examination which is time consuming, did not in general accurately indicate the physiological status of the individual fish.

While failure to undergo FOMO and spawning is the most commonly encountered disruption to the reproductive cycle of captive fish, reproductive failure can occur at other stages of the ovarian cycle. Nutritional deficiencies have been reported to disrupt the reproductive cycle in captive fish though these in general have been relatively easy to overcome (Izquierdo 2001). The lack of suitable environmental cues can result in perturbation of the vitellogenic process in some species (Peter 1981). The stress of capture or captive conditions can disrupt the reproductive cycle through the production steroid hormones (Stone and Forteath 1994). Typically in these scenarios the response of females to traditional endocrine intervention has been poor and overcoming this difficulty has proved to be problematic. These difficulties have in some cases been eliminated by providing captive conditions more closely resembling the natural environment of the fish to provide the appropriate stimulus (Crim 1991). Where this has failed or the appropriate stimuli are unknown novel hormonal intervention approaches such as sustained release delivery systems utilizing pellet implants have been employed (Marte et al 1987).

Environmental Factors Controlling Final Maturation and Spawning

Researchers have consistently emphasized the importance of water temperature in controlling the maturation and spawning of Macquarie perch. The role of other environmental factors has received much less attention. There are many reports of Macquarie perch making 'spawning runs' up rivers. Largely these have been reported as movements out of lakes a short distance upstream into inflowing rivers into areas with suitable substrates for spawning. Wharton (1968) reported such movements out of Lake Eildon as occurring once river temperatures exceeded 16.5 °C while Gooley and McDonald (1988) reported movements out of Lake Dartmouth as occurring when maximum temperatures ranged from 14 to 18 °C. These conflicting observations suggest that while temperature may be an important factor stimulating migration, it may not be the only one.

Historically there are accounts by professional fishermen of Macquarie perch making upstream migrations associated with major spring flooding in large rivers such as the Murray. Within the Mitta Mitta River system there are accounts of the species accumulating at rock barriers and moving upstream and out of the river itself into feeder streams during spring (Trueman 2007). Observations of wild spawning of Macquarie perch have been made over a number of consecutive seasons in the Hughes Creek near Seymour. The lower sections of the Hughes Creek near Avenel is largely silted and of sand substrate. In the 1995 and 1996 seasons individuals and groups of fish could be seen moving upstream towards a section of creek 15 km upstream of Avenel where the gradient increased. Here the creek entered a gorge and formed distinct pools over a boulder, pebble and sand substrate. The clarity of the water allowed individual fish to be observed as well as acts of spawning.

In 1996 male fish appeared to arrive and accumulate at these spawning beds on the 16-17th of September when water temperatures were 13 °C. This was several weeks prior to the gravid females, numbers of which were apparent by the 7th of October when temperatures had risen to 15 °C. Acts of spawning were observed over the following three weeks at temperatures from 15 – 18 °C (W. Trueman, pers. obs). These observations were supported by catches of anglers upstream of the Avenel area where predominantly male fish were taken early in spring and subsequently female fish a few weeks later. During the 1990's Macquarie perch were a popular target of local anglers in that water. Since then a ban on the taking of the species from that water has been introduced to protect the population. Similarly NFA members have observed male Macquarie perch to accumulate in spawning areas in the Yarra River between Yerring and Templestowe some weeks before the females and then become more difficult to angle (R. Lewis & N. Thorne, NFA, pers. com.).

It is possible that water temperature singularly and acting alone may not control the migration of Macquarie perch and associated ovarian development. Studies on the reproductive cycle of the species in Lake Dartmouth indicate that testicular and ovarian maturation are initiated at different times (Appleford et al 1998). This observation may be explained simply by the reproductive systems in the two sexes responding to different set points in temperature or it may imply that different stimuli may be involved for the two sexes.

Stream flows and flooding may also play a role in the reproductive process. Lake (1967) suggested that a substance such as petrichor released into the water from the soil during flooding may have been responsible for inducing final maturation and spawning in golden and silver perch. It is plausible that a similar mechanism triggering migration or maturation in Macquarie perch could exist. Flooding associated with high flow rates could be detrimental by dislodging spawned eggs resulting in their mortality and providing a negative stimulus to adults. However flood events could create ideal situations for the recruitment of larvae, supported by the fact that populations have shown excellent recruitment during the filling phase of impoundments. A strategy of migration induced by flooding and then spawning after the flood peak is a plausible one.

Pheromones and social interactions have been reported to be important agents controlling migration, final maturation and spawning in a range of fish species (Jalabert 2005). It is possible that pheromones emitted by males may influence the reproductive processes in female Macquarie perch. There are numerous historical accounts of anglers being able to detect a distinctive odour of aggregations of the species and even dogs reacting to their presence (Trueman 2007). This area has

previously received no attention from researchers for what has been reported to be at times a gregarious or social species of fish.

Cadwallader and Rogan (1978) reported that, during the course of their study, individual tagged Macquarie perch in Lake Eildon in almost every case returned to the same river each year for spawning. This high fidelity suggests the possibility that in addition to migration that homing may take place. Homing has been reported in many species of migratory fish with imprinting by olfactory cues occurring at the juvenile stage. The possibility that alterations to the environment, such as changes in vegetation, have altered olfactory cues may have impacted upon Macquarie perch populations seem unlikely but given the scant knowledge of this area in Australian fish cannot be dismissed altogether.

Macquarie perch spawning bed in the Hughes Creek. (Photo courtesy of Stephen Kerris.)

Female Maturity and Oocyte Assessment

The success of endocrine intervention to induce spawning in Murray-Darling fish species such as golden and silver perch has been demonstrated to be dependent upon the state of maturity of the female fish. In these species changes in the gross appearance of the oocytes, in terms of oil/yolk droplet size and transparency, while not directly indicating the physiological status of the female, have been developed into reliable empirical protocols for spawning induction (Rowland 1983, 1984).

Most reports to date have described the appearance of Macquarie perch oocytes prior to hormonal intervention as being opaque and showing limited variation (Wharton 1973, Gooley & McDonald 1988, Ingram et al 1993). Some histological description of the oocytes have been provided (Sheik-Eldin et al 1995, Appleford et al 1998) but the preparation methods do not lend themselves to samples collected from females earmarked for hormone injection with minutes of examination.

Variations in the gross appearance of oocytes are apparent between fish, though these are usually subtle. In most fish during the spawning season there appears to be very little variation in the size of the oocytes within individual wild fish or between fish of different sizes from the wild. Differences in appearance are apparent between fish from different individual sites, oocytes varying from a white to a strong lemon colour. Often, a small proportion of oocytes are less opaque, revealing limited internal structure. Rarely, females are encountered with semi-transparent oocytes with yolk and oil droplets apparent. Certainly, in the vast majority of female Macquarie perch encountered the progressive changes in oocyte appearance during the reproductive season apparent in species such as golden and silver perch are not apparent.

Overseas workers investigating the reproductive physiology of fish for controlled reproduction for aquaculture have reported the use of a number of substances to increase the transparency of oocytes to reveal internal structure. Glycerol has been used with success though the clearing process requires several hours making its application to spawning trials impractical. The Serra clearing solution has been used in a range of investigations and has the advantage that it acts rapidly, within a period of minutes (Goetz & Bergman 1978; Kestemont 1991; Dasgupta et al 2001). The 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 these structures translucent. It is particularly useful for discerning internal features in fish whose oocytes are opaque which do not lend themselves to the use of other protocols such as the size and distribution of oil droplets. Its main disadvantage appears to be that it can cause distortion of the oocytes of some species through osmotic shock.

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, the freshwater catfish *Tandanus tandanus*, and the Macquarie perch. The solution was modified to a new ratio consisting of ethanol: formalin: glacial acetic acid at 2:3:1 or 3:3:1 diluted with twice the volume of the mixture with water to reduce distortion of the oocytes through osmotic shock. No doubt the effectiveness of the preparation could be improved through appropriate buffering (Trueman 2006).

When applied to the oocytes of these species, clearing took place within a few minutes revealing oil and yolk droplets. Eventually, the germinal vesicle (GV) became visible as an oval brown structure which ultimately became bleached by the solution but remained visible. To accurately assess GV position it was found necessary to roll oocytes individually so as to remove the effect of perspective due to the orientation of individual oocytes (Figure 1). The solution has also proved useful in highlighting the position of the GV 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 without harm while the process takes place.

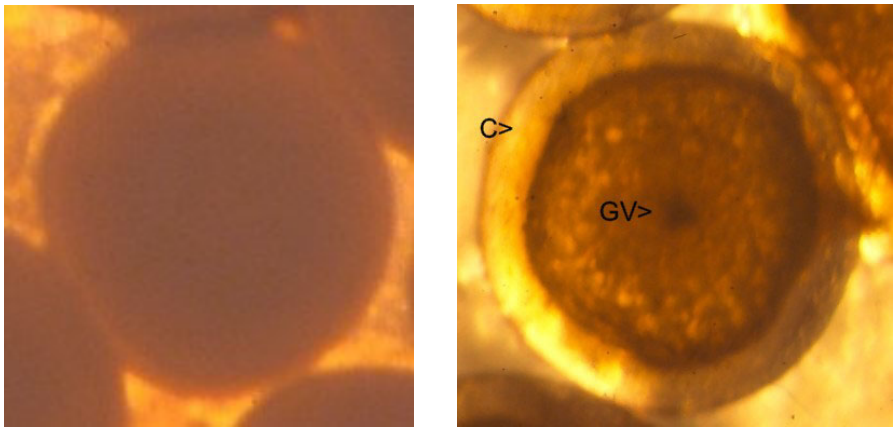

Figure 1. Macquarie perch oocyte before (left) and after (right) treatment with the clearing solution. The future chorion (C) is obvious and the dark germinal vesicle (GV) is visible in the centre of the oocyte.

Overseas workers have developed a number of systems for assessing oocyte maturity in terms of GV position. Some systems are largely descriptive, while others have divided the process into a series of discrete stages. An index incorporating the relative frequency of GV position in oocyte samples has also been used (Kestemont, 1991; Yueh and Chang 2000; Dasgupta et al 2001).

In the mid 1990's the author applied the modified clearing solution to the oocytes of Macquarie perch sourced from a range of locations through the reproductive season. These included live wild fish sourced from Lake Dartmouth, the Mitta Mitta, Gibbo and Yarra Rivers and the Hughes Creek by angling. Also examined were fresh ovaries donated by anglers sourced from wild fish taken from Lake Dartmouth, the Yarra River, the Hughes Creek and Polly McQuinns Weir on Seven Creeks.

These investigations also utilised the oocytes of fish sourced from captive environments including broodstock held by Wartook Native Fish Culture and a number held in small privately owned farm dams. Female Macquarie perch in these small earthen ponds of approximately 0.1 Hectares or less had been present for periods ranging from seven months to nearly ten years. The ponds were well stocked with yabbies and small shrimp and some were regularly fertilized to promote the development of food sources. They were removed with a seine net during the spawning season and examined. Access was gained to a large (approximately 2 Ha) farm dam built across a gully supporting a small, perennial creek. It had been stocked by an angler some years previously with Macquarie perch sourced from Lake Dartmouth. These fish were collected with gill nets and also examined.

From this work the appearance of Macquarie perch oocytes in their natural state and after treatment with the clearing solution was classified into a series of descriptive stages. These stages have been numbered to match descriptions provided by West (1990) for fish oocytes in general and by Appleford et al (1998) for those of Macquarie perch:

Stage 1

These are tiny, previtellogenic oocytes present in ovarian samples throughout the reproductive season. The clearing solution revealed the presence of the GV in a slightly eccentric, central position. These match the description of oocytes in the perinucleolar stage as described by West (1990) and Appleford et al (1998).

Stage 2 (Figure2)

Oocytes are totally opaque and show variations in size. When treated with clearing solution the primary envelope or chorion (future egg shell) is relatively thin and the germinal vesicle is central to very slightly eccentric. Oil and yolk droplets are small and cannot be differentiated from each other. Oocytes at this stage are commonly encountered in samples from fish from late summer through to the time of spawning but predominate in autumn and winter. This matches previous descriptions of the yolk-vesicle and early-vitellogenic stages.

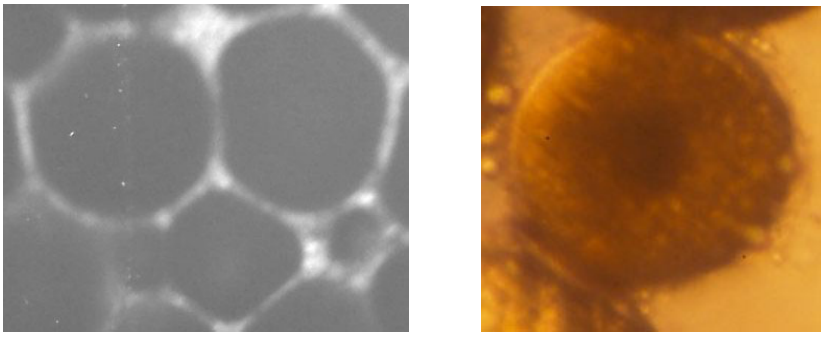

Figure 2. The left photo depicts a smaller stage 2 oocyte (bottom) with two larger stage 3 oocytes (top) prior to clearing. The right photo is of a stage 2 oocyte after clearing.

Stage 3A (Figure 3)

Oocytes are totally opaque, larger (approximately 1.5 mm diameter) and of more uniform appearance. After treatment the chorion appears to be thicker than the previous stage though this varies between individual fish. The GV is still more or less central, though typically slightly eccentric. The oil droplets are slightly larger than the yolk droplets and appear to be concentrated towards the centre, with the yolk droplets visible around the periphery. In wild fish oocytes at this stage predominate in samples taken in the weeks leading up to spawning. This stage appears to define the completion of vitellogenesis.

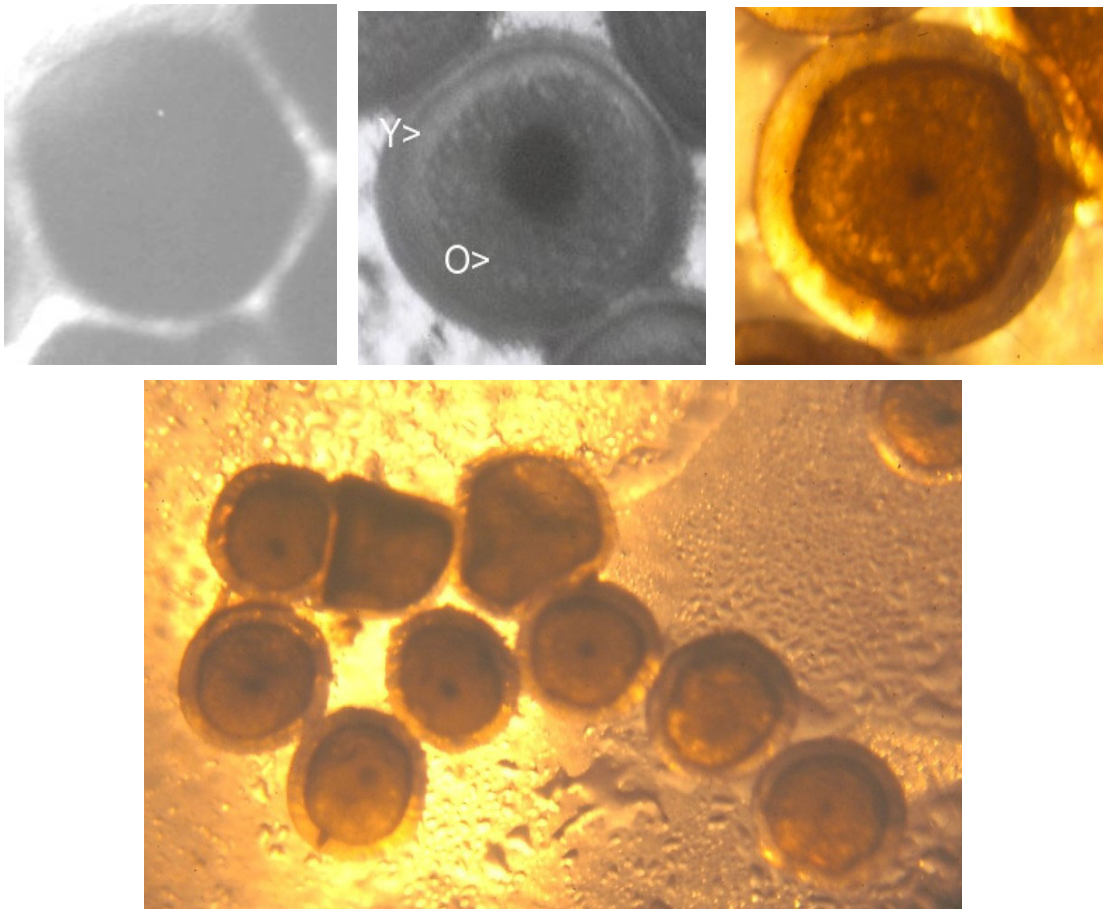

Figure 3. Oocytes at stage 3A prior to clearing (top left) and after (top centre and right). In the top centre photo the oocyte has not completed the clearing process, giving the impression of a large GV. The larger size of the oil droplets (O), concentrated towards the centre, compared to the yolk droplets (Y) visible on the periphery is obvious. This is less apparent in the colour photo though was obvious to the eye. Differences in the thickness of the chorion between oocytes can be seen. The bottom photo illustrates how, dependant upon orientation, the GV in stage 3A oocytes may appear slightly eccentric. Also visible are a few, misshapen atretic oocytes.

Stage 3B (Figure 4)

Oocytes show increased transparency although remain largely opaque. Oil/yolk droplets may just be discerned with strong illumination. The chorion is partially visible without the clearing solution as a clearer boundary layer around the oocyte. This clarity provides the oocytes with a slightly bluish hue under certain lighting angles. In the clearing solution the chorion is thick and the GV is often in a semi-peripheral position, having migrated about 50% towards the chorion. It may be necessary to roll individual oocytes to correctly ascertain its position as they may orientate themselves with the GV in an apical position. Oil droplets are significantly larger than the yolk droplets. Fish containing a majority of oocytes at this stage are infrequently encountered during the spawning season and typically close to the time of spawning.

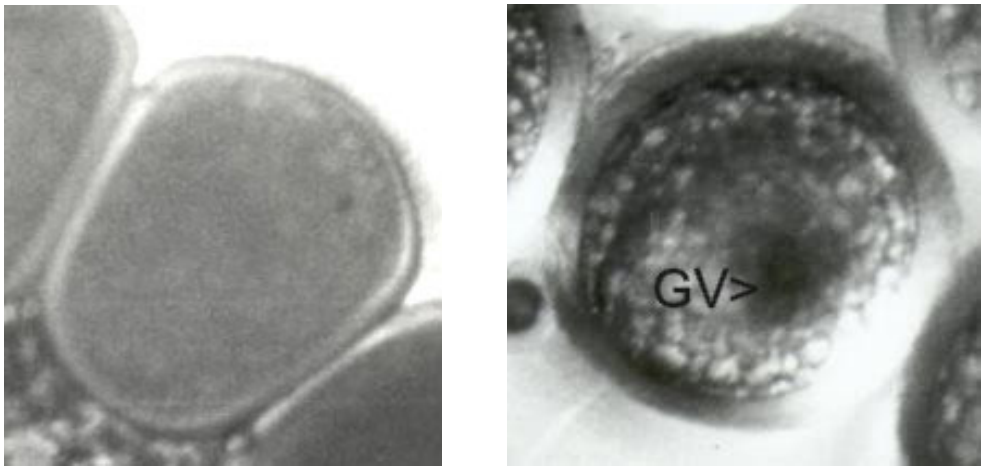

Figure 4. Stage 3B oocytes prior to (left) and after clearing (right). The germinal vesicle (GV) is partially polarized.

Stage 4A (Figure 5)

Oocytes exhibit increased transparency and oil droplets may be discerned, but without great clarity. In the clearing solution the GV is peripheral but usually intact. The oil droplets show some polarisation and have coalesced to form a few dozen larger droplets. In wild fish it was rare to encounter fish with a majority of oocytes at this stage. Oocytes with this appearance appear to be into the transition of what Appleford et al (1998) described being as 'gravid' and 'hyaline' though in preservative oocytes at the previous stage may show some transparency.

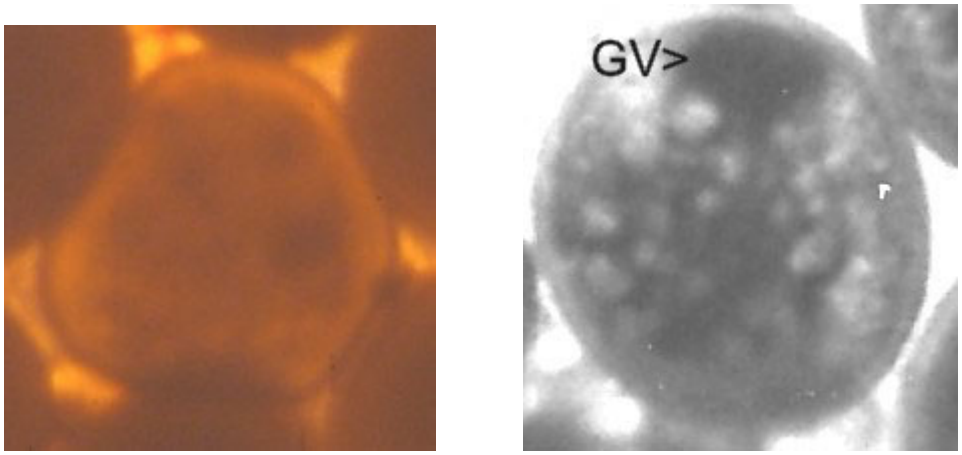

Figure 5. Stage 4 oocytes prior to (left) and after clearing (right). The germinal vesicle (GV) is fully polarized.

Stage 4B (Figure 6)

Oocytes are almost translucent with a group of under a dozen polarized oil droplets present at one pole. They may take on a light grey appearance due to surface roughness evident on the chorion. In samples from some fish oocytes treated with the clearing solution reveal a peripheral brown patch being the dispersed GV and cortical alveoli. Typically the oil droplets are of relatively uniform size. Fish containing stage 4B oocytes appear to be close to ovulation and spawning.

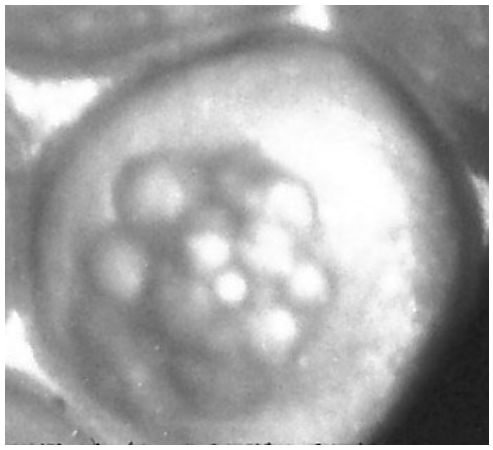

Figure 6

Stage 5 (Figure 7)

In most samples of oocytes taken in the weeks up to and including spawning a small proportion of oocytes are encountered which are atretic and have commenced the resorption process. Typically these oocytes are semi-translucent, larger in appearance and somewhat flaccid. The clearing solution reveals an absence of a GV. The oil droplets may be, in the early stages, dispersed throughout the cytoplasm and in the latter stages misshapened. Treatment with the clearing solution results in distortion of the oocyte, suggesting a breakdown of internal integrity.

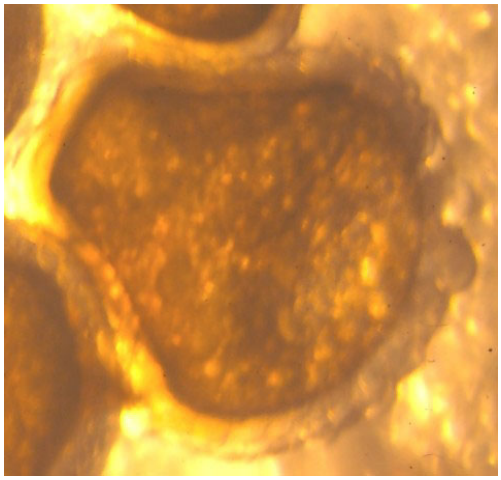

Figure 7

Pattern of Oocyte Development

Wild Fish

Female Macquarie perch were captured by angling from the Gibbo and Mitta Mitta rivers at the opening of the season in mid December 1995. It was found that the larger females over 800 g were spent, but many smaller females containing maturing oocytes were still present, particularly in the Gibbo river arm near the confluence of the inflowing river water. Females as small as 350 g contained advanced oocytes. This contrasts with the observations of Appleford et al (1998) who found females to be mature when over 1 kg in weight. However at the time of their study (pre 1982) Lake Dartmouth was in its filling phase resulting in rapid growth rates which would tend to increase the body weight at maturity.

In general most gravid females captured in Lake Dartmouth itself contained oocytes the majority of which were at stages 2 or 3A though a few fish some had progressed to stage 3B (Figures 8 & 9). In the inflowing rivers females were captured containing oocytes from stages 3A to 4A and with a few individual fish containing oocytes at stage 4B (figures 10 & 11). It was noted that in about one third of the gravid females captured that the ovaries exhibited asynchronous development, the oocytes being at a diverse range of stages, from stage 2 through to stage 4A. A substantial proportion of gravid females showed evidence of the commencement of resorption of oocytes at this time.

Macquarie perch were captured by angling from the Yarra River in the Wonga Park area over several seasons during the months of October to December. Females as small as 280 g were found to be mature and appeared destined to spawn. Most females captured had ovaries containing oocytes at stages 2 and 3A with a few being encountered at stage 3B. All females captured had ovaries exhibiting synchronous development of oocytes, the variations seen in many Lake Dartmouth fish not being apparent. Females caught were either gravid or spent, with no resorbing females encountered.

In early October 1995 when the water temperature was 14 ºC two female Macquarie perch captured by anglers from the Hughes Creek were inspected one of which had clearing oocytes with large oil droplets and was therefore undergoing final maturation. The other had opaque oocytes which are commonly encountered with the species. At that time the creek was falling after recent flooding due to heavy rain. On October 29 five females were examined one of which was spent, three undergoing resorption (indicated by the presence of misshapen oocytes and excess fluid in ovarian samples) and one had large opaque oocytes that had undergone slight clearing and was probably undergoing final maturation. The water temperature was recorded as being 17 °C.

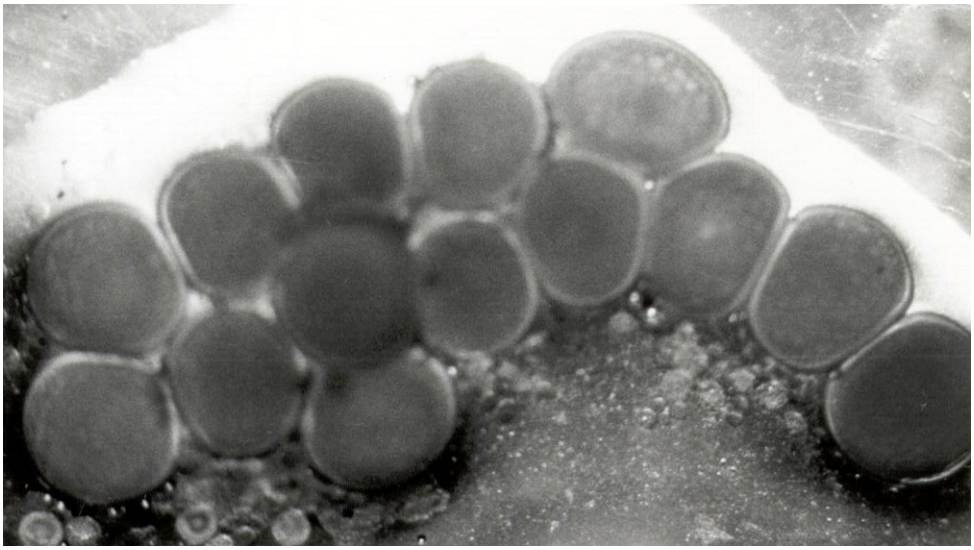

Figure 8. Oocyte sample of a Macquarie perch sourced from Lake Dartmouth before clearing and utilizing strong illumination. The oocytes appear to be relatively uniform with an obvious chorion and hints of internal droplet structure. One oocyte is significantly larger and clearer than the rest.

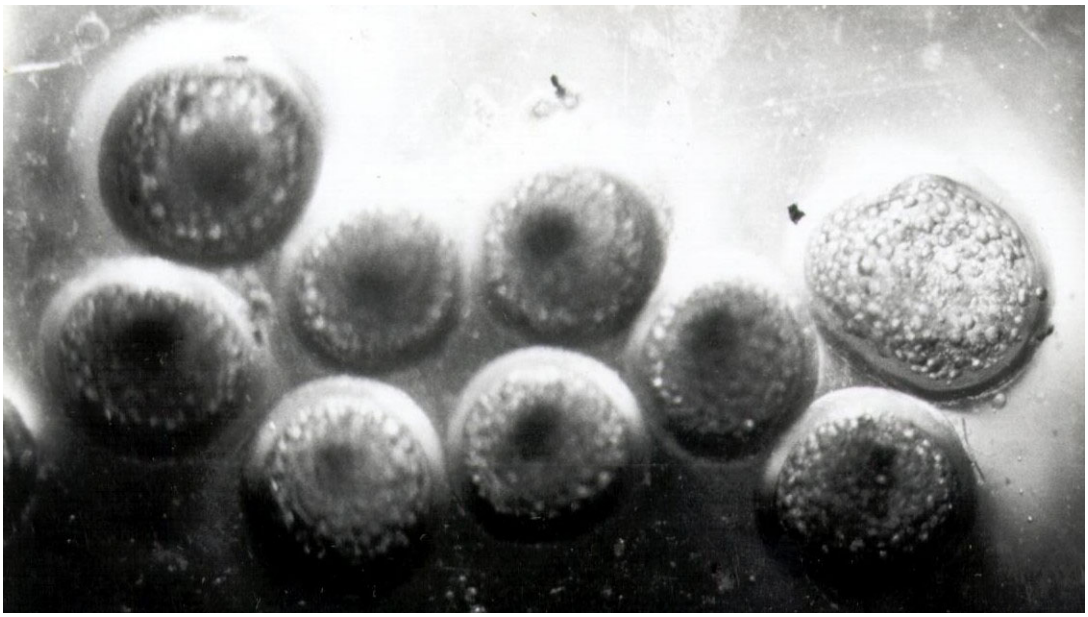

Figure 9. Oocyte sample from the same Macquarie perch as in Figure 8 after clearing. Many oocytes contain GV in sub-peripheral positions. Some appear to have central GV, but have not as yet been rolled to ascertain their correct position. This female was successfully induced to ovulate with *Ovaprim*® and produced viable ova. The large oocyte in the previous figure appears to be atretic showing disruption to its internal structure, lack of a GV and distortion in the clearing solution.

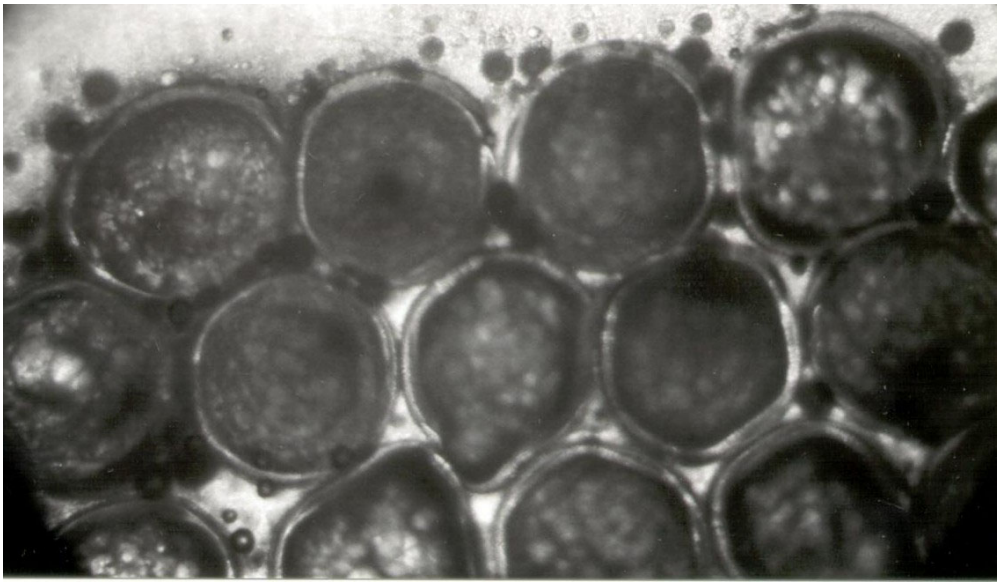

Figure 10. Oocyte sample from a Macquarie perch captured in the Gibbo River. The oocytes are semi-translucent with oil droplets visible under strong illumination.

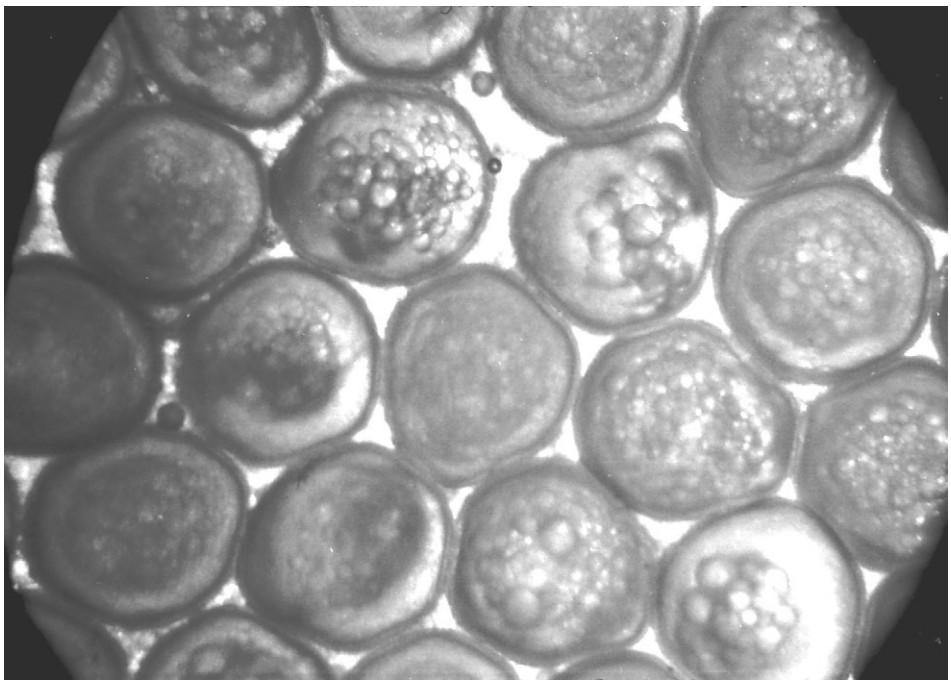

Figure 11. Oocyte sample from the same female as in Figure 10 after treatment with the clearing solution. Many oocytes are at stage 4A with oil droplets becoming polarized and GV in the peripheral position. Some oocytes have progressed to stage 4B indicated by coalescence of oil droplets and GV breakdown. A few oocytes are atretic indicated by disruption to internal structures. This fish was successfully induced to ovulate with HCG.

Captive Fish

Females removed from the small ponds when bottom temperatures had reached 17 °C contained oocytes of variable size and appearance. No fish contained oocytes that had progressed to having sub-peripheral GV. In the majority of oocytes the GV were central, but there were variations in the size of the oocytes indicating asynchronous development (Figure 12). One large female of 1.2 kg however contained oocytes which were of uniform size and appearance. They were lemon-white in colour, approximately 1.5 mm in diameter and contained a central GV.

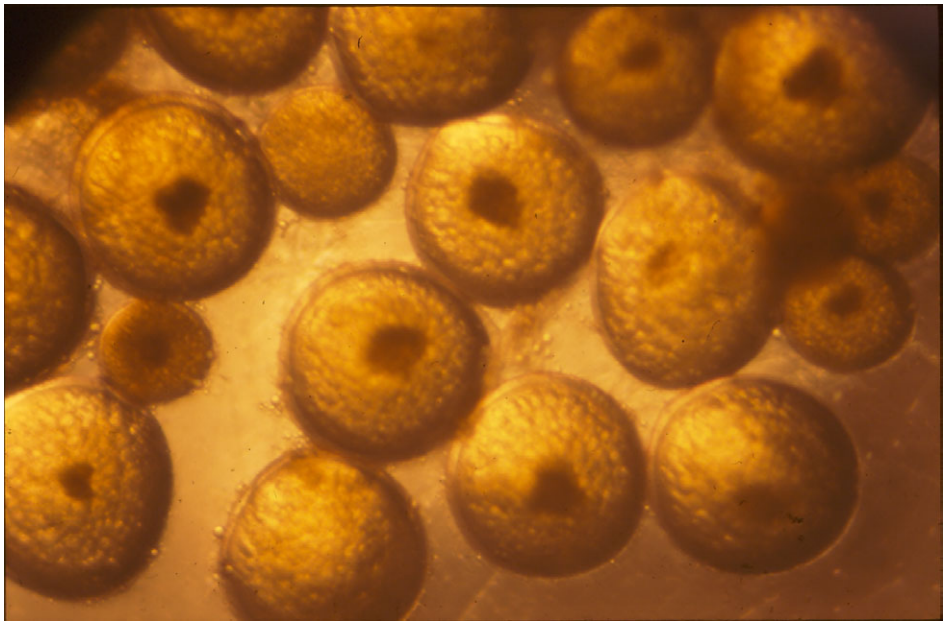

Figure 12. Oocyte sample taken from a Macquarie perch held in a small pond after clearing. The oocytes show variations in size, indicating asynchronous development, and none have progressed56 to having sub-peripheral GV. This fish received treatment with *Ovaprim*® resulting in partial ovulation. None of the ova produced proved to be viable.

Female Macquarie perch were gill netted from the farm dam in late October at a time when water temperature at a depth of 2 m was recorded at 15 °C and the inflow into the dam at 18°C. These fish were examined and most were found to contain oocytes of large uniform appearance with central GV (Figure 13 & 14). These females were utilized in most of the trials described below. Two females had ovaries in which the majority of oocytes had GV in the sub-peripheral position (Figures 15 & 16).

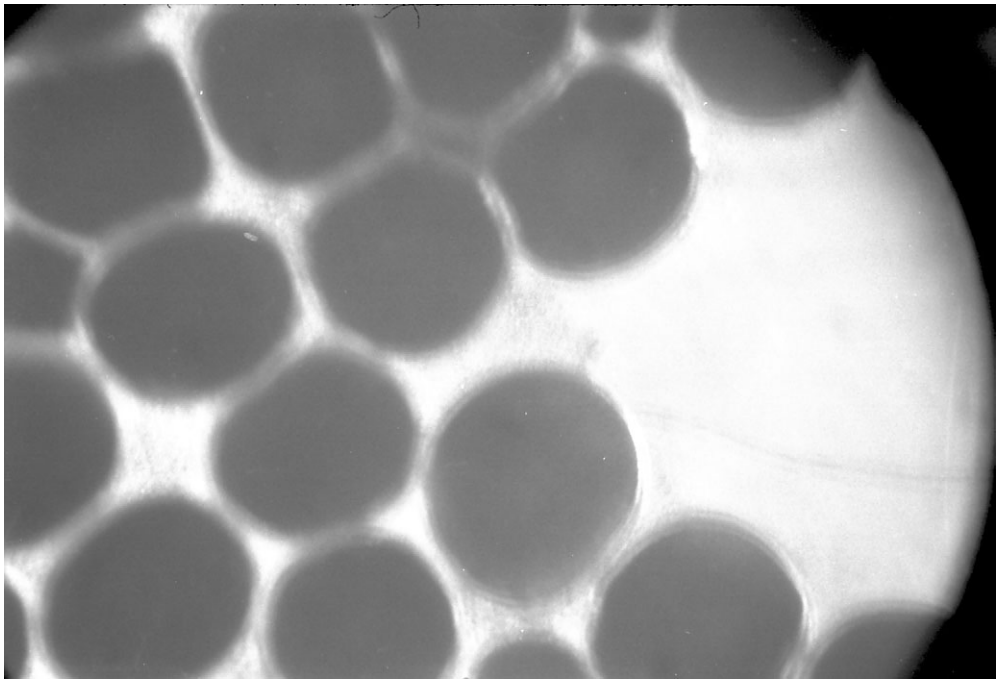

Figure 13. Oocyte sample of a Macquarie perch sourced from the farm dam before clearing and utilizing strong illumination. Most oocytes are opaque with some suggestion of the chorion being present.

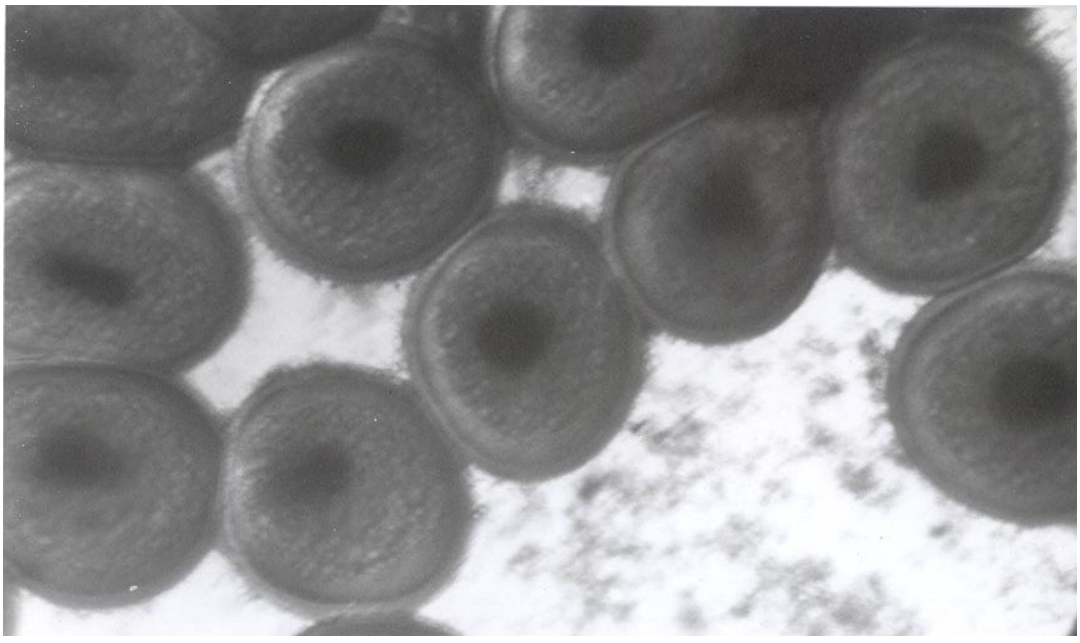

Figure 14. Oocyte sample from the same fish as in Figure 13. Most exhibit well developed chorions and GV in central to slightly eccentric positions. This female was treated with *Ovaprim*® and ovulated semi-opaque ova which proved to be unviable.

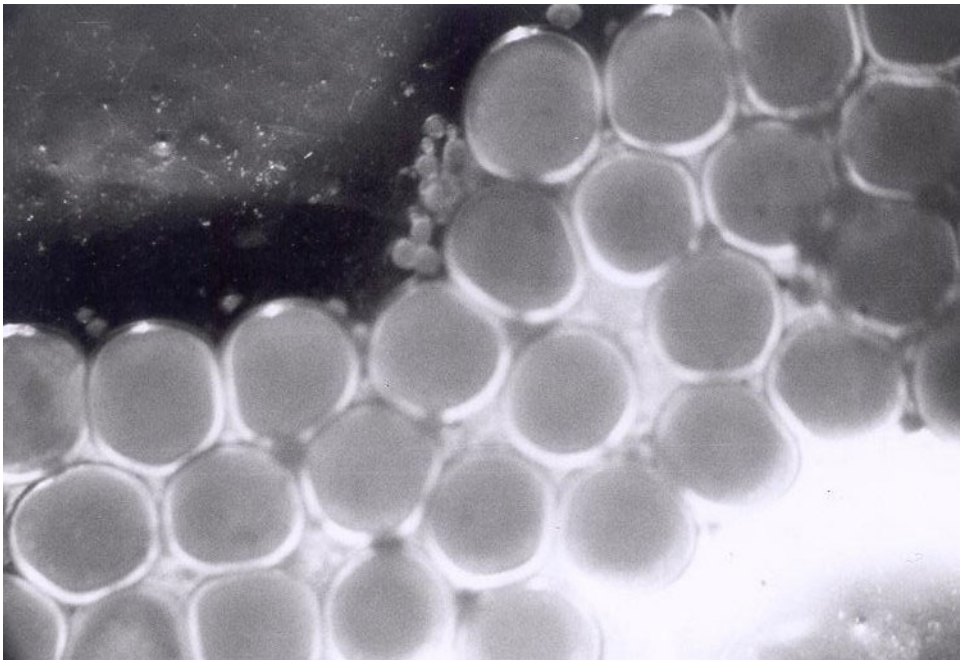

Figure 15. Oocyte sample of a Macquarie perch sourced from the farm dam before clearing. They appear to be relatively uniform in appearance with an obvious chorion and hints of internal droplet structure.

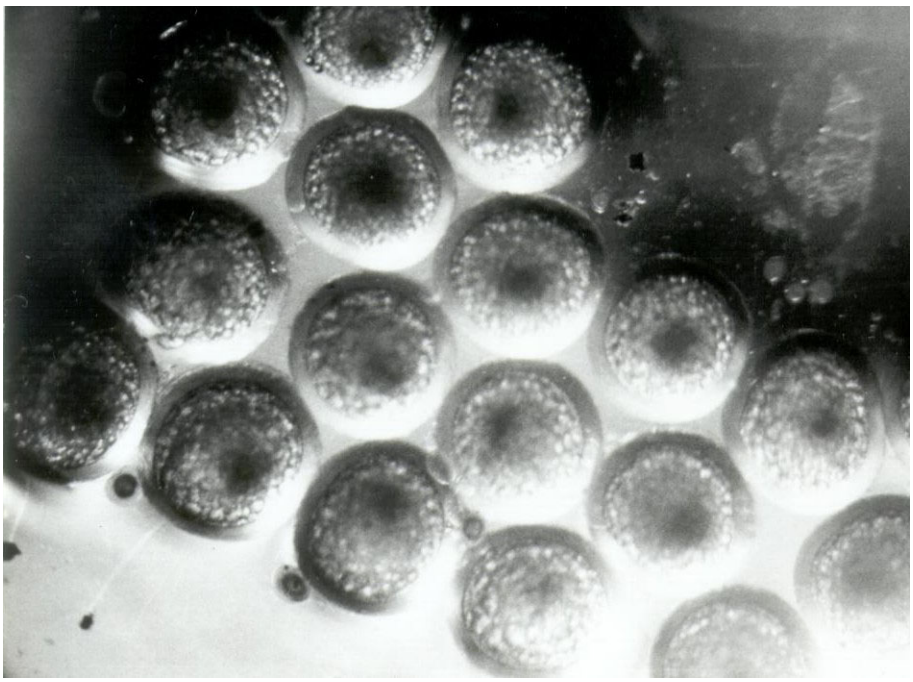

Figure 16. Oocyte sample from the same Macquarie perch as in Figure 15 after clearing. Many oocytes contain GV in sub-peripheral positions. Some appear to have central GV, but have not as yet been rolled to ascertain their correct position. This female was successfully induced to ovulate with *Ovaprim*® and produced viable ova

Discussion on the Ovarian Cycle

Caution has to be exercised in interpreting these observations due to the lack of suitable controls and the relatively small number of fish examined. It appears that in most wild females and those sourced from the large dam that oocyte development progressed from stage 2 to stage 3A by the spring months. However, the majority of fish held in the small ponds showed inconsistent progression to stage 3A and usually asynchronous development of the oocytes. The rarity of captive fish with oocytes at stage 3B stage suggests that either this phase of oocyte development is brief or alternately that captive conditions may impede progression to this stage. However, the relative scarcity of wild fish encountered with oocytes at this stage combined with these observations suggests that females rapidly progress from stage 3A to 3B upon which the oocytes may remain in that state for a relatively short period of time.

No female to date has been encountered containing oocytes with GV between the sub-peripheral and peripheral positions suggesting that the transition between these stages is rapid. Females containing oocytes with fully polarized GV at stage 4 were rarely encountered and those that were had been captured from spawning aggregations in the rivers. This may suggest that, after rapid progression from stage 3B, incorporating the completion of GV migration, oocytes may pause briefly at stage 4A with polarized GV awaiting a final stimulus.

These observations are supported by those of NFA members capturing broodstock Macquarie perch from the Yarra River. In recent years they have used the clearing solution to assess maturity and have found most oocytes during the spawning period to have central GV with a few fish containing GV in the sub-peripheral position. Fish containing oocytes with fully migrated GV appear to be rarely encountered.

Spawning Trials

Broodstock

Macquarie perch sourced from the ponds, farm dam, and the Mitta Mitta, Gibbo and Yarra Rivers were utilized in a series of trials to assess the activity of a range of hormones in promoting the completion of vitellogenesis, final maturation and ovulation. The trials incorporated the use of females containing oocytes at stage 3A, 3B or 4A with GV in the central, sub- peripheral or peripheral positions. Females containing oocytes of largely uniform appearance were usually selected. Additional trials with females containing asynchronous oocytes encompassing a range of stages were also undertaken.

Wild Fish Spawning Trials

(a) HCG and *Ovaprim*®

Wild female Macquarie perch (weight range 500 – 800 g) captured by angling on the same day were involved in a comparative trial investigating response to hormonal treatment at different oocyte stages. The water temperature on the day of capture was recorded at 16 °C. The fish were selected on the basis of having oocytes of largely uniform appearance with the majority being at a specific stage of development. The females were treated with *Ovaprim*® (Syndel Laboratories Ltd.) at a dose of 0.5 mL/kg or HCG (*Chorulon*®, Intervet) at 1000 i.u./kg via intraperitoneal injection within five hours of capture.

After injection, females were placed in aquaria with males injected with *Ovaprim*® at the same rate as the females. The fish were examined 36 hours after injection and thereafter at intervals dependant upon the appearance of the oocytes. Where artificial fertilisation of ova was attempted the dry method was used. Fertilised eggs were incubated in aquaria provided with a flow of water. Fertilisation rates were estimated by removing five batches of approximately 100 eggs, examining them microscopically to determine their viability, and averaging the result. The results are presented in the Table 1 below.

(b) Trials with Wild Fish Containing Asynchronous Oocytes

Two females with asynchronous oocytes captured in the Gibbo River arm of Lake Dartmouth, containing oocytes from stage 2 to stage 4, were injected with hormones to determine if viable ova could be produced from such fish. One female was treated with *Ovaprim*® and the other with HCG as described previously. Both fish ovulated within 44 hours of injection and the ova produced were fertilized. It appeared that either or both the stage 3B or 4A oocytes had completed normal maturation and development. Stage 4B or 5 oocytes had become enlarged and flaccid while early stage oocytes had partially cleared. A small proportion (<20%) of the eggs completed development and hatched.

Table 1. Results of Trials with HCG and Ovaprim® on Wildfish.

Oocyte Description	Hormone	Result
Nearly all oocytes at stage 3A with central GV and thick primary envelope. A small proportion of oocytes slightly clearer.	HCG	Oocytes had enlarged and become clearer at 47 hours. By 73 hours partial ovulation had occurred, but the ova appeared to be covered by an opaque membrane. Ova were artificially fertilised but failed to develop.
Nearly all oocytes at stage 3A with central GV and thick primary envelope. A small proportion of oocytes slightly clearer.	*Ovaprim*®	Oocytes larger and clearer at 36 hours. Ovulation occurred at 49 hours, ova still semi-opaque. Ova were artificially fertilised but failed to develop.
Most oocytes at stage 3A with central GV and thick primary envelope. Some oocytes were slightly clearer and had commenced stage 3B with GV positioned 1/3 towards periphery.	*Ovaprim*®	Ovulation occurred at 44 hours but oocytes had cleared only slightly. Ova were artificially fertilised but failed to develop.
Most oocytes were at stage 3B and were opaque with sub-peripheral GV. A small proportion had central GV.	*Ovaprim*®	Ovulation occurred at 47 hours, the ova were fertilised, most commenced cell division, 74% completed gastrulation and hatched.
Approximately 2/3 of oocytes were at stage 3B and were opaque with sub-peripheral GV. The balance were at stage 3A with a central GV.	HCG	Ovulation occurred at 43 hours and oocytes had cleared significantly but were not translucent. Ova were artificially fertilised but failed to develop.
Approximately 3/4 of oocytes at stage 4A with GV intact and at the peripheral position. The balance were at stage 4B, but appeared large and flaccid.	*Ovaprim*®	Ovulation occurred at 40 hours, the ova were fertilised, many commenced cell division, 56% completed gastrulation and hatched.
Most oocytes at stage 4A with GV intact and at the peripheral position. The rest were at stage 4B, but appeared large and flaccid.	HCG	Ovulation occurred at 37 hours, the ova were fertilised, most commenced cell division, 43 % completed gastrulation and hatched.
Most oocytes were at stage 4B, no GV was apparent.	*Ovaprim*®	At 31 hours oocytes were larger, clear and appeared overripe. Partial ovulation occurred at 58 hours and all ova were flaccid and had a single large oil droplet. They were fertilised and cell division occurred in a small proportion, less than 10% hatched.

Captive Fish Spawning Trials

(a) Large Farm Dam

Female Macquarie perch (weight range 650 – 1100 g) were captured with a gill net from the farm dam in late October adjacent to the inflowing creek water which was at a temperature of 18 °C. The owner of the dam had observed an aggregation of fish in previous days in the vicinity of where the creek entered the dam. After capture the fish were examined and most were found to contain oocytes of largely uniform appearance with central GV. One or two females contained asynchronous oocytes at various stages of development. In addition two females containing a majority of oocytes with GV in the sub-peripheral position were captured.

The rationale for these trials was to replicate the work previously done with the wild fish for comparative purposes. As for the wild fish trials, females were selected on the basis of having oocytes of largely uniform appearance with the majority being at a specific stage of development. The females were treated with HCG or *Ovaprim*® as described for the wild fish trials within three hours of capture. The results are presented in the Table 2 below.

Table 2. Results of Trials with Captive Fish Sourced from the Large Farm Dam

Oocyte Description	Hormone	Result
Nearly all oocytes at stage 3A with central GV and thick primary envelope. A small proportion of oocytes slightly clearer.	HCG	Oocytes had enlarged and become clearer at 50 hours. By 62 hours partial ovulation had occur, but the ova were opaque. No attempt was made to fertilise the ova.
Nearly all oocytes at stage 3A with central GV and thick primary envelope. A small proportion of oocytes slightly clearer.	*Ovaprim*®	Oocytes larger and clearer at 39 hours. Ovulation occurred at 50hours, ova semi-translucent. The ova were artificially fertilised but failed to develop.
Most oocytes were at stage 3B and were opaque with sub-peripheral GV. The balance were at stage 3A with a central GV or atretic.	*Ovaprim*®	Ovulation occurred at 40 hours, the ova were fertilised, many commenced cell division, 57% completed gastrulation and hatched.
Most oocytes were at stage 3B and were opaque with sub-peripheral GV. The balance were at stage 3A with a central GV.	HCG	Ovulation had occurred at 50 hours but oocytes had cleared only slightly. Eggs were artificially fertilised but failed to develop. No attempt was made to fertilise the ova.

(b) Small Ponds

Female Macquarie perch (weight range 400 – 1200 g) were removed from the small ponds when bottom temperatures had reached 17 °C and contained oocytes of variable size and appearance. No fish contained oocytes that had progressed to having sub-peripheral GV. In the majority of oocytes the GV was central, and in many there were variations in the size of the oocytes, with fish generally showing asynchronous development. One large female of 1.2 kg and several smaller fish however contained oocytes which were of uniform size and appearance. The oocytes were a lemon-white colour, approximately 1.5 mm in diameter and contained central GV.

A number of females, encompassing fish with both synchronous oocytes at stage 3A and asynchronous oocytes, were injected with *Ovaprim*®. Some fish ovulated, some partially ovulated and others failed to ovulate. Apart from partial clearing of the oocytes, *Ovaprim*® failed to promote significant internal maturation in the oocytes, even though in some cases ovulation occurred. A number of females from the small ponds were included in some of the trials described below.

Other Hormone Trials

(a) FSH Treatment

Two females sourced from a small pond, containing asynchronous oocytes with central GV were injected with mammalian follicle stimulating hormone (FSH) (*Folligon*®, Intervet) at a rate of 100 i.u./kg and examined 48 hours later. In one fish it was observed that some oocytes had increased slightly in transparency. After treatment with the clearing solution the oil droplets in these oocytes had increased in size and the GV had moved to varying degrees from the central position. This fish received a second treatment of *Folligon*®. The fish was again examined at 68 hours and at that time it was injected with *Ovaprim*® at 0.5 mL/Kg.

At 84 hours the more advanced oocytes had developed fully polarized GV while others had commenced GV migration (Figure 17 below). At 96 hours the female had partially ovulated and the ova were artificially fertilised. It was apparent that the ovulated ova varied in their stage of maturity, but a proportion underwent normal development and approximately 2000 larvae hatched (Figure 18 below).

The trial was repeated with a female sourced from the large dam containing oocytes with central GV. This fish was injected with FSH at a rate of 500 i.u./kg and when examined twenty four hours later it was found that the oocytes had cleared slightly, and in some the GV had migrated to the sub-peripheral position. The fish was then injected with *Ovaprim*® at 0.5 mL/Kg and ovulation had taken place by 48.5 hours after the first injection. The ova generally appeared slightly flaccid and showed variations in the number and size of oil droplets. They were stripped, artificially fertilised and a few thousand completed normal development and hatched.

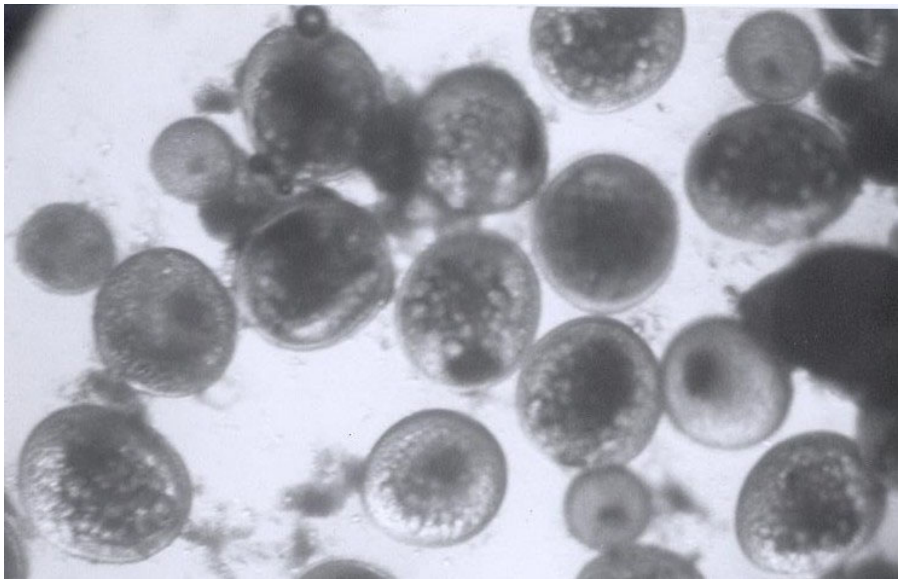

Figure 17. Oocytes from a Macquarie perch containing asynchronous oocytes 84 hours after initial treatment with *Folligon*® and injection of *Ovaprim*® at 68 hours.

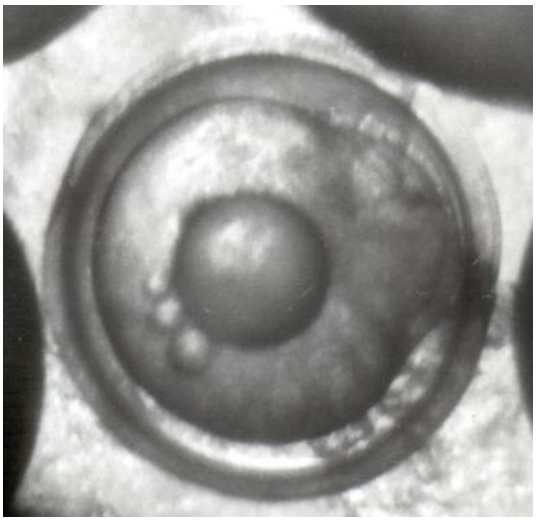

Figure 18. Fertilised egg of a Macquarie perch produced through treatment with FSH.

(b) Long Term treatment with LHRHa

Various workers have reported the use of sustained delivery of LHRHa (GnRHa) to promote maturation and spawning in cultured fish (Marte et al 1987). This approach has proved to be useful in promoting ovarian development, and has been particular useful when applied to batch spawning species such as Barramundi, *Lates calcarifer*.

Two female perch, one from the large dam and the other from a pond, were implanted with cholesterol pellets containing 100 μg/pellet of LHRHa (des-Gly^{10}, [D-Ala^{6}]-LHRH Ethylamide, Sigma Product No. L4513), utilizing the method described by Lee et al (1986), into the shoulder musculature. Both females contained oocytes of uniform appearance with central GV at stage 3A. After implantation the fish were placed in a small pond of 0.1 Ha and examined 2, 4 and 6 weeks after implantation. At 2 weeks a slight increase in oocyte transparency and size in both fish was visible, but no change in GV position. By 6 weeks it was apparent that both fish had commenced resorption of the oocytes.

(c) Prostaglandin PGF2α

Prostaglandins are substances involved in reproductive behaviour and ovulation in many vertebrates including fish. Stacey (1976) reported that injection of 5 mg/kg of the prostaglandin F2α (PGF2α) induced spawning behaviour in goldfish. PGF2α has also been used to induce ovulation in fish oocytes in both in vitro studies and in fish. Jalabert and Szollosi (1975) observed *in vitro* ovulation of rainbow trout oocytes incubated with PGF2α at 1 and 5 μg/1. Goetz and Theofan (1979) reported that PGF2α concentrations as low as 0.16 μg/ml induced ovulation *in vitro* of yellow perch oocytes. There are also reports of injections of this prostaglandin inducing ovulation in fish either alone or after pre-treatment with gonadotrophins or GtRHa (Goetz et al 1991).

Ingram et al (1994) suggested that prostaglandins be trialled in Macquarie perch and Sheikh-Eldin et al (1996) inferred that problems in the metabolic pathway synthesizing prostaglandins may have been responsible for spawning failure in captive Macquarie perch. Consequently trials were conducted investigating the effectiveness of a prostaglandin in inducing ovulation in Macquarie perch.

A female sourced from the Gibbo River containing a majority of oocytes at stage 4A with the GV in the peripheral position was treated with PGF2α (*Lutalyse* ®, Upjohn) by intraperitoneal injection to give an effective dose of 5 mg/kg within 6 hours of capture. Within 30 minutes the fish was observed to adopt a head down position and a spermiating male was placed in the tank. When examined 39 hours after injection a significant proportion of the oocytes had ovulated. However, while the oocytes had increased slightly in transparency, polarization and coalescence of oil and lipid droplets had not taken place to any noticeable degree. No attempt was made to fertilise the ova. A second female with oocytes at stage 3A was also treated and behaved in a similar manner with an hour of injection. Oocytes increased slightly in diameter but again gross changes in internal structure were not apparent. Partial ovulation occurred but no attempt was made to fertilise the ova.

A female sourced from the farm dam containing uniform large oocytes at stage 3A with central GV was injected with PGF2α at the same rate as the wild fish. The female was examined 48 hours later and it was found that the oocytes had increased in size slightly and had become semi-opaque. After injection this female also adopted a head down position and male appeared to actively court the female. At 24 hours the oocytes had cleared noticeably and the fish was injected with *Ovaprim*® at 0.2 mL/Kg. When examined at 48 hours the oocytes had not changed their appearance. At 70 hours numerous ova were observed in the tank that were flaccid and contained fine oil droplets indicating that coalescence of oil droplets prior to ovulation had not taken place.

(d) Treatment with Arachidonic acid

The importance of suitable nutrition in fish reproduction is well known with a range of lipids, eicosanoids and polyunsaturated fatty acids being reported to play important roles in the reproductive process. It has been suggested that problems in one of these metabolic pathways may have been responsible for spawning failure in Macquarie perch (Sheikh-Eldin et al 1996). Fatty acids have been demonstrated to play an important role in reproductive processes in fish, with arachidonic acid in

particular being an important component in the metabolic pathways of ovarian steroidogenesis and in the synthesis of $PGF_{2\alpha}$ (Berndtson et al 1989, Van de Kraak & Chang, 1990). In vitro trials indicate that it stimulates ovarian testosterone production at concentrations equivalent to 32.65 –3.265 mg/L (Van de Kraak & Chang, 1990).

In order to investigate the hypothesis of a deficiency in the biosynthetic pathway encompassing arachidonic acid impacting upon final oocyte maturation and ovulation a number of trials were conducted whereby female Macquarie perch containing stage 3A oocytes were injected with a 'priming' dose of arachidonic acid, followed 24 hours by treatment with *Ovaprim*®. Arachidonic acid (Sigma product# A3925, 90% free acid) was initially dissolved in a small volume of ethanol followed by the addition of physiological saline and injected via the intraperitoneal route at a rate of 10 mg/kg.

In the first trial the female when examined 24 hours after injection it was noted that the oocytes had increased in size and the chorion was noticeably clearer. There was also evidence of dissolution of tissue and the presence of lipid droplets around the oocytes. However after treatment with the clearing solution it was apparent that no movement of the GV had occurred or changes in droplet size and structure. The fish was injected with *Ovaprim*® at 0.5 mL/kg and had ovulated when checked at 36 hours. The ova were semi-opaque and no attempt was made to fertilise them. In a second trial another female was similarly treated with arachidonic acid, but without treatment with *Ovaprim*® and monitored every 12 hours. At 24 hours the oocytes had increased in size and cleared slightly and changes in the appearance of the follicles suggested that the oocytes were destined to ovulate. The fish partially ovulated at 31 hours but the ova were again semi-opaque and unviable.

DISCUSSION

The results of the investigations reported herein may provide an insight as to where the difficulties lie in historic attempts at the artificial propagation of the Macquarie perch. Previously, oocyte appearance has not been a useful tool for assessing the physiological status of female perch and the likelihood of successful induced ovulation and spawning. Changes in oocyte appearance in the species are subtle and subjective. The use of the Serra clearing solution revealed internal changes in oocyte structure apparent in species with less opaque oocytes such as the golden perch. In terms of gross changes in oocyte morphology the clearing solution makes apparent the progressive enlargement and coalescence of oil droplets used reliably as a protocol for hormone-induced spawning in some other Australian native fish species.

From the limited trials outlined in this report, it appears that females containing oocytes with a central GV are not responsive to treatment with HCG or *Ovaprim*®, at least in terms of producing viable ova. Progression to the sub-peripheral phase appears to indicate a change in physiological status to where the fish become more responsive to *Ovaprim*® and HCG though in the latter the ova produced may not viable. Oocytes with peripheral GV are responsive to intervention with both *Ovaprim*® and HCG. As a direct indicator of the physiological status of female Macquarie perch GV position may be a more reliable and powerful tool then gross morphological features such as oocyte clarity and oil droplet size.

Reproductive Failure

Examples of reproductive failure in female Macquarie perch were encountered in both captive and wild fish. Using GV position as a criterion it appears that female Macquarie perch can experience several types of disruption to the reproductive process. The first type of failure appears to be an inability to acquire maturational competence, indicated by failure of the GV to move from the central position. No fish were encountered with oocytes at stage 3B that were held in the small ponds. The two females captured at that stage from the large dam have been the only captive fish in that state encountered by the author. Descriptions of the gross appearance of the oocytes of captive Macquarie perch reported in previous studies suggest that in these cases there may also have been a failure to progress from stage 3A to 3B. An alternate explanation may be that the sub-peripheral phase is relatively short and so the probability of encountering a female in that state is low.

Asynchronous development, where oocytes do not develop in synchrony but progress at variable rates, appears to be another type of perturbation to the reproductive cycle of female Macquarie perch. In most captive fish held in small ponds oocyte development appeared to be inconsistent and asynchronous, the majority of females containing oocytes at stages 2 and 3A and not progressing further. This phenomenon was less common in females sourced from the large dam where vitellogenesis was more consistent, with the majority of oocytes in most fish progressing to stage 3A. Asynchronous development of oocytes has also been observed by the author in silver and golden perch in fish held in very small ponds and farm dams (W. Trueman, pers. obs.). Either nutritional inadequacies or extreme variations in environmental conditions such as temperature, which such small water bodies are subject too, are possible explanations. However the asynchronous development observed in oocytes of wild Macquarie perch in Lake Dartmouth combined with the reports of decline in body condition suggests that this form of perturbation to the reproductive cycle could be nutritionally induced.

Sheikh-Eldin et al (1995) reported a similar phenomenon in tank held fish, with oocytes reportedly varying in size and failing to complete final maturation. Similarly the variations in oocyte description provided by Ingram et al 1994 plus the instances of partial ovulation reported suggests that asynchronous development may have taken place in the pond held fish at Narrandera. In the case of these studies and the fish held in small ponds reported here there appears to be also a failure to progress to stage 3B indicated by movement of the GV to a sub-peripheral position. In both the small ponds and the large dam females captured late in the reproductive season had commenced resorption, few had progressed past stage 3A and none past stage 3B. These observations suggest the existence

of two distinctive perturbations to the ovarian cycle of Macquarie perch confounded under the general observation of failure to complete normal development reported in the published literature.

Asynchronous development was also observed in some fish sourced from Lake Dartmouth in December 1995, with some individuals containing oocytes at stages 2, 3A, 3B and 4A. However, in this case some of the oocytes had progressed through vitellogenesis and some degree of final oocyte maturation. This is further evidence to suggest that two discrete phenomenon are acting to disrupt the reproductive cycle, the first creating asynchronous development being apparent in these wild fish while the second preventing the acquisition of maturational competence being absent in the Lake Dartmouth fish.

A number of female Macquarie Perch taken by anglers from the Hughes Creek in October 1995 as well as some fish captured from Lake Dartmouth contained ovaries with oocytes that had commenced the atretic process. This appears to be a distinctive type of failure causing a cessation of the reproductive process at any stage. In the case of Hughes Creek oocyte development in individual fish had progressed to stages 3A and 3B prior to the onset of atresia. At Lake Dartmouth significant numbers of females were encountered that had reached stages 3B and 4A only then to have commenced the atretic process. In many cases oocyte development had been consistent with most oocytes in individual females having reached a similar stage of development before the onset of atresia.

At the opening of the angling season at Lake Dartmouth in December 1995 all female Macquarie perch captured over 800 g were found to be spent or contained oocytes undergoing resorption, though many fish under this size had not spawned. While numbers of females were caught by angling, relatively few males were captured or observed, even in the rivers. It is possible that most males may have completed spawning and as a consequence the remaining females may have commenced resorption due to an inability to find mates. Alternately a change in environmental conditions may have triggered resorption of the oocytes. The causative agent that stimulated resorption of oocytes in some Hughes Creek fish in 1995 remains unknown. Possibilities include environment factors or an inability to find mates, possibly as a result of low population density created by limited suitable habitat and through angling pressure in the more accessible stretches.

Induction of Ovulation

Caution has to be exercised in interpreting the results of the spawning trials reported herein due to the limited number of fish involved, though in some cases the trials were subsequently repeated and yielded similar results. The work with both wild and captive Macquarie perch suggest that the production of viable ova utilizing HCG is only likely to occur if oocytes have progressed to stage 4A containing peripheral GV. Females containing stage 3B oocytes treated with HCG ovulated but produced unviable ova while its use with females containing predominantly stage 3A oocytes resulted in partial ovulation or failure altogether. These observations provide a possible explanation for the variable results obtained by Gooley and McDonald (1988) using HCG. In this study while females with stage 3A and 3B oocytes were captured in relatively static water in the river arms of Lake Dartmouth, the few fish captured at stage 4A were either in the top of the arms adjacent to the rivers or in the rivers proper in flowing water. This lends support to their observation that fish on their final spawning run were the ones that responded successfully to HCG and produced fertile ova.

The results using *Ovaprim*® suggest a wider window for using this hormone for spawning induction than with HCG. It is apparent that females containing oocytes at stage 3B with partially polarized GV have the potential to produce viable ova if injected with *Ovaprim*®. This may explain the at times relatively high success (>40% fertilisation) reported with this hormone for the production of viable ova using broodstock captured adjacent to the wall of Lake Dartmouth many kilometres away from inflowing rivers in the early 1990s (Gray et al 2000) compared to the earlier work with HCG. The observation that female Macquarie perch appear to respond to HCG and *Ovaprim*® at different stages of oocyte development may suggest a difference in the response of endocrine pathways at the follicular level or problems of species specificity in response to HCG as reported in other fish (Zohar 1989).

Ovaprim® appears to be a convenient and satisfactory tool for inducing ovulation in female Macquarie perch and producing viable ova provided that oocytes have commenced GV migration. The problem is obtaining broodstock at this stage of development. The proportion of fish encountered at this stage in the wild is relatively low, but if numerous fish are collected towards the period of spawning then sufficient females may be encountered to maintain a breeding program. However if the fish are relatively scarce or the time/location of spawning uncertain then the probability of encountering receptive females may be so low as to ensure ongoing failure.

In general it is believed that once vitellogenesis is completed that LH triggers FOMO and it is at this time that traditional endocrine intervention techniques such as injection of pituitary extracts, HCG or LHRHa successfully induce ovulation and spawning in fish. While asynchronous development appears to be a problem in captive Macquarie perch it is not universal. The fundamental problem in the artificial propagation of Macquarie perch appears to be the failure to acquire maturational competence at the end of the vitellogenic process. In this species it appears that maturational competence is acquired only once GV have become partially polarized, suggesting some type of post-vitellogenic process. A similar phenomenon has been reported in other species with a succession of phases exhibiting refractory responses to various hormones prior to maturational competence (Wen-Shiun Yueh and Ching-Fong Chang 2000). It has also been reported that disruptions to the reproductive process after the completion of vitellogenesis can impact upon the ability of female fish to undergo GV migration and FOMO (Mylonas et al 1998).

Over the past decade a series of papers have suggested that nutritional problems may be the cause of reproductive problems in captive female Macquarie perch. Similarly this scenario has been implicated in the lack of spawning success after hormone injection of wild sourced fish from Lake Dartmouth and the decline of the wild population (Sheikh-Eldin et al 1995; Sheikh-Eldin et al 1996; Gray et al 2000). Certainly the role of adequate and appropriate nutrition, and in particular fatty acids, in the successful induced spawning of finfish has been extensively reported and is generally acknowledged (Bruce et al 1999; Izquierdo et al 2001).

It is entirely plausible that tank held female Macquarie perch could suffer nutritional deficiencies not only through an unsuitable diet, whether natural live food or prepared pellet, but also through the impacts of captivity which could alter feeding behaviour. The production of steroid stress hormones has been demonstrated to have a negative impact upon reproductive processes (Stone and Forteath, 1994) and could also modify processing and storage pathways affecting ovarian development. The captive environment rather than the food source as the cause of fatty acid deficiency is suggested by the observation that Macquarie perch concentrate n – 6 fatty acids in their tissues generally relative to their dietary supply and that wild and captive fish differ in their ability to do this (Sheikh-Eldin et al 1996).

However an inadequate or inappropriate diet appears to be a less satisfactory explanation for incomplete ovarian development in pond held fish. The diet of wild Macquarie perch has been relatively well studied (Cadwallader and Eden 1979, Lintermans 2006) and they are best described as generalist insectivores and certainly do not exhibit the characteristics of having a specialist or restricted diet. The presence of stunted Macquarie perch in rocky streams containing a relatively depauporate invertebrate food supply such as the Seven Creeks reproducing successfully supports this contention. Other native species such as golden perch with unspecialized diets successfully undergo ovarian development in ponds suggesting that under good conditions this type of environment should provide adequate nutrition.

Fatty acid deficiencies have been suggested as a potential cause for failure of hormone induced ovulation to produce viable ova in Macquarie perch, with the pathways in prostaglandin production implicated. The results of the trials with $PGF2\alpha$ suggest that this particular metabolic pathway is not the bottleneck in the artificial propagation of Macquarie perch. The response of wild and captive females with stage 3A oocytes was similar to that of females treated with HCG containing similar oocytes except that expulsion of the oocytes was generally more complete. In both cases oocytes hydrated and partially cleared but failed to undergo the normal processes associated with FOMO such as coalescence of yolk droplets to form a yolk mass and coalescence and polarization of oil droplets. The female containing stage 4A oocytes with polarized GV exhibited a similar response. These

results suggest that the role of PGF2α in the reproductive endocrinology of Macquarie perch is similar to that reported in other teleosts, i.e. rupture of the follicle, expulsion of the ova and stimulation of behaviour associated with spawning (Stacey 1976; Stacey and Goetz 1982; Kagawa et al 2003).

The responsiveness of stage 3A Macquarie perch oocytes to treatment with arachidonic acid has previously been reported for *in vitro* studies of oocytes of other fish species (Van Der Kraak and Chang 1990; Sorbera et al 2001). The effect of arachidonic acid mirrors that of treatment with PGF2α with the process of ovulation being stimulated without the necessary concurrent internal development of oocytes associated with normal FOMO being completed. Since arachidonic acid is a precursor in the formation of PGF2α the similarity in response could be attributed to conversion of arachidonic acid to PGF2α initiating that particular pathway in the reproductive process.

However arachidonic acid is not only a precursor for PGF2α but also for production of prostaglandin PGE2 by the granulosa cells of the follicle. While PGF2α production is stimulated by LH and is involved in FOMO, PGE2 production is regulated by FSH and initiates ovarian production of testosterone and its ultimate conversion to oestrogen. As such PGE2 appears to play an important role in the process of vitellogenesis and pre-maturational development (Mercure and Van Der Kraak 1993; Sorbera et al 2001). It can be speculated that if a deficiency of arachidonic acid was interfering with the completion of vitellogenesis that treatment with arachidonic acid should have initiated completion of this processes. That it stimulated ovulation may have been the result of a dose dependant response or that the metabolic changeover to the PGF2α pathway had already had taken place.

The results using HCG, *Ovaprim*®, LHRHa, PGF2α, and arachidonic acid on oocytes with central GV are similar with a general failure to initiate normal FOMO. However the ability of *Folligon*® to stimulate GV migration in captive fish is notable. *Folligon*® or pregnant mare serum is a mixture of mammalian FSH and CG with the former predominating. Trials conducted by the author in the early 1980's employing low doses of HCG on captive fish failed to illicit the type of response seen with *Folligon*® suggesting that it is caused by the FSH component. While there are differences in both the structure and mechanism of action of piscine and mammalian FSH, sections of the molecules are highly conserved and their roles in some aspects of oocyte development appear to be parallel (Santos et al 2001; Jalabert 2005).

Although the proportion of viable ova produced was relatively low, the clear response of oocytes to *Folligon*® treatment suggests the potential of it or a more specific endocrine agent for the controlled reproduction of captive Macquarie perch. It appears that this approach may have been used successfully elsewhere with captive fish (Walker 2006). Recently NFA has had some success in promoting GV migration with *Folligon*® followed by treatment with *Ovaprim*® to induce FOMO and in some cases produce viable ova (Rob Radavicius, personal communication). These results tend to suggest that the past failures in the controlled reproduction of captive Macquarie perch may in part be due to the captive conditions failing to provide the necessary stimuli via an endocrine priming process for oocytes to acquire maturational competence.

Recent works report the purification of piscine FSH and it would appear to be a worthwhile trial to treat female Macquarie perch with such extracts to ensure completion of vitellogenesis and development of maturational competence by stimulating 17β-estradiol production. Direct treatment with 17β-estradiol in other species has not proved successful due to the feedback mechanisms involved (Saligaut et al 1998). However other hormones in recent years have been demonstrated to play roles in ovarian steroidogenesis including growth hormone, prolactin, activin, IGF-1 and insulin. These, in addition to FSH and PGE2, may offer opportunities in promoting the development of maturational competence in Macquarie perch oocytes (Singh et al 1988; Van de Kraak et al 1990; Kagawa *et al* 1994; Jalabert, B., 2005). In particular the recently reported successful use of prolactin to stimulate 17β-estradiol synthesis and insulin to terminate meiotic arrest and stimulate the initiation of GV migration (Tan et al 2005; Dasgupta et al 2001) offers considerable potential.

The factors responsible for stimulating GV migration to the sub-peripheral position in Macquarie perch remain unclear. It could be that, provided the nutritional status of the female is satisfactory and the appropriate environmental cues are present, the process is a regular occurrence and a natural

progression in oocyte development in the species. Alternately it may be that a particular environmental stimulus is required to initiate progression from stage 3Ato 3B and this may be indicated by the process being rapid, occurring within 48 hours, as suggested by the trials with *Folligon*®. Observations of wild impoundment fish indicate that in some cases this takes place during upstream migration. While speculative, such stimuli could include nutritional status, water temperature, olfactory stimuli or possibly behavioural or pheremonal cues provided by males or aggregations of fish. The full elucidation of the factors controlling the maturation process is not only important for the controlled reproduction of the species but in understanding the types of environmental disruptions that could impact upon wild populations.

The observations of synchronous oocyte development to stage 3A and in some cases 3B in Macquarie perch sourced from the large farm dam suggests that under some captive conditions normal ovarian development can take place. The scarcity of captive fish under these conditions observed with partially polarized GV could be due to a number of reasons. The first may be that in general such environments do not provide the necessary stimuli for development of maturational competence. In the case of the farm dam in this study at the time of capture water was inflowing into the dam and this may have provided adequate stimulus for some fish. The example of the running ripe female captured from the farm dam at Strathbogie by the late C. C. Kipping supports this hypothesis.

An alternate explanation for the rarity of encountering females at stage 3B in ponds could be that this stage is relatively brief or that if, having reached this stage, appropriate conditions do not exist for FOMO and spawning then resorption commences. Golden and silver perch have relatively broad windows of opportunity lasting perhaps several months for spawning induction. In these species FOMO can take place over a period of many months being cued by flooding (Lake 1967). It may well be that Macquarie perch differ from these species in that the developmental process is highly synchronized with a narrow window existing in which to obtain individual females in suitable condition for spawning within both wild and pond populations.

The capture of the females from the farm dam with stage 3B oocytes and the successful induced ovulation and production of ova of relatively high viability from this fish is of great significance and is the first example reported for captive Macquarie perch. The inflowing water appears to be a significant difference compared to previous work. Ultimately this still leaves a major bottleneck in the development of methods for inducing ovulation in captive Macquarie perch. Large ponds or dams are more likely to provide suitable nutrition for broodstock and less stress than captivity in tanks. However, they may not provide adequate or suitable stimuli for the development of maturational competence or that its occurrence is unpredictable and brief in this situation. Tank systems have environments which in theory are easier to manipulate and therefore provide suitable environmental conditions for FOMO provided these conditions are known. They appear to be problematic in providing a suitable environment for normal oocyte development in Macquarie perch whether this is due to nutritional, stress or other environmental agents.

It is notable that to date no real attempt has been reported in which an effort has been made to replicate under outdoor conditions a more natural environment for female Macquarie perch to stimulate normal ovarian development and FOMO. The closest appears to be Wharton's (1973) trial though the inflow to that pond was minimal and the fish may have already commenced resorption of their oocytes at the time of this trial. A large pond or raceway with a sizeable inflow of water would appear to be a useful approach. Incorporated into the design could be a 'spawning pool' a short distance upstream of the main pond, with a barrier provided between the two. Broodstock could be implanted with some type of tracking device and monitored for accumulation at the barrier. Flows and temperatures could be varied to stimulate upstream movement and individual females sampled for ovarian development. Spermiating males could possibly be placed upstream of the barrier to investigate the possibility that substances produced by the males induce upstream movement of females. In the event of natural spawning a removable substrate, such as synthetic turf, could be utilized to harvest fertilized eggs.

The results of hormone trials reported here, while perhaps indicating the responsiveness of female Macquarie perch to various hormones during different phases of the ovarian cycle, cannot be considered conclusive. The number of fish involved was small and while some trials were later replicated others were not simply due to the lack of fish, facilities and the time involved. With the

current conservation status of the Macquarie perch, sourcing large numbers of stock for trials is difficult with the Yarra River and perhaps Lake Dartmouth being the only options, at least in the Victorian context.

An alternate approach is to investigate the development and responsiveness of Macquarie perch oocytes to endocrine agents utilizing the *in vitro* culture approach successfully applied to other fish species overseas (Yueh and Chang 2000; Dasgupta et al 2001). By sacrificing a single fish at a given stage of development, oocytes could be exposed to a range of hormones and substrates such as arachidonic acid and insulin and monitored. By replicating the work with oocytes sourced from females at different stages of maturity a more complete picture could be rapidly elucidated from a small number of fish. In addition this approach would eliminate the effects of stress hormones produced by the captive conditions in spawning trials. It has the advantage that a large number of trials with various hormones over a range of doses could be completed during the course of one reproductive season. An equivalent *in vivo* study would require many years of trials and require large numbers of fish. This approach represents the most time and cost efficient option for developing a comprehensive understanding of the potential of endocrine intervention to induce oocyte maturation and ovulation in Macquarie perch.

Such an investigation would be well suited to a post graduate student at a Melbourne-based university. The fish required could be sourced from the Yarra River population and as the spawning areas are known collecting fish at various stages of oocyte development is likely to be successful. Alternately, fish could be sourced from Lake Dartmouth and the Mitta Mitta River in which they congregate prior to spawning. The very small number of fish required for such an investigation would be of no detriment to either population and would provide rapid results. Such a project possibly could also be undertaken out of Sydney utilizing the Shoalhaven or Nepean populations of the eastern form of Macquarie perch or in Canberra utilizing the translocated population in the Queanbeyan River.

A parallel study investigating the environmental stimuli involved in promoting FOMO and spawning in the Yarra River population could be a worthwhile contribution to knowledge of the reproductive biology of the species. The fitting of radio tracking devices would determine the movements of fish in the Yarra system during the reproductive season and allow correlation of these movements with environmental factors. Given the size and accessibility of the Yarra River population it appears to be an underutilized resource for research. A priority should be the installation of data loggers measuring a range of factors on the Yarra River to provide a daily record of conditions. Similarly data loggers should be installed on the rivers flowing into Lake Dartmouth and possibly Hughes Creek in Victoria and the Queanbeyan River in NSW as ongoing research is conducted in these waters.

At the present time the only organization actively pursuing the artificial propagation of Macquarie perch is NFA on a volunteer basis with limited facilities. To date progeny produced by their efforts out of prudence have been restricted to release within the Yarra River catchment by regulatory authorities due to genetic considerations. This is of concern given the current conservation status of the species and the importance of creating new populations to provide a buffer against extinction as well as helping to meet the Murray-Darling Basin Commission's goals for restoring native fish populations.

It is well documented that the individuals used to establish the Yarra River population through translocation were sourced within the Murray-Darling basin initially from the King Parrot Creek and subsequently from the Goulburn and Broken Rivers (Wilson 1857, Cadwallader 1981). Therefore the Yarra River population does not pose the speciation issues present with the east coast populations in New South Wales. Given the fact the Yarra River population was in part created by the translocation of over 20,000 fish in the twentieth century (Cadwallader 1981) it should have as broad a genetic base as the Lake Dartmouth population. Fish from Lake Dartmouth have been previously used for the creation of new populations through artificial propagation of juveniles or by translocation of adults. The Dartmouth fish originated from a small relic population in the Mitta Mitta River which flourished during filling of the impoundment. It could be argued that both populations have experienced historic genetic bottlenecks but remain the best genetic resources for creating new populations within the Victorian and possibly national contexts.

Officers of the Victorian Fisheries and Game Department load juvenile Macquarie perch captured from the Goulburn River at Tahbilk for translocation to the Yarra River, c1930. Photo courtesy J. O. Rhodes.

The current approach of NFA sourcing multiple pairs of wild fish for use as broodstock from the Yarra River appears to provide the opportunity to produce juveniles meeting the type of genetic guidelines outlined in the recovery program for the trout cod. It would seem that if success similar to that in 1994 were to be repeated that such an opportunity should be used to advantage to use the progeny to establish another population within the Murray-Darling basin. Unfortunately NFA does not have the facilities to rear large numbers of fry. A cooperative approach with larvae hatched by NFA and reared at the Snobs Creek or Narrandera facilities would be pragmatic. It is acknowledged that, as NFA's work is largely ad hoc and unpredictable due to its dependence upon the availability and condition of the adult fish as well as volunteers, this provides problems in coordination with such major facilities. However at the present time this option represents the only opportunity to produce juvenile Macquarie perch for conservation purposes.

NFA is also limited in its ability to produce Macquarie perch larvae by the difficulty in capturing mature females suitable for spawning induction as these fish can be difficult to angle at this time. One option may be to issue a permit to NFA to take a reasonable number of fish with nets from the Yarra River during the spawning season. This would improve the opportunity to capture fish receptive to hormonal intervention. NFA is already permitted to capture and retain the fish by angling and the use of fyke nets, under strict conditions, could improve the probability of success. Alternately the fish could be captured as part of a monitoring program by a government agency. The NFA hatchery facility at La Trobe University is in close proximity to the Yarra River Macquarie perch population and as such is ideally suited to rapid handling of fish destined for induced spawning with a minimum of stress.

Similar scope possibly exists for a parallel cooperative program between Environment ACT and NSW DPI to produce juvenile Macquarie perch through the capture of a small number of adults from the Queanbeyan population. The installation of environmental data loggers on both the Yarra and Queanbeyan Rivers would allow collection of data such as water temperature, flows, etc. A monitoring program whereby female Macquarie perch are captured and undergo ovarian assessment in terms of germinal vesicle position on site could then allow a more reliable correlation between

environmental factors and final maturation. In the case of the Queanbeyan fish a small number of females with sub-peripheral germinal vesicles could be retained for spawning induction with the larvae produced reared at the Narrandera facility. The resulting juveniles could be used to extend the Queanbeyan River population upstream as advocated by Lintermans (2006) or population enhancement elsewhere in the Murrumbidgee catchment.

Historically, of the native freshwater species, Macquarie perch was only second to Murray cod in terms of its importance to recreational angling in north-eastern Victoria. This fact and concerns for its conservation justify ongoing, coordinated research into artificial propagation of the species. It is hoped that the information contained within this report will stimulate renewed interest into the development of techniques for the production of juvenile Macquarie perch so that the species can be restored to its former status.

Recommendations

1. Future attempts at monitoring ovarian maturity and inducing spawning Macquarie perch should employ the Serra clearing solution and report oocyte development in terms of germinal vesicle position;
2. A tertiary institution should develop and conduct *in vitro* trials investigating the responsiveness of Macquarie perch oocytes at various stages to a range of endocrine agents and substrates. The work should encompass trials over a range of concentrations to determine the responsiveness of oocytes to these agents, particularly in promoting termination of meiotic arrest and initiating germinal vesicle migration. Trials should incorporate novel endocrine agents such as insulin, piscine prolactin, piscine FSH and prostaglandin PGE_2 as well as substrates such as arachidonic acid;
3. Data loggers should be installed on waters containing significant populations of Macquarie perch such as the Yarra River in order to comprehensively monitor a range of environmental parameters potentially controlling FOMO of Macquarie perch;
4. Consideration should be given to granting permission to NFA to capture reasonable numbers of Macquarie perch from the Yarra River using fyke nets for oocyte monitoring and spawning trials. Alternately government agencies could capture the fish and assist in the monitoring process;
5. NFA and government agencies should liaise and develop a contingency for the rearing of Macquarie perch larvae should NFA produce a significant quantity of viable ova. Such planning should identify and prioritise target waters for the release of fingerlings outside the Yarra system;
6. Government agencies should fund and initiate programs into the controlled reproduction of Macquarie perch. Such work should attempt to replicate natural conditions outdoors and investigate the effect of temperatures and flows on Macquarie perch behaviour and oocyte maturation.

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A REVIEW OF ATTEMPTS AT THE ARTIFICIAL PROPAGATION OF THE MACQUARIE PERCH *Macquaria australasica*

WITH RECOMMENDATIONS FOR FUTURE ACTION