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Induced Ovulation of the Australian Eel-tailed Catfish *Neosilurus ater* (Perugia) with Ovaprim

M.S.H. CHEAH[1] and C.L. LEE[2]

[1]*Central West College of TAFE*
PMB 103
Geraldton, WA 6531
Australia

[2]*Fisheries WA*
PO Box 71
Broome, WA 6725
Australia

Abstract

The effect of Ovaprim on induced ovulation of eel-tailed catfish, *Neosilurus ater*, was studied. Twenty gravid females with a body weight of 582±25 g were randomly assigned to five treatment groups. Females were treated with either saline (control), 0.25, 0.50, 0.75, or 1.00 mL·kg^{-1} body weight (BW) of Ovaprim. The oocyte diameters prior to hormone administration ranged from 1.91-2.01 mm and the oocyte diameters for all experimental fish were similar (P>0.05). No ovulatory response was observed when the fish were treated with either 0 or 0.25 mL·kg^{-1} body weight (BW) of Ovaprim. However, all of the fish that were treated with 0.50, 0.75 or 1.00 mL·kg^{-1} body weight (BW) of Ovaprim ovulated. The latency periods and the working fecundities per 100 g BW ranged from 20-23 hours at 28 °C and from 2,877-3,001 eggs, respectively, and the values for the three treatments were similar (P>0.05). The fertilization rates and the hatching rates ranged from 77.5-89.9% and 75.6-82.0%, respectively. All the rates for the three successful treatments were not significantly different (P>0.05).

Introduction

Aquaculture in tropical Australia is a relatively new activity and has tremendous potential for development because the warmer year round temperatures are conducive for the growth of aquatic organisms. The major species of finfishes that is cultured is *Lates calcarifer*, which is locally known as barramundi. Besides barramundi, other finfishes such as freshwater catfishes also have the potential for development. Even though catfish is farmed extensively in Asia and the United States of America (USA), its culture in Australia especially in tropical Australia is virtually non-existent. There are many species of catfishes in Australia and among them the eel-tailed catfish (*Neosilurus ater*, Plotosidae) has been identified to be of

aquaculture importance (Cheah et al. 1993). This species is commonly known as Butter Jew, Narrow-fronted Tandan or Black Catfish and is found in northern Australia and central-southern New Guinea.

The colour of *N. ater* is typically blackish or dark brown and the belly is white. However, adults collected from the Manton Dam, Northern Territory (NT), Australia are golden along the sides throughout the year (Cheah 1999). Lake (1978) reported that they can attain 47 cm in total length and 2.0 kg in weight. Besides being caught from the wild for the aquarium trade, its potential as a food species is virtually untapped. Cheah et al. (1993, 1999) reported that the percentage dress-out of this fish or the fillet was 42.6% of the body weight and that the fillet was pinkish white, it does not have any intramuscular bones and as a consequence makes it suitable for most cooking styles.

For the culture of this species to be successful, several problems such as seed production and nutrition will have to be addressed. Orr and Milward (1984) studied the reproduction and development of *N. ater* at Ross River, northern Queensland. In the Northern Territory, breeding is known to occur early in the wet season and the fishes were observed to move upstream to spawn during monsoonal flooding in early February (Larson and Martin, 1990).

Several hormones such as acetone-dried carp pituitary, human chorionic gonadotropin and Luteining Hormone-Releasing Hormone (LH-RH) and more recently Ovaprim have been used to induce breeding of other catfishes under hatchery conditions and a recent review on this matter has been published (Lin and Peter 1996). Ovaprim is a mixture of the analogue of salmon gonadotropin-releasing hormone (sGnRHa) and a dopamine antagonist domperidone (Leelapatra 1988, Nandeesha et al. 1990, Goudie et al. 1992). The administration of Ovaprim is based on the 'Linpe' method (Peter et al. 1988a) which was developed after extensive research on the combined effects of the LH-RH analogue sGnRH in combination with a dopamine antagonist such as domperidone or pimozide on the Chinese loach (Lin et al. 1987a, 1988), common carp (Lin et al. 1987b, 1988) and chinese carps (Peter et al. 1987, 1988 a,b). Ovaprim which is marketed by Syndel Laboratories Ltd. of Canada has been successfully tested on Thai carp *Puntius gonionotus* in Thailand (Leelapatra 1988), the Indian major carps (Nandeesha et al. 1990, 1991), channel catfish *Ictalurus punctatus* in the USA (Goudie et al. 1992), walking catfish *Clarias batrachus* in Malaysia (Cheah and Yeo, 1994), *Macquaria australasica* in Australia (Ingram et al. 1994) and sand whiting *Sillago ciliata* also in Australia (Battaglene 1996).

The dosages of Ovaprim that successfully induced ovulation in the following fishes were: *P. gonionotus* (1.0 $mL \cdot kg^{-1}$ BW of Ovaprim), Indian major carps (0.4-1.2 $mL \cdot kg^{-1}$ BW of Ovaprim), *I. punctatus* (0.5 $mL \cdot kg^{-1}$ BW of Ovaprim), *C. batrachus* (0.75-1.00 $mL \cdot kg^{-1}$ BW of Ovaprim), *M. australasica* (0.13-0.75 $mL \cdot kg^{-1}$ BW of Ovaprim) and *S. ciliata* (0.5 $mL \cdot kg^{-1}$ BW of Ovaprim).

As there is insufficient reports on the induced breeding of *N. ater*, we began a study on the effect of Ovaprim on induced ovulation of *N. ater* at

the hatchery of the Aquaculture complex, Northern Territory University, Darwin, Australia in 1993. This report discusses the results of our trials.

Materials and Methods

Twenty gravid females measuring 39.21±0.68 cm in total length, 34.71±0.69 cm in standard length and weighing 582±25 g were caught from the Manton Dam, N.T., Australia using gill nets. The males and females were transported to the hatchery for the experiment. Samples of oocytes were collected prior to the administration of the hormone by using a catheter and the diameters of at least 20 oocytes from each fish were determined using an eye piece micrometer.

The females were randomly assigned to five treatment groups and were treated with either saline (0.9% NaCl, control group), 0.25, 0.50, 0.75, or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim while all the twenty males were administered with 0.50 $mL \cdot kg^{-1}$ BW of Ovaprim at the same time as the females were treated. Ovaprim contains the synthetic GnRH analog [(D-Arg^6,Trp^7,Leu^8,Pro^9Net)-LH-RH] and domperidone dissolved in distilled water at 20 $\mu g \cdot mL^{-1}$ and 10 $\mu g \cdot mL^{-1}$, respectively. A pair of fish was then put together in a 500 L cylindro-conical fibre glass tank containing 300 L of dechlorinated tap water.

Approximately 12 hours after the administration of Ovaprim, the females were checked for their ovulatory response and non-ovulating fish were checked again every three hours. The details of the methodology for the stripping of eggs and fertilization have been described previously (Cheah et al. 1990a, 1990b, Cheah and Yeo 1994).

Egg incubation was done by putting 20 mL of water-hardened eggs into an incubator which was made from a black polypot. The incubator was submersed to a depth of approximately 15 cm in a shallow fibre glass tank containing dechlorinated water. The flow rate of dechlorinated water from the header tank to the incubator ranged from 150-200 $mL \cdot min^{-1}$. The incubators were prepared prior to the breeding season and a brief description is presented. The dimensions of the incubator were as follows: upper internal diameter - 19.5 cm, lower internal diameter - 15.5 cm, height - 19.5 cm. The incubator had eight holes along the periphery of the bottom section of the sidewalls and each hole measured approximately 2 cm in width and 1.8 cm in height. The bottom of the incubator was removed using a sharp knife leaving an open space, which had a diameter of approximately 13 cm. The bottom space and the holes were then covered with a nylon fabric which had a mesh size of approximately 2 mm X 2 mm. The fabric was glued to the incubator with silicone sealant and left to dry overnight.

Data for the following aspects were collected: ovulatory response, latency period, ovulatory egg diameter, water-hardened egg diameter, number of ovulated eggs per g, number of water-hardened eggs per mL, working fecundity (number of eggs per 100 g BW), number of water-hardened eggs per mL, fertilization rate and hatching rate. The data were then subjected to analysis of variance (ANOVA) and multiple range tests using the Statview

512+ package. The fertilization and hatching rates were transformed using arcsine transformation prior to running the ANOVA tests.

Results

Sexual dimorphism

Sexual dimorphism in *N. ater* was observed to be restricted to the shape of the urinogenital papilla. The male urinogenital papilla is conical and raised, whereas that of the female is stouter and slightly recessed. These findings were similar to that reported by Orr and Milward (1984).

Oocyte diameters, ovulatory response and latency period

The oocyte diameters prior to hormone administration ranged from 1.91-2.01 mm (Table 1) while the oocyte diameters for all the experimental fish were similar ($P>0.05$). Table 2 shows the ovulatory response of the fish receiving different dosages of Ovaprim. There was no ovulatory response in the fish that were treated with saline or 0.25 $mL \cdot kg^{-1}$ BW of Ovaprim. However, all of the fish that were administered with either 0.50, 0.75 or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim ovulated. The latency period ranged from 20-23 hours after the administration of Ovaprim at 26-30 °C and there were no significant differences in the latency periods in the females that ovulated.

Table 1. Summary of the data on induced ovulation of *Neosilurus ater* administered with various dosages of Ovaprim. (mean ± sd; *n*=4).

Parameter	Dosage of Ovaprim ($mL \cdot kg^{-1}$ BW of Ovaprim)				
	0	0.25	0.50	0.75	1.00
TL (cm)	39.1±0.8	39.1±2.4	39.2±0.3	38.3±4.0	40.2±4.6
SL (cm)	34.6±0.6	34.7±2.1	34.9±0.5	33.7±3.5	35.6±4.2
BW(g)	584.7±44.5	581.8±65.2	585.8±51.9	543.9±164.0	615.5±219.6
P.O.D. (mm)	2.01±0.15	1.97±0.11	1.91±0.13	1.96±0.14	1.93±0.11
L.P. (hours)			20±3	23±6	22±3
O.E.D.(mm)			$2.13±0.12^{b}$	$2.05±0.12^{a}$	$2.16±0.13^{b}$
W.H.E.D.(mm)			$2.21±0.14^{a}$	$2.33±0.08^{b}$	$2.33±0.09^{b}$
N.O.E.			$287±14^{b}$	$289±10^{b}$	$265±13^{a}$
N.W.E.			110±11	113±14	111±8
W.F.			2,987±684	2,877±796	3,001±329
F.R.(%)			77.5±16.9	89.9±5.6	88.4±6.7
H.R.(%)			75.6±15.3	78.7±7.1	82.0±15.3

TL - Total length, SL - Standard length, B.W. - Body weight, P.O.D. - Preinjection oocyte diameter, L.P. - Latency period, O.E.D.- Ovulatory egg diameter, W.H.E.D. - Water-hardened egg diameter, N.O.E.- Number of ovulated eggs/g), W.F. - Working fecundity (no. of ovulated eggs/100g BW), N.W.E. - Number of water-hardened eggs/mL), F.R. - Fertilization rate, H.R. - Hatching rate.
Values in the horizontal row which are followed by different superscript(s) are significantly different ($P<0.05$).

Ovulatory egg diameters and water-hardened egg diameters

Table 2. The ovulatory response of *Neosilurus ater* administered with Ovaprim.

Ovaprim (mL/kg BW)	Number of fish		
	Ovulated	Non-ovulated	Total
0	0	4	4
0.25	0	4	4
0.50	4	0	4
0.75	4	0	4
1.00	4	0	4

At the time of ovulation, the egg diameters of fish that were treated with 0.50, 0.75 and 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim were 2.13 ± 0.12 mm, 2.05 ± 0.12 mm and 2.16 ± 0.13 mm, respectively, and the egg diameters in fish that were treated with either 0.50 mL or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim were similar ($P>0.05$) but larger than in fish that were treated with 0.75 $mL \cdot kg^{-1}$ BW of Ovaprim. The water-hardened egg diameter of fish that were treated with 0.50, 0.75 and 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim were 2.21±0.14 mm, 2.33±0.08 mm and 2.33±0.09 mm, respectively, and the water-hardened egg diameter of fish treated with either 0.75 or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim were similar ($P>0.05$) but larger than in fish treated with 0.50 $mL \cdot kg^{-1}$ BW of Ovaprim ($P<0.05$).

Number of ovulated eggs per gram, working fecundities, and number of water-hardened eggs per mL

The number of ovulated eggs per gram in fish that were treated with 0.50, 0.75 and 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim were 287±14, 289±10 and 265±13, respectively, and the egg number in fish that were treated with 0.50 or 0.75 $mL \cdot kg^{-1}$ BW of Ovaprim were similar ($P>0.05$) but significantly higher than those in fish treated with 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim. The working fecundity per 100g BW was calculated by using the formula [(Total number of ovulated eggs/BW of the female in g) X 100g]. The working fecundities for fish in the three successful treatments ranged from 2,877-3,001 eggs and there were no significant differences among the three treatments ($P>0.05$). The number of water-hardened eggs per mL in fish that were administered with 0.50, 0.75 or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim were 110±11, 113±13, 111±8, respectively, and the numbers of eggs in the three treatments were similar ($P>0.05$).

Fertilization and hatching rates

The fertilization and hatching rates for the three successful treatments ranged from 77.5-89.9% and 75.6-82.0%, respectively, and both the fertilization and hatching rates were not significantly different between treatments ($P>0.05$). The incubation period ranged from 36-48 hours at 28-31 °C. The total length, standard length and weight of the newly hatched larvae were 5.08± 0.05 mm, 4.87±0.10 mm and 0.0019±0.0001 g, respectively.

Discussion

Oocyte diameters

In this study, the oocyte diameters prior to hormone administration ranged from 1.88-2.02 mm. In comparison, the oocyte diameters of several species of catfish were: *C. gariepinus* (0.7-1.06mm) (Inyang and Hettiarachchi 1994), *C. batrachus* (1.09-1.15 mm) (Cheah and Yeo 1994), *C. macrocephalus* (1.49-1.59 mm) (Tan-Fermin et al. 1997), *T. tandanus* (2.28-3.05 mm) (Davis 1977b), *A. graeffei* (11.0-13.7 mm) (Rimmer 1985). The oocyte diameters of *N. ater* were larger than those of *C. gariepinus*, *C. batrachus* and *C. macrocephalus* but smaller than those of *T. tandanus* and *A. graeffei*. Passive parental care in fishes is demonstrated by the females where yolk is deposited in the eggs to ensure the survival of the larvae up to the first feeding stage. Larger eggs usually have more yolk reserves and tend to be more developed at first feeding when compared to larvae that hatched from smaller eggs. Moreover larvae which have a relatively larger yolk would have more resources to develop its external and internal organs for the detection, capture, ingestion, digestion and assimilation of its prey. Since the oocyte diameters of *N. ater* are intermediate between those of *C. batrachus* and *T. tandanus*, it was surmised that the time of first feeding of *N. ater* would be between those of *C. batrachus* and *T. tandanus*.

Ovulatory response

The ovulatory response in this study was positive when the fish were administered with 0.50, 0.75 or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim. It appears that *N. ater* requires lower dosages for induced ovulation as compared to the other catfishes. Cheah et al. (1994) reported that ovulation in *C. batrachus* was achieved only when Ovaprim was administered at the level of at least 0.75 $mL \cdot kg^{-1}$ BW of Ovaprim. In the other catfishes such as *I. punctatus*, *C. gariepinus* and the Chinese catfish (*C. fuscus*), ovulation occurred when they were treated with 1.0 $mL \cdot kg^{-1}$ BW of Ovaprim (Richard Bradshaw, pers. com., Goudie et al. 1992). In contrast, studies in India have shown that most of the Indian carps such as mrigal (*Cirrhina mrigala*), catla (*Catla catla*)and rohu (*Labeo rohita*) ovulated when they were treated with 0.50 $mL \cdot kg^{-1}$ BW of Ovaprim and in some cases, the fishes ovulated when they were treated with Ovaprim at levels lower than this amount (Nandeesha et al. 1990). In the case of sand whiting (*S. ciliata*), administration of 0.5 $mL \cdot kg^{-1}$ BW of Ovaprim induced ovulation but not spawning in 75% of the females (Battaglene 1996).

Latency period

The latency period of *N. ater* ranged from 20-23 hours at 26-30 °C after administration of Ovaprim. The latency period was similar to those of Ovaprim treated *C. fuscus* (20-24 hours; Richard Bradshaw, pers. com.) but

longer than those of Ovaprim treated *C. batrachus* (12-15 hours; Cheah and Yeo, 1994), acetone-dried carp pituitary treated *C. gariepinus* (11-16 h, Hogendoorn 1979) and LH-RHa treated *C. macrocephalus* (16-20 h, Tan-Fermin 1997). In contrast, the latency period of Ovaprim administered channel catfish was 92±39 h when water temperatures ranged from 21-29 °C (Goudie et al. 1992). The latency period or response time is often related to water temperature and the period decreases with an increase in temperature (Clemens and Sneed 1962). For the best results in induced breeding of *C. gariepinus*, Hogendoorn and Vismans (1980) recommended the stripping of eggs at 21, 11 or 7 h after hormone administration at 20, 25 and 30 °C, respectively.

Ovulatory egg diameters and water-hardened egg diameters

The egg diameters at ovulation ranged from 2.05-2.16 mm in *N. ater* while that in *C. batrachus*, ranged from 1.24-1.29 mm (Cheah and Yeoh 1994). This indicates that more nutrients had accumulated in the eggs of *N. ater* as compared to those in *C. batrachus* eggs. In comparing the preinjection oocyte diameters and the ovulatory egg diameters, it can be seen that oocytes generally enlarge during final oocyte maturation as a result of hydration. Following fertilization, the eggs of most fishes absorb more water and the chorion (egg membrane) separates from the cortex which results in the appearance of the perivitelline space (Blaxter 1969). The water-hardened egg diameters of *N. ater* ranged from 2.21-2.33 mm while those in the seasonal tributary of Ross River, northern Queensland was 2.6 mm (Orr and Milward 1984). The differences in egg sizes could be attributed to genetically controlled factors of the two geographically distinct populations and/or to a lesser extent the effects of the natural diet of the fish.

Working fecundities

The working fecundities of wild *N.* ater in this study and the estimated fecundities of the same species in a seasonal tributary of Ross River, Queensland ranged from 2,877-3,001 and 2,200-2758 eggs per 100g BW, respectively indicating that the fish in this study had produced a greater quantity of smaller eggs than their counterparts in Queensland. The working fecundities of *N.* ater were much higher than those for Ovaprim treated *C. batrachus*, which ranged from 1,687-2,190 eggs per 100g BW even though the eggs of the latter were smaller (Cheah and Yeo 1994). Ovaprim treated albino and pigmented channel catfish were reported by Goudie et al. (1992) to have working fecundities which ranged from 199±32 eggs per 100g BW and 435±140 eggs per 100g BW, respectively, indicating that the working fecundity of *N.* ater was much higher. From the aquaculture point of view, the higher fecundity of *N.* ater is an advantage should its culture become commercialized. For comparison, the fecundities of other catfish species were: *T. tandanus* (296-1440 eggs per 100 g BW) (Lake 1967a, b,

Davis 1977a), *C. gariepinus* (2084-3260 eggs per 100 g BW) (Salami et al. 1994, 1996), *C. macrocephalus* (2,000-9,000) eggs per 100 g BW) (Tan-Fermin et al. 1997). If the working fecundities of hatchery maintained broodstock can achieve higher values than those obtained in this study in the future remains to be seen.

Fertilization and hatching rates

The fertilization rates for the three successful treatments in this study ranged from 77.5-89.9% and these values were much higher than those reported for Ovaprim treated *C. batrachus* (Range: 22.4-47.3%, Cheah and Yeo 1994). Several species of fishes have also been successfully bred with Ovaprim in Thailand and the fertilization rates for big head carp (*Aristichthyes nobilis*) was 50% while those in *C. mrigala*, *L. rohita* and *P. gonionotus* were 90% (Leelapatra 1988). Following the success in Thailand, the use of Ovaprim was widely tested in India on the Indian major carps such as *C. catla*, *L. rohita* and *C. mrigala* and the fertilization rates ranged from 70-99% (Nandeesha et al. 1990). Recently in Australia, *S. ciliata* was induced to ovulate with Ovaprim and the fertilization rates ranged from 33-98% (Battaglene 1996). The fertilization rates achieved in this study were comparable to those reported for other Ovaprim treated fishes and as such, its use has a tremendous potential in Australia and elsewhere.

The hatching rates for the fish in the three successful treatments in this study ranged from 75.6-82.0% and these rates were also much higher than those reported for Ovaprim treated *C. batrachus* (Range: 17.1 to 26.9%, Cheah and Yeo 1994). In comparison, the hatching rates of Ovaprim treated *S. ciliata* in Australia mainly ranged from 83-96% (Battaglene 1996) while those for Ovaprim treated *L. rohita* it was 94% in India (Harker 1992). Since there were no significant differences in the fertilization and hatching rates between treatments in this study, one would surmise that the administration of 0.50 $mL \cdot kg^{-1}$ BW of Ovaprim would be adequate. The use of Ovaprim for induced breeding of fishes including *N. ater* is anticipated to become more widespread considering the ease of administration and the good results achieved in terms of fertilization and hatching rates.

Conclusion

Administration of 0.50, 0.75 or 1.00 $mL \cdot kg^{-1}$ BW of Ovaprim was successful in inducing ovulation in the Australian eel-tailed catfish, *Neosilurus ater*. The fertilization and hatching rates ranged from 77.5-89.9% and 75.6-82.0%, respectively, and both rates for the three treatments were not significantly different ($P>0.05$). The total length, standard length and weight of the newly hatched larvae were 5.08±0.05 mm, 4.87±0.10 mm and 0.0019±0.0001 g, respectively.

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