Nippon Suisan Gakkaishi 58 (9), 1681–1685 (1992)

Induction of Ovulation by HCG Injection in the Tropical Walking Catfish *Clarias batrachus* Reared under 23–25°C

Muhammad Zairin Jr.,[*1] Kiyoshi Furukawa,[*1] and Katsumi Aida[*1]

(Received March 7, 1992)

A series of trials on induced ovulation were conducted using female walking catfish to examine conditions of maturity under warm water conditions. For this purpose, females were maintained at 23–25°C for a long period, either under 12L12D or natural photoperiod. Using 12–37 month-old females, induction of ovulation was achieved with a single injection of HCG (0.2, 0.4, 0.8, 1.6, or or 3.2 IU/g BW). In all the trials, HCG treatment succeeded in inducing ovulation at dosages of 0.4 IU/g BW or higher. Oocytes larger than 770 μm responded to HCG and ovulated. These results indicate that female walking catfish can maintain conditions of maturity for an extended time under warm water conditions, and can respond to HCG irrespective of photoperiod.

The tropical walking catfish *Clarias batrachus* is a freshwater teleost widely cultured in Southeast Asian countries. This species possesses an interesting feature in its reproductive cycles, which appears to be influenced by the amplitude of local temperature which they inhabit.[1] In India, where annual temperature variation is large, this species is an annual spawner, as revealed through observation of oocyte development[2] and elucidation of annual profiles of plasma sex steroids.[3] On the other hand, in more tropical countries such as Indonesia and Thailand, where temperature is generally warm and annual fluctuations are minimal, the spawning season coincides with the rainy season.[4] The final stimulus for natural spawning seems to be associated with a rise in water level and an inundation of marginal area, either as a result of direct rainfall or inflow of water from upstream sources as has been reported in the African catfish.[5] Under cultured conditions, spawning in the walking catfish can be induced by the manipulation of water levels.[6,7],[*2] Recent reports[1,8] show that the ovulation of this species can be induced during non-spawning season not only by environmental manipulation, but also by hormonal stimulation such as the injection of carp pituitary homogenate or human chorionic gonadotropin (HCG).

These results suggest that the walking catfish can maintain mature conditions throughout the year under tropical conditions and can respond to spawning stimuli at any time. In order to confirm this hypothesis, a series of induced ovulation trials were conducted using catfish maintained under warm temperature for an extended period.

Materials and Methods

Fish Stock

1. Grop I: Walking catfish fingerlings were originally obtained from The Faculty of Fisheries, Bogor Agricultural University, Bogor, Indonesia in 1988. These fish were approximately 2 months in age and 3 cm in total length. Fish were reared for 2 months at a density of 25 fish per aquarium in 80 *l*-aquaria provided with a bottom gravel filter. During this rearing period fish were maintained under natural photoperiod at 27°C until reaching about 50 g in body weight (BW) and 10 cm in total length.

Thereafter, fish were transferred to the Fisheries Laboratory of The University of Tokyo at Maisaka, Shizuoka Prefecture, due to increased growth and space requirements. Fish were reared in indoor concrete ponds (dimensions: length × width × depth = 3 × 1.5 × 0.7 m) supplied with running freshwater of deep-well origin.

[*1] Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo, Tokyo 113, Japan (ムハマド ザイリン, 古川 清, 会田勝美: 東京大学農学部).

[*2] Muhammad Zairin, Jr. 1990. Physiological studies on maturation and spawning of channel catfish (*Ictalurus punctatus*) and walking catfish (*Clarias batrachus*). Master Thesis. Faculty of Agriculture, The University of Tokyo. 39 p.

During this period, fish were subjected to 12L12D photoperiod (Group Ia) or natural photoperiod (Group Ib). Being of deep-well origin, water temperature was nearly constant (23–25°C) throughout the year. Up to an age of one year, fish were fed twice daily with commercially available trout dry pellets (Chubu Shiryo, Co.) at a ration of 2–3% of BW. One- to 2-year-old fish were fed once daily at about 1.5% of BW. Fish above 2 years old were fed every two days at 1.5% BW, but feeding was intensified to once daily 2 weeks prior to the start of the experiments.

2. Group II: Second generation fish (of those transported from Indonesia in 1988) were also used as experimental material. One month after hatching, fingerlings (Group 1 offsprings) were transferred to indoor concrete ponds (3 × 1.5 × 0.4 m) supplied with running freshwater of deep-well origin (23–25°C), and reared under 12L12D (Group IIa) or natural photoperiod (Group IIb). Other rearing conditions were the same as those of Group I.

3. Group III: Fish used in this experiment were also directly transported from Bogor, Indonesia, in 1990 at an age of about 2 months old. After arrival, fish were reared in an outdoor concrete pond (3 × 1.5 × 0.4 m) under natural photoperiod at 23–25°C until reaching an age of 8 months.

Table 1. Results of induced ovulation by HCG injection in walking catfish maintained under 23–25°C

Group	Fish age (months)	HCG dose (IU/g BW)	Number of fish		Body weight (g)	Date of trial
			Used	Ovulated		
Ia	13	0.6	3	3	450–475	Aug., 1989
	17	0.8	14	14	325–675	Nov., 1989
		Control	10	0	325–675	
	22	0.8	5	5	500–800	Mar., 1990
Ib	31	0.8	10	10	525–1125	Feb., 1991
		Control	5	0	550– 875	
	37	0.8	10	10	600–1200	Aug., 1991
		Control	5	0	650–1250	
IIa	13	0.8	6	6	300– 400	Apr., 1990
	18	0.8	4	4	350– 500	Aug., 1990
	21	0.8	5	5	450– 500	Nov., 1990
		0.7	3	3	400– 450	
		Control	3	0	375– 425	
	24	1.6	10	10	475– 775	Mar., 1991
		0.8	10	10	500– 650	
		0.4	10	10	400– 775	
		0.2	10	0	425– 750	
		Control	10	0	400– 725	
	25	1.6	3	3	525– 550	Apr., 1991
		0.8	3	3	350– 575	
		0.4	3	3	375– 650	
		0.2	3	0	400– 550	
		Control	3	0	400– 725	
	29	0.8	19	19	325– 475	Aug., 1991
		Control	9	0	375– 525	
IIb	12	0.8	10	10	275– 325	Aug., 1991
		Control	5	0	225– 350	
	13	3.2	8	8	275– 325	Sep., 1991
		1.6	8	8	300– 375	
		0.8	8	8	325– 375	
		0.4	8	8	275– 325	
		0.2	8	0	250– 350	
		Control	8	0	300– 425	
III	15	0.8	13	13	275– 500	Aug., 1991
		Control	4	0	275– 325	

Thereafter, they were reared in a slightly larger concrete pond (3×1.5×0.7 m) under the same conditions. Other rearing conditions were the same as those of Group I.

Induced Ovulation

Female walking catfish at various ages (12–37 months old) were selected from the stock ponds, and given a single muscular injection of HCG (0.2–3.2 IU/g BW, see Table 1) just below the beginning of the dorsal fin, after anesthetizing fish with 600 ppm 2-phenoxyethanol (Wako, Japan). In order to meet the required dose, the original hormone was diluted with 0.6% NaCl solution. After injection, fish were maintained in indoor concrete ponds or outdoor containers supplied with running freshwater (23–25°C), either under 12L12D or natural photoperiod. Fish did not receive feed during the experiment. Ovulation was checked at 20, 24, and 30 h after the HCG injection by applying gentle pressure on the fish's belly.

Measurement of Oocyte Diameter

In the trial using 25-month-old fish in Group IIa, ovulated eggs were stripped, and all fish were dissected. Small pieces of ovarian tissue were fixed overnight with Bouin's fixative, and then preserved in 70% alcohol. Measurement of oocyte diameter (about 200 oocytes/fish) were carried out under conditions of preservation in 70% alcohol.

Results

Induced Ovulation

Results of induced ovulation following HCG injection are summarized in Table 1.

Fish in Group IIa and IIa were reared under 12L12D photoperiod. Fish age ranged from 13 to 29 months old, and trials covered all seasons. In these trials, HCG injection at dosages of 0.4 IU/g BW or higher succeeded in inducing ovulation in all the treated fish. This indicates that ovulation can be induced at any time in fish reared under 12L12D at 23–25°C. Ovulation was observed 20–30 h after the HCG injection of 0.4 IU/g BW, and 20–24 h after the injection of higher doses.

Fish in Group Ib, IIb, and III were reared under natural photoperiod. Fish age ranged from 12 to 37 months old. Trials were conducted in winter and summer. Again, all fish treated with HCG at dosages of 0.4 IU/g BW or higher ovulated. This indicates that ovulation can be induced either in winter or summer in fish reared under natural photoperiod at 23–25°C. Ovulation was observed 20–30 h after the HCG injection of 0.4 IU/g BW, and 20–24 h after injections of higher doses.

Critical Size of Oocytes for Ovulation

In order to determine the critical size of mature oocytes which can respond to gonadotropin, various doses of HCG were injected using 25-month-old females in Group IIa. The results of this experiment are shown in Table 2. This table shows clear changes in size composition of oocytes after HCG injection. Oocyte size composition in the control group indicated that mature females have a wide size range of vitellogenic oocytes. A single injection of 0.2 IU HCG/g BW lowered the percentage of oocytes ranging from 770 to 880 μm in diameter, and

Table 2. Changes in size composition of ovarian oocytes following HCG treatment in walking catfish

Oocyte diameter (μm)	Size composition of ovarian oocytes (%)*				
	Control	0.2 IU HCG/g BW	0.4 IU HCG/g BW	0.8 IU HCG/g BW	1.6 IU HCG/g BW
110– 220	16.4±3.8	17.0±12.0	20.0±6.1	14.6±1.7	23.9±2.5
220– 330	21.3±3.7	24.8± 5.4	29.3±1.8	36.0±5.5	35.4±2.1
330– 440	9.5±0.4	11.0± 2.6	14.1±3.6	20.1±0.4	14.8±2.3
440– 550	7.0±1.8	7.3± 0.8	7.5±0.8	7.8±0.4	8.4±0.6
550– 660	4.7±0.7	4.7± 1.5	4.4±0.8	5.2±1.0	5.6±0.4
660– 770	6.4±1.7	4.7± 0.4	5.4±0.7	7.1±1.8	5.5±0.4
770– 880	17.4±2.6	7.7± 0.8	5.9±0.8	6.3±0.5	4.8±0.6
880– 990	14.7±3.9	18.6± 3.9	8.5±3.0	1.4±0.8	1.3±0.5
990–1100	2.6±1.1	4.3± 1.4	4.9±4.4	0	0.2±0.1

* Expressed as Mean±SEM.

increased the percentage of those in the 880–990 μm and 990–1100 μm classes. However, this dosage level is not great enough to induce ovulation, since no fish ovulated at this dose. With a dosage increase to 0.4 IU/g BW, a small amount of oocytes ovulated, the percentage of oocytes ranging from 770 to 990 μm decreased, and the percentage of oocytes ranging from 990 to 1100 μm increased. At a dosage of 0.8 IU HCG/g BW, the percentage of oocytes larger than 770 μm decreased sharply due to ovulation. At a dosage of 1.6 IU HCG/g BW, the pattern of changes in oocyte size composition was similar to that of 0.8 IU/g BW treatment, but more oocytes were ovulated.

Discussion

In this investigation, we have succeeded in inducing ovulation by injecting HCG in female walking catfish which were maintained at 23–25°C under 12L12D or natural photoperiod for an extended period. These results indicate that fish used in this investigation possessed mature oocytes which were capable of responding to HCG. Since ovulation was induced in fish maintained under both 12L12D and natural photoperiod, photoperiod does not seem to influence gonadal maturation in this species; rather, water temperature is probably a major factor in maintaining conditions of maturity. Endocrine requirements for the maintenance of maturity under constant warm water conditions are under investigation, and will be reported separately.

In other investigations, under either 12L12D or natural photoperiod condition, walking catfish began to mature at an age of 9 months.* However, spontaneous spawning did not occur in the stock ponds, indicating that environmental or hormonal stimuli are necessary for the induction of ovulation. In fact, manipulation of water level[6,7] or hormonal treatment such as injection of carp pituitary homogenate or HCG[1,8] are known to be effective in inducing ovulation. The present studies also indicate that HCG is effective in inducing ovulation. All fish treated with HCG at dosages of 0.4 IU/g BW or higher ovulated 20–30 h after the treatment. None of fish in the control and 0.2 IU/g BW groups ovulated. At 0.4 IU HCG/g BW, the quantity of ovulated eggs was small and practically negligible, whereas a large number of ovulated eggs were observed in the groups treated with 0.8 and 1.6 IU HCG/g BW. These results indicate that these fish possessed oocytes which had already completed vitellogenesis, and that the number of ovulated eggs is dose-dependent. Zonneveld *et al.*[8] recommended the dose of 3 IU HCG/g BW for inducing ovulation in this species. In *Clarias gariepinus* and *C. macrocephalus*, similar doses were recommended.[9,10]

Regarding oocyte size composition in the control group, individuals in this group exhibited a wide size range of vitellogenic oocytes. Injection of 0.2 IU HCG/g BW decreased the percentage of oocytes in the 770–880 μm class, and increased the percentage of 880–990 μm class oocytes. However, this dose was not sufficient to induce ovulation, since no fish ovulated at this dose. With the dosage increased to 0.4 IU/g BW, a small amount of oocytes in the 990–1100 μm class ovulated, and the number of oocytes in the 770–880 and 880–990 μm classes decreased (entering into the next class). In fish treated with 0.8 IU HCG/g BW, the percentage of oocytes larger than 770 μm decreased sharply due to ovulation. In fish treated with 1.6 IU HCG/g BW, the pattern of the changes in oocyte size composition was similar to that of the 0.8 IU/g BW treated group, but more oocytes were seen to have ovulated. These results indicate that oocytes larger than 770 μm in diameter are responsive to HCG injection and undergo final oocyte maturation and ovulation.

Acknowledgements

We express our thanks to our colleagues at the Faculty of Fisheries, Bogor Agricultural University, Indonesia, for their kind help in supplying catfish fry stock. We thank M. N. Wilder for correcting the English. This study was partially funded by a Grant-in Aid for Scientific Research from the Ministry of Education, Science, and Culture.

References

1) N. Zonneveld, Rustidja, W. J. A. R. Viveen, and W. Mudana: Induced spawning and egg incubation of the Asian catfish, *Clarias batrachus*. *Aquaculture*, 75, 41–47 (1988).

* Muhammad Zairin, Jr. 1990. Physiological studies on maturation and spawning of channel catfish (*Ictalurus punctatus*) and walking catfish (*Clarias batracthus*). Master Thesis. Faculty of Agriculture, The University of Tokyo. 39 p.

2) G. K. Lehri: Cyclical changes in the ovary of the catfish *Clarias batrachus* (L.). *Acta Anat.*, **69**, 105–124 (1968).
3) S. Singh and T. P. Singh: Seasonal profiles of sex steroids in blood plasma and ovarian tissue of *Clarias batrachus*. *Gen. Comp. Endocrinol.*, **65**, 216–224 (1987).
4) A. Sidthimunka, J. Sanglert, and O. Pawapootanon: The Culture of catfish (*Clarias* spp.) in Thailand, in "FAO Fisheries Reports, 44(5)" (ed. by T. V. R. Pillay), 1968, pp. 196–204 (1968).
5) M. N. Bruton: The breeding biology and early development of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias* (*Clarias*). *Trans. Zool. Soc. Lond.*, **35**, 1–45 (1979).
6) W. Tarnchalanukit: Induced spawning of walking catfish, *Clarias batrachus*, by water level regulation, in "Proc. of the Third Int. Symp. on Reprod. Physiol. of Fish" (ed. by D. R. Idler), St. John's, New Foundland Canada, 1987, p. 138.
7) C. F. Knud-Hansen, T. R. Batterson, C. D. McNabb, Y. Hadiroseyani, D. Dana, and M. Eidman: Hatchery techniques for egg and fry production of *Clarias batrachus* (Linnaeus). *Aquaculture*, **89**, 9–19 (1990).
8) N. Zonneveld, A. C. Wilbrink, A. Soeprijanto, W. J. A. R. Viveen, and Y. Nursalam: Induced spawning of the Asian catfish—*Clarias batrachus*—by means of HCG. The Second Asian Fisheries Forum, Tokyo, 1989, pp 587–590.
9) E. H. Eding, J. A. L. Janssen, G. H. J. Klein Staarman, and C. J. J. Richter: Effects of human chorionic gonadotropin (HCG) on maturation and ovulation of oocytes in catfish—*Clarias lazera* (C & V), in "Proc. Int. Symp. Reprod. Physiol. Fish. PUDOC Wageningen", the Netherlands. 1982, p. 185.
10) M. F. A. Mollah and E. S. P. Tan: HCG induced spawning of the catfish—*Clarias macrocephalus* (Gunther). *Aquaculture*, **35**, 239–245 (1983).