OBSERVATION ON LARVAL DEFORMITY DURING INDUCED SPAWNING OF WALKING CATFISH, *CLARIAS BATRACHUS* (ACTINOPTERYGII: SILURIFORMES: CLARIIDAE), AT DIFFERENT COMBINATIONS OF HUMAN CHORIONIC GONADOTROPIN DOSE AND LATENCY PERIOD

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Abstract. Five combinations of human chorionic gonadotropin (hCG) dose and five latency periods were evaluated to observe the pattern of deformed larvae among the hatchlings in walking catfish, *Clarias batrachus* (L.). A higher percentage (7%–12%) of deformed larvae were observed at 1000, 2000, and 5000 IU $\cdot$ kg^{-1} with 14–23 h latency combinations. The results of the study indicated that 3000 IU $\cdot$ kg^{-1} with 14–23 h and 4000 IU $\cdot$ kg^{-1} with 14–17 h latency combinations were preferable to reduce the deformed larvae (4%–6%) among the hatchlings during induced spawning of the catfish.

Keywords: *Clarias batrachus*, deformed larvae, latency period, human chorionic gonadotropin, hCG

The walking catfish, *Clarias batrachus* (L.), is a potential species for aquaculture. Its production has been documented at few occasions (Areerat 1987, Thakur and Das 1986). Availability of quality seed is one of the important pre-requisites for successful culture of any species. So induced spawning is always advisable for getting optimum quantity and quality of the stocking material.

The breeding performance and egg quality of *C. batrachus* has been reported by the use of different dose of inducing agents with latency combinations in several occasions (Zonneveld et al. 1988, Sahoo et al. 2005, 2007). The deformed larvae like bend trunk and tail, acephalic and tunicate larvae are often encountered along with the normal larvae during breeding operations in carp (Rath et al. 1995) and catfish (Sahoo et al. 2004). The quantity of deformed larvae in *Silurus glanis* was observed as high as 50% during hatchery production (Linhart and Billard 1995). The deformity in larvae is originated due to poor egg quality, impact of environment or due to fertilization of over-ripped eggs. The over-ripening of eggs occurs due to delayed in stripping of ovulated eggs. This condition is sometimes happened while working in the hatchery condition. The increase in number of deformed larvae affects the production, quality of larvae and the profitability of a hatchery. No information is available on the pattern of deformed larval production while using human chorionic gonadotropin (hCG) as an inducing agent during induced breeding of *C. batrachus*. Hence the present study is aimed to communicate the pattern of deformity among the hatchlings during its induced spawning at different dose of hCG and latency period combinations.

C. batrachus broods were raised in earthen ponds (0.01 ha) at the Institute and were fed with pelleted feed containing 30% crude protein at 2% of their body weight, daily. The female broods of 120–130 g weight range were selected for induced breeding during July–August. The female broods were selected as suggested earlier (Sahoo et al. 2005). The hCG was purchased and reconstituted in 1 mL of solvent provided with the pack. That was further diluted with normal saline solution to get required concentrations of injectable hCG. The groups of females were injected with 1000, 2000, 3000, 4000, and 5000 IU hCG per kg body weight, and stripped after five latency periods, 11, 14, 17, 20, and 23 h in 25 (5 × 5) different combinations. Each group of fish comprised of five females and, was considered for each dose level and latency period combination.

The testes were removed from three male fish, incised and squashed in normal saline solution (0.89% NaCl) to get sperm suspension. A drop of sperm suspension was

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checked under microscope for sperm motility only once of every time before fertilizing the eggs of the female exposed to particular latency and dose combination. The motility of the sperm was initiated by the use of water supplied to hatchery and was found to be more than 80%. At the end of desired latency periods the eggs of individual females were stripped into clean dry plastic Petri dishes.

From each female, three samples of 300 mg egg were weighed to its nearest mg and the individual samples were mixed gently with 4–5 drops of sperm suspension. Thereafter the eggs were washed thoroughly with water and released into the plastic incubation tray of 5-L capacity, provided with flow through of water (0.2 L · min^{-1}) till hatching. After complete hatchings, the total number of deformed larvae was counted among the total hatchling on the basis of bent trunk and tail, short tail, headless and rudimentary tail. The mean deformed larvae in triplicate trays were recorded and expressed as percent of deformed larvae per female. The mean percent of deformed larvae in all five females was considered as the rate of deformed larvae under a particular latency period and hormone dose combination.

Hatchery water temperature, pH and dissolved oxygen were 27–28.5°C, 6.8–7.5 and 5.8–6.7 ppm, respectively. Statistical analysis of the data was performed using two-way analysis of variance (Snedecor and Cochran 1967) that included effects due to the dose of inducing agent and latency period. Treatment effects were considered significant at $P < 0.05$.

The percentage of deformed larvae among the hatchlings during induced spawning of *C. batrachus* at various doses of hCG and latency periods is presented in Table 1. There were no deformed larvae found in females injected with 1000–5000 IU · kg^{-1} and stripped at 11 h post-injection, since the eggs did not hatch. Whereas the deformed larvae (Figs. 1, 2) were observed in all other combinations during induced spawning and they appeared as high as 11%–12% in certain combinations. The injection of low dose (1000 and 2000 IU · kg^{-1}) to female fish and stripping at 14–23 h post-injection produced higher percentage of deformed larvae, which were similar to each other ($P < 0.05$). At these combinations, it was difficult to strip the females indicating incomplete ovulation. The ejection of

Table 1

Pattern of deformed larvae percentage among the hatchlings during induced spawning
of *Clarias batrachus* at different dose of hCG and latency period combinations

Latency period [h]	hCG per kg of body weight				
	1000 IU	2000 IU	3000 IU	4000 IU	5000 IU
11	0^{a}_{w}	0^{a}_{w}	0^{a}_{w}	0^{a}_{x}	0^{a}_{w}
14	$8.60 \pm 3.60^{a}_{v}$	$8.00 \pm 2.04^{a}_{v}$	$5.20 \pm 0.58^{ab}_{v}$	$4.20 \pm 0.37^{b}_{w}$	$9.60 \pm 0.50^{a}_{v}$
17	$7.40 \pm 3.02^{ab}_{v}$	$7.60 \pm 1.96^{ab}_{v}$	$6.20 \pm 0.48^{b}_{v}$	$4.60 \pm 0.67^{b}_{w}$	$10.60 \pm 0.50^{a}_{v}$
20	$9.40 \pm 2.44^{a}_{v}$	$8.80 \pm 0.66^{a}_{v}$	$5.00 \pm 0.31^{b}_{v}$	$8.40 \pm 0.50^{ab}_{v}$	$11.20 \pm 1.11^{a}_{v}$
23	$8.00 \pm 2.02^{ab}_{v}$	$9.00 \pm 0.70^{ab}_{v}$	$5.40 \pm 0.50^{b}_{v}$	$10.00 \pm 0.50^{a}_{v}$	$11.80 \pm 0.80^{a}_{v}$

The value bearing different superscripts in the row differs significantly ($P < 0.05$); The value bearing different subscripts in the column differ significantly ($P < 0.05$).
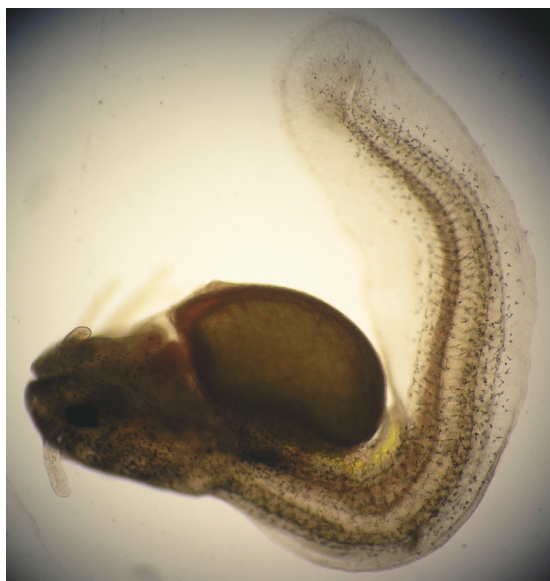

Fig. 1. *Clarias batrachus* hatchling showing bend trunk
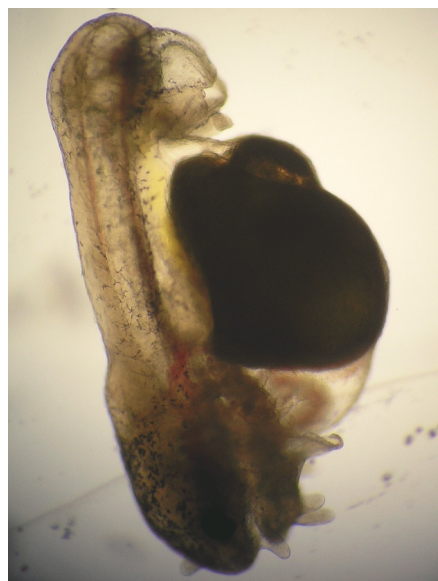

Fig. 2. *Clarias batrachus* hatchling showing fused tail

unripe eggs cannot be ruled out during the stripping of incomplete ovulated females. So the production of abnormal larvae at these combinations might have originated due to fertilization of unripe ova, which agrees to the previous report (Richter and van den Hurk 1982). In a separate study, higher percentage of deformed larvae was also appeared while injecting lowest dose of an inducing agent in this catfish during spawning induction (Sahoo et al. 2005). The higher percentage of abnormal larvae ranging from 8%–12% was also observed when the females were injected 4000 and 5000 IU · kg^{-1}, and stripped at 20–23 h and 14–23 h post-injection respectively. The eggs obtained by stripping in these combinations might have remained longer period in ovocoel after ovulation. These ovulated eggs were also exposed to hypoxic condition, led to over ripening (Ohta et al. 1996). Hence the high fraction of abnormal larvae at these combinations may have originated from fertilization of over-ripped eggs in this catfish, as also reported in *Pangasianodon hypophthalmus* (cf. Legendre et al. 2000) and *Silurus glanis* (cf. Linhart and Billard 1995). The reduction in permeability of essential ions (Lam et al. 1978), error in the chromosomal distribution (Saito et al. 1993), and chromosomal aberration (Yamazaki et al. 1989) are some of the causes reported during aging phenomenon responsible for abnormal larval production. As few as 4%–5% of deformed larvae were observed when the female was injected with 4000 IU hCG and stripped at 14–17 h post-injection. The deformed larval production was also 5%–6% only at 3000 IU dose for an extended period of 23 h latency.

In practice, it is therefore recommended that the hCG injection of 3000–4000 IU · kg^{-1} body weight in combination with 14–17 h post-injection is the best to reduce the possibilities of over-ripening of ovulated eggs. Further prolonging of latency up to 23 h at 3000 IU hCG dose will be helpful to get reduced number of deformed larvae during breeding operation. So fertilization of ovulated eggs obtained in these combinations ensures larger number of normal larvae production during spawning induction.

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