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Comparative Ovulation in Clariidae using Crude HCG from Early Pregnancy Urine

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ABSTRACT

Members of Clariidae do not spawn in captivity. Culture of these species in captivity must necessitate induce spawning of the eggs. Synthetic hormones have been known to assist this process. This paper used purified crude HCG from early pregnancy urine to assay ovulation in three species of Clariidae. Purification and extraction of crude HCG was carried out by the techniques involing alcohrl precipitation, ion exchange chromatography and gel filteration. Biological activity of the purified homogenate was tested on three species of clarridae: *Heterotranchus longifilis, clarias gariepinus and clarias anguillaris* at a dose 2.5i.u/gram body weight. Eggs were stripped from fish after a latency period of 12-20hours. *H. longifilis* gave the highest number (percentage) of stripped eggs and showed significant difference with clutch size (PLO.05) when compared with *C. gariepinus* and *C. anguillaris* No significant difference (P > 0.05) was observed in fecundity between the two *Clarias* species. However all the three species showed high fecundity when treated with the homogenate. The results show positive implications for the culture of the species in captivity.

Key words: Ovulation, Clariidae, HCG

The members of clariidae are highly esteemed food fishes in Africa (Inyang et al 1997). With increasing exploitation of wild stocks, there is an obvious decrease in the wild stocks supporting the protein base of African inhabitants. It is estimated that the members of Clarridae and Cichlidae support 50% of protein intake of rural inhabitants in Africa (FDF 1990).

With the increasing depletion of stocks in the wild, a recourse to the culture of the species in captivity becomes inevitable. Although the cichlids breed in captivity, the propagation of the more cherished members of Clariidae are hindered due to the inability of the species of this family to ovulate and spawn in captivity. This is because the stimulation of the gonadotropin hormone (FSH and LH) in the clariids are linked to environmental factors such as flooding in rivers. Literature is replete with the use of hormone preparations such as HCG and pituitary extracts to induce ovulation.

Anibeze (1998) reported the purification and extraction of crude HCG from early pregnancy urine to induce ovulation in *Heterobranchus longifilis.* The report showed that comparable success was achieved when compared with the pituitary hormone stimulation which is known to have been long standardized. The report found that 2.5iu/gram body weight of the crude HCG gave the highest fecundity in *H. longifilis.* This paper presents information on the comparative ovulation in three species of Clariidae adjudged to be the most important members of this family in terms of biomass supporting the protein base of Africans. The species include *Heterobranchus longifilis* (Valencieness), *Clarias gariepinus* (Burchell) and *Clarias anguillaris* (Linnaeus).

MATERIALS AND METHOD

Pooled early morning urine samples were collected from the ante-natal ward of Bishop Shenahan Hospital, Nsukka. Purification and extraction of crude Human chorionic Gonadotrophin (HCG) was carried out by the method described by Anibeze (1998) and Bell et al (1969) which involved alcohol precipitation, ion exchange chromatography and gel filteration.

The purified homogenate of crude HCG was administered to 30 gravid but non-ovulating *H. longifilis, C. gariepinus* and *C. anguillaris.* 30 each of the three species were earlier selected with distended stomach which is indicative of their gravid state. They were non-ovulating and kept in three separate tanks and left to acclinatise for two weeks. The fish were fed ad libitum with Pfizer fish fed.

After acclimatization, fish were injected with 2.5iu/g body weight intraperitoneally between the dorsal and rayed fins.

Beginning 6hours after injection fish were checked hourly for ovulation by the application of gentle pressure in the antero-posterior direction of the abdomen. Weight of stripped eggs were taken and number counted after each batch of stripped eggs have been immersed in Gilson's reagent to loosen the mesenteries and free the eggs for counting. Counting was done directly and values expressed as percentages for each species. Analysis of variance was used to compare the values of the these species at 95% confidence limit (ie P=0.05).

RESULTS AND DISCUSSION

The result showed that 2.5iu/gram body weight was able to induce ovulation in all the three species. However the number of stripped eggs showed variability (Table 1). Ovulation was highest in H. longifilis. The differences in the number of stripped eggs was significantly higher in *H. longifilis* when compared with *C. gariepinus* and *C. anguillaris* ($P<0.05$). However the difference between the two C***larias*** species was not statistically significant ($P>0.05$)

The latency period between injection of hormone and ovulation was similar in the three species and ranged from 12 to 12hours.

TABLE 1: The Effect of Crude HCG on Ovulation of *H. longifilis*, *C. gariepinus* and *C. anguillaris*

Species	Body weight(g)	No. of striped Eggs per female kg(x1000)
H. longifilis	1659 $\pm$ 1075	56.8
C. gariepinus	1211 $\pm$ 755	44.6
C. anguillaris	1206 $\pm$ 811	43.5

Table 2: The Effect of Crude HCG on Percentage (number) Ovulated Fish

Species	No. of Ovulated fish	No. of non-Ovulated fish
H. longifilis	90 (27)	10 (30)
C. gariepinus	81 (24)	19 (6)
C. anguillaris	83 (25)	17 (5)

In this study purified crude HCG was able to successfully induce ovulation in the three species of Clariidac (Table 1). The appreciable percentages of ovulated fish (Table 2) is indicative of the fact that purified extract of crude HCG from early pregnancy urine has a high efficiency in inducing ovulation in the clariids. The implication for the culture of these species is that appreciable quality oocytes can be obtained from these species even during their "off season" for spawning. This work corroborates Anibeze (1998) on a single species of this family. Also it is in line with the high HCG biological activity recorded in the mouse uterine weight assay using purified extracts of crude HCG from early pregnancy urine (Bell et al 1969, Lytle & Haskel 1964)

The number/percentages of ovulated fish which is highest in *H. longifilis* is in line with the high fecundily recorded for this species over the other two clariids (Anibeze 2002, Teugels et al 1990). The non significant difference recorded for *C. gariepinus* and *C. angullaris* is also in line the observed natural fecundities of the two species. Available data have shown that both osteolgical and morphometric parameters have always been significantly higher in *Heterobranchus* when compared with the other Clariids (Tengels et al 1990, Anibeze 2002, Legendre 1986, hecht & Lublinkhof 1985)

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