EFFECTS OF HUMAN CHORIONIC GONADOTROPIN (HCG), TOAD AND *Clarias* PITUITARY HORMOGENATES ON SPAWNING IN THE CATFHISH: *Clarias lazera* (C&V) AND *Clarias anguillaris* (LINNE)

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ABSTRACT

Experiment on induced spawning of *Clarias lazera* and *Clarias anguillaris* using human chorionic gonadotropin (HCG), freshly prepared toad and *Clarias* pituitary hormogenates were carried out.

Clarias pituitary hormogenates induced spawning in *C. lazera* and *C. anguillaris* at dosage levels of 0.27–0.46 mg/150 g body weight or 2 glands/fish of equivalent weights. HCG induced spawning in *C. anguillaris* at 500 i.u/500 g body weight but failed in *C. lazera*. Toad pituitary was not successful at even a higher dosage level of 0.60 mg/150g body weight. The implications of these results are discussed.

Spawning occurred in the HCG (and *Clarias* pituitary treated females in less than 12 hours after injection and subsequent examination of ovaries of the spawned fish showed incomplete spawning. Furthermore, fertilization occurred, following spawning in the piscine pituitary hormone treated male and female fish but failed in the HCG (treated pair. A mean fertilization rate of 50–90% was recorded. Possible explanations of these observations are advanced.

The hatching time of 24–48 hours and a mean hatching rate of 75–80% were recorded. A high larval mortality of up to 95% was observed in the post yolk-sac stage after 8 days. The need for the development of appropriate larval food for *Clarias* species in culture practice is stressed.

INTRODUCTION

Hormonally induced ovulation and spawning have been carried out in different parts of the world. Experiments with the channel catfish, *Ictalurus punctatus* have shown tht spawning was induced by the injection of pituitary materials from carp, buffalo fish, flat head catfish, channel catfish or gar into the females (Gray, 1976). Ramaswani and Sundararaj (1956) have induced ovulation in gravid *Heteropneustes fossilis* by injecting hormogenate of the catfish pituitary glands. Success was also obtained with pituitary hormogenate and some mammalian hormones (Ramaswami, 1962; Sundararaj and Goswami, 1977). Partially purified salmon gonadotropin (SG–100) brought about ovulation in the hypophysectomized *Heteropneustes fossilis* (Sundararaj *et al*, 1972). Similarly, de Kimpe and Micha (1974) have suggested that the rise of water level as stimulus for *Clarias lazera* to spawn can be replaced by a hormonal injection. Tests with pituitary suspensions and DOCA (Deoxycorticosterone acetate) have been successful, whereas human chorionic gonadotropin (HCG) and luterinizing hormone (LH) gave negative results in inducing spawning in *Clarias lazera* (de Kimpe and Micha, 1974; Nwoko, 1983, Adigun *et al*, 1984).

In the present study, HCG, Toad and *Clarias* pituitary hormogenates were comparatively used on *C. lazera* and *C. anguillaris* so as to determine their relative potency in inducing spawning in the two fish species.

MATERIALS AND METHODS

Collection and Care of Fish

Specimen of *C. lazera* and *C. anguillaris* were collected from fishermen in the Anambra River Basin in mid-February. The fish were captured by bailing out of water from family ponds – a dry season (February/March fishing method in the basin (Awachie, 1975; Awachie and Ezenwaji, 1981; Ezenwaji, 1982). This method guarantees fish capture with minima? injuries to the fish. The fish were transported a distance of about 130 kilometers in FAO type standard fish carrying tanks, to the experimental station (Zoological Garden, University of Nigeria, Naukka) The catfish were kept in concrete tanks and fed on domestic waste for a period of four months.

Preparation of Pituitary Hormogenates

Pituitary glands were removed from mature fish and toad respectively. The fish or toad was weighed on a torsion balance to the nearest gramme and immediately killed by a blow on the head. Careful removal of the roof of the buccal cavity exposed the rounded whitish mass – pituitary gland, located ventrally at the base of the cerebrum. This was then excised with fine forceps weighed on a mettler balance and stored prior to use in saline-alcohol (10% absolute alcohol in 0.6% Nacl). The glands were later ground in groups of 4, 8 and 12 respectively in pyrex test-tubes using glass pistle, hormogenized in 2 ml Saline-alcohol and then centrifuzed using Sanetzki (T30) centrifuge. Each hormone concentrate (the supernatant) was then collected in 2 ml stringe for use.

Female Treatment

Gravid females (mean body weight 130g) were chosen for this study. Each femal was weighed and placed in separate (80x40x40cm) aquarium tank containing 70 litres of water and palm fronds at the bottom as substrate for the attachment of spwaned eggs. The fish was allowed to acclamatise for one day. Ovarian development was determined by in vivo siphoning of intra-ovarian eggs using cannula according to Shedadeh *et al* (1973 b) and females with mean egg diameters in excess of 800 u were selected for use. Fish weights and initial mean egg diameters are shown in Tables 1 and 2. In the absence of established guidelines, dose rates were chosen arbitrarily at the beginning of the study and subsequently modified according to initial results. Injections were given in the evening (17:00 to 18:00 hours) and at the base of the caudal peduncle of each fish.

In order to determine the effect of single dose injection in each female, in vivo egg samples were taken before each injection. Egg diameters were measured and the dose rates (Unit/150 g body weight) and injection schedules are listed in Tables 1 and 2.

Male Treatment

The males used in this study were taken from the same captive populations as the females. The body weights of both males and females were equally matched and were injected with equal dosage of hormones.

The male was placed with each injected female when the latter began to exhibit abdominal distension (ovulation) (Shehadeh et al, 1973b). At this time, aeration was increased in the tank to help in mixing of eggs and sperm. Spawning and fertilization of released eggs were permitted to proceed naturally in the tanks. Each experiment was in three replicates. The female and male catfish were removed from the spawning tanks after spawning and natural fertilization was allowed.

Table 1 – Spawning by exogenous hormone treatment in *Clarias lazera* (C&V)

Group	*Mean Body Wt. (g)*	*Hormone Treatment*	*Dose Inject. Unit/150 g Body (Wt.)*	*No. of Injections.*	*Initial Mean Egg Diameter (u)*	*Latency Period (Mean)*	*Fecundity (Estimated After Spawning (mean)*	*Fertilization Rate (% Range)*	*Hatching Range*	*Mortality After 8 days*	
										Egg Yolk	*Natural Food*
1	130	HCG	20 i.u	1	900						
2	135	HOG	300 i.u	1	960						
3	150	HOG	500 i.u	1	880						
4	122.5	Toad Pituitary (3 glands)	0.26 mg	1	890						
5.	131.6	Toad Pituitary (5 glands)	0.44 mg	1	936						
6.	134	Toad Pituitary (7 glands)	0.61 mg	1	1050						
7.	135	*Clarias* Pituitary (1 gland)	0.12 mg	1	985						
8.	130	*Clarias* Pituitary (2 glands)	0.29 mg	1	950	11 hrs 10 mins	3,120	80–95	90–95	90–93	40–70
9.	142	*Clarias* Pituitary (3 glands)	0.46 mg	1	1120	10 hrs 30 mins	3,566	90–95	85–100	90–95	40–70

Table 2 – Spawning by Exogenous hormone treatment in *C. anguillaris* (Linne)

Group	*Mean Body Wt.(g)*	*Hormone Treatment*	*Dose Inject. Unit/150 g Body (Wt.)*	*No. of Injections.*	*Initial Mean Egg Diameter (u)*	*Latency Period (Mean)*	*Fecundity (Estimated after Spawning (mean)*	*Fertilization Rate (% Range*	*Hatching Range*	*Mortality After 8 days*	
										Egg Yolk	*Natural Food*
1	125.5	HCG	200 i.u	1	999						
2	120	HCG	300 i.u	1	842						
3	132	HCG	500 i.u	1	947	10 hrs 45 mins	5,800	None			
4	126.5	Toad Pituitary (3 glands)	0.23 mg	1	894						
5	128	Toad Pituitary (5 glands)	0.41 mg	1	1030						
6	138	Toad Pituitary (7 glands)	0.60 mg	1	999						
7	128.6	*Clarias* Pituitary (1 gland)	0.13 mg	1	1105						
8	126.5	*Clarias* Pituitary (2 glands)	0.27 mg	1	947	11 hrs 26 mins	2,150	55–80	75–100	80–90	40–55
9	130.8	*Clarias* Pituitary (3 glands)	0.40 mg	1	894	11 hrs 32 mins	2,600	75–96	80–100	85–95	45–60

Determination of the Number of Eggs Spawned

The weight of the females were taken before the injection and after spawning. The drop in weight was used to calculate the numher of eggs spawned using the equation for estimating the fecundity of *Clarias lazera* by Hogendoorn (1977). Total number of eggs = 66.6xfemale body weight(s).

Estimation of Percentage Fertilization

The number of unfertilized and fertilized eggs were estimated by counting 10 to 12 hours after fertilization. The unfertilized eggs were white while the fertilized eggs were transparent (de Kimpe and Miche, 1974). The percentage fertilization was estimated from the number of unfertilized eggs by the equation:

$$\% \text{ fertilization} = \frac{N - n}{N} \times 100$$

N = Total No. of eggs

n = No. of unfertilized eggs.

RESULTS

The results of the effects of various dosage levels of human chorionic gonadotropin (HCG) and pituitary materials on spawning are summarized in Tables 1 and 2. Both HCG and *Clarias* pituitary homogenates proved to be potent spawning agents for *C. anguillaris.*

With a high dose of 500 i.u of HCG/150g body weight; *Clarias anguillaris* spawning naturally producing a mixture of mature and immature eggs. Similar dosage and even more failed to elicit spawning in *C. lazera.* There was no fertilization as the eggs degenerated after 4 days.

All the females injected with different dosages of toad pituitary homogenates neither ovulated nor spawned throughout the period. However, spawning occurred in females treated with *Clarias* pituitary at a minimum 0.27 mg/150g body weight dosage level. Examination of the ovaries confirmed that spawning was incompiete even at a higher dosage levels. Spawned fertilized eggs measured 1313 + 10 u.

The "latency period" (i.e. the time before spawning after injection of the effective dose) varied between 10 hours 30 minutes and 11 hours 30 minutes. During this time, the females exhibited gradual abdominal distension and protrusion of the cloacal region. This distension was apparently due to egg hydration (ovulation) (Shehadeh*et al,* 1973a).

Observations on the Spawning Behaviour, Hatching and the Early Larval Stages

During the latency period, and as each female began to exhibit abdominal distension, the male became more active and tended to remain in close contact with the female. Occasionally, the male would spin around the female or nudge her cloacal region. When female eventually shed her eggs the male was always closely applied to her sides with the caudal fin near the femals's cloaca. As spawning commenced, the bodies of the males vibrated rapidly and the caudal fin tended to disperse the eggs. Sperm was not visible during natural spwaning. Fertilization rates ranged between 50% and 90%.

Most of the sheded eggs due to their adhersive outer covering were attached to palm fronds provided as substrate. The unattached eggs at the bottom of each aquarium seldom hatched. Hatching occurred from 24–48 hours after spawning at temperatures of 26–27oC and pH of 6.9–7.1. Hatching success was estimated to be 75 to 80%.

The hatchings on emergence wriggle downwards into the gravel bottom only to re-emerge after two days, swimming vigorously in the water column. The larvae at this stage is transparent and yolk ladden.

Larvae were fed on two separate diets - ground boiled egg yolk and natural food (plankton) filtered out of the concrete pond. Initial larval feeding started just before final yolk resorption on the third day when larvae were seen clustering around egg-yolk deposits or darting around for food in the plankton feed tanks. The darting behaviour of larvae for planktonic food seems a predatory habit and suggests a preference for zooplankton which incidentally was in short supply.

Larval mortality as high as 95% was recorded after 8 days in the egg-yolk feed tanks. Most dead larvae were seen entangled in the mesh produced by mucor that had grown around each egg yolk deposit.

DISCUSSION

The efficacy of HCG in the induction of spawning in *C. anguillaris* in this study confirms similar findings in the catfish, *Clarias batrachus* (Ramaswami and Sundararaj, 1956) and Indian catfish, *Heteropneustes fossilis* (Sundararaj and Goswami, 1966) and in the channel catfish, *Ictalurus punctatus* (Sneeds and Clemens, 1969). On the other hand, the failure of HCG to induce spawning in *C. lazera*, a closely related species to *C. anguillaris*, collaborates the findings of De-Kimpe and Micha (1974) and Adigun *et al* (1984). The reason for this is not known. In the present study also, a comparatively high dose (500 i.u./150g body weight) of HCG was required to induce spawning, compared to 700 i.u/450g body weight in the case of *Ictalurus punctatus* (Sneeds and Clemens, 1969), 100 i.u for *Heteropneustes fossilis* (Sundararaj and Goswami, 1966) and 250–300 i.u. for *C. batrachus* (Ramaswami and Sundararaj, 1956). Thus the spawning response of different catfish species to HCG may be dose-related as well as species specific.

Of interest is the non-occurrence of fertilization in the HCG spawned eggs and the reason for such a phenomenon remain unclear. It is however, tempting to speculate that HCG may have caused only spawning (abortion) in *C. anguillaris* (Nwoko, 1983), without bringing about final maturational changes preceding spawning in both the male and female fishes. In such a case, the action of HCG in *C. anguillaris* is similar to that of Deoxycorticosterone acetate (DOCA) in *C. lazera* which only induced spawning without affecting eocyte maturation (De Kimpe and Micha, 1974).

Toad pituitary hormogenate failed to induce spawning in *C. lazera* and *C. anguillaris* at even 0.6 mg or 7 pituitary glands/150g body weight. The reasons for this failure are not clear and further investigation is deserable. The successful spawning runs achieved in *C. lazera* and *C. anguillaris* when injected with freshly prepared pituitary homogenates confirm earlier reports on the use of exogenous fish pituitary material to induce spawning in catfishes (Ramaswami and Sundararaj, 1956; Sundararaj and Goswami, 1966; De Kimpe and Micha,, 1974; Gray, 1976; Nwoko, 1983 and Adigun *et al*, 1984). The spawning time of less than 12 hours, in all the treatment after injection, in this study is in agreement with the 10–16 hours range reported by De Kimpe and Micha (1974) for *C. lazera*.

Of significance however, is the fact that a higher dose of 0.40mg or 3 pituitary glands/150g body weight yielded partial spawning in both *C. lazera* and *C. anguillaris*. Two possible reasons can be adduced for this phenomenon. Firstly, it is possible that the "captivity syndrome" aided by the limited space provided in the aquarium may have hampered the vigorous movement of the catfish during spawning resulting in partial spawning. A second possibility which can be verified may be that *Clarias* sp. do not shed all the eggs in one spawning-run which presupposes that the species spawn intermittently and probably at definite intervals over a period of time. The later argument is butressed by the examination of ovaries of female *C. anguillaris* and *C. lazera* from the wild during the late spawning season (August–September), which varied from deflated sacs forming amorphous mass to fully turgid sacs from which fewer or no eggs had apparently been shed. Similar observations for *C. gareipinus* (Bruton, 1979) and *C. albopunctatus* (Ezenwaji, 1982) after the spawning season lends credence to this possibility.

The observed hatching time for eggs of between 24 to 48 hours after fertilization was within the range (minimum of 24 hours) recorded for *C. lazera* (De Kimpe and Micha, 1974). The high larval mortality recorded in 8 days after hatching may have resulted from mal-transformation from the yolk-sac stage to the internal feeding runs after the resorption of the yolk sac. This situation in the present circumstances could be attributed to the use of inappropriate or poor food quality for initial larval feeding (Jones et al, 1974). This highlights the importance of adequate food for catfish larval rearing. Alternatively, a detailed investigation on the development of appropriate techniques for the production of plankton preferred by *Clarias* larvae is thus recommended.

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