Comparative study of hatching rates of African catfish (*Clarias gariepinus* Burchell 1822) eggs on different substrates.

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Abstract

The choice of incubation substrate materials is one of the major factors to consider in the production of African catfish (*Clarias gariepinus*) fingerlings. There was significant difference between the hatching rates of the catfish eggs incubated on natural substrates: Nile cabbage (*Pistia stratiotes*), water hyacinth (*Eichhornia crassipes*), pond weed (*Ceratophyllum dermasum*) roots, and green grass leaves (*Commelina Sp.*) and the artificial substrates: sisal (*Agava sp.*), plastic, papyrus (*Cyperus papyrus*), kakaban mats and concrete slabs. Ranking of the natural substrates by performance indicated that *Pistia* roots were the best with mean hatching rate of 66.2 ± 3.62%, the green grass leaves were second with a mean of 54.0 ± 3.46%, water hyacinth roots were third with mean rate of 49.7 ± 3.16%, while the *Ceratophyllum* roots were fourth with a mean of 13.0 ± 2.37%. The ranks for the artificial substrate showed that the concrete slabs tied with the sisal mats as the highest having mean rates of 18.6 ± 2.8% and 18.6 ± 2.0% respectively, papyrus was second with a mean rate of 12.2 ± 1.2%, kakaban was third with a mean rate of 11.8 ± 1.9% while the plastic mats were the last with mean rate of 4.0 ± 0.7%. The natural substrates performed better than the artificial substrate. The best performing natural substrates were those with floating ability and thin fibrous roots that seemed to allow higher aeration of the eggs during incubation. There were minimal costs incurred in the usage of the natural substrates.

Introduction

The fish of the family Clariidae is endemic to Africa. *Clarias gariepinus* currently but mistakenly, synonymized with *C. mossambicus, C. lazeras* and *C. senegalensis* ranges from Natal and the orange River in South Africa through central, west east and north Africa where it is under culture (Teugels, 1986). The widespread distribution is a reflection of their ability to tolerate a wide range of environmental parameters. *C. gariepinus* adapt well to artificial environments, and has rapidly gained status as premier aquaculture species Hetch, Uys and Britz (1988). Early in the twentieth century, colonists realized that *Clarias* might have an economic value since they were highly prized by the locals and catches demanded high market prices. By the early 1950's Belgian workers started *Clarias* culturing techniques in the former Belgian Congo.

Clarias species are noted to have a rapid growth, a high reproductive potential and sturdy resistance to environmental variations. *Clarias* has been bred in the South and central Africa with varying degree of success (Clay, 1977; Msiska, 1981; and Hogendoorn, 1990). The potential for culture of *Clarias* is enormous. It comes at a time when tilapias have given discouraging results due to their uncontrolled breeding, diluted genetic progeny and stunted growth. A reliable supply of good quality fry resulting from high fecundity as well as good natality is an essential prerequisite to aquaculture development in Kenya. The Government of Kenya, Fisheries Department (1998) report show that the

culture of *Clarias gariepinus* is still low as indicated by production data over the years. In the years 1995 to 1998 the total aquaculture production was 14 metric tones and *C. gariepinus* contributed only 2% of this. In comparison, tilapia production accounted for 45% over the same period. Maithya, *et al.,* (2000) pointed out the great potentials of aquaculture in Kenya while Owiti, (2000) emphasized the need to reactivate aquaculture in Kenya. The issue of the catfish low production can be tackled by addressing the factors limiting aquaculture development including the main problem of seed production.

Demand for *C. gariepinus* fingerlings in Kenya both in aquaculture and as baits in capture fisheries have substantially increased in the last few years. The Fisheries Department estimates that for aquaculture activities, there is a demand of about 10 million *C. gariepinus* fingerlings per year, while the demand *for C. gariepinus* in the Lake Victoria capture fisheries is about 18 million fingerlings per year. This brings to a total of about 28 million *C. gariepinus* fingerlings per year, Government of Kenya (1998).

The government supplies about 5 million fingerlings per year through the Fisheries Department and the Lake Basin Development Authority (LBDA) farms. This leaves an estimated deficit of about 23 million fingerlings. There is therefore a need to increase their supply. This is only possible through 'simple-and- easy-to-adopt' protocols on efficient seed production and management for small-scale fish farmers.

The African catfish naturally spawns in floodplains during wet season in response to the rise in water levels (Pillay, 1990). Seed collection from the wild is however unreliable and limited only to rainy seasons. Under culture conditions, ovulation of the species can be induced by environmental manipulation and/or hormonal stimulation.

Presently, catfish seed production in Kenya has had limited success due to the high mortality of eggs and larvae. This has been attributed to among other factors, the use of unsuitable incubation substrates, e.g., mud, sand or concrete surfaces are commonly used despite the poor results. The hatching rates are usually as low as 25% (Obuya *et al.,* 1995), which is, far much below the 50-70 % recorded in well-managed hatcheries in other countries (de Graaf *et al.,* 1995). Moreover, there still is a poor survival rate of eggs and larvae to the fingerling stage, mainly as a result of inadequate nutrition during the early nursing phases along with careless nursery management practices. Successful hatching of fish eggs and careful feeding of larvae during the early stages of their development is essential for better larvae survival. This experiment aimed at realizing simple and low cost management practices for mass production of *Clarias* fingerlings.

Materials and Methods

This study was carried out at Kibos Fish Farm in Kisumu District, Nyanza Province. The farm is located along the Kisumu – Miwani road about 10 km from Kisumu Town. Mature brood stocks (females of about 300g to 500g and males of over 200g) were collected from the wild in Kano plains and stocked in Chwele, Yala and Kibos Fish Farms, all which are within the Lake Victoria Basin. They were then transferred from the farms and stocked at Kibos in a pond of about 300 m^2.

A few sample of the brood stock were sacrificed to obtain pituitary glands. The pituitaries were pulverized in a porcelain mortar, mixed with a physiological solution (9 g of de-iodised sodium chloride in one litre of distilled water), to make a pituitary suspension. The suspension was then injected intra-muscularly into the dorsal muscle of the experimental female fish, as outlined in de Graaf *et al.* (1996). Randomly selected, ready to spawn, females were used.

Eggs were obtained by stripping the females while milt was obtained by sacrificing the males. One male was used to fertilize eggs from three females. The milt was diluted with the physiological solution and was added to the stripped eggs, which were fertilized by adding equal volume of clean water to activate the sperms (de Graaf *et al.,*1995). A bird's feather was used to spread the eggs evenly on the substrate in bunches of 600-1200 eggs.

The eggs were then incubated on different substrate materials; *kakabans*, sisal, papyrus and plastic (nylon) mats all with equal surface area of 1350 cm^2. These were put in flow-through concrete troughs. Concrete slabs were used as control due to the prevalent use of concretes in the region. The same set up was done for the other set of substrates i.e., root fibres of Nile cabbage (*Pistia stratiotes)*, water hyacinth (*Eichhornia crassipes*), pond weed (*Ceratophyllum dermasum*) roots, and green grass (*Commelina Sp*.) leaves. The DO, PH, conductivity and temperature were monitored on a daily basis. The percentage hatching was obtained by using the formula outlined below,

$$\textit{Hatching rate} = \frac{\textit{No. of live hatchlings}}{\textit{No. of incubated eggs}} \textit{ X 100}$$

as stipulated in Viveen *et al.,* (1985). Costs of using each of the hatching practices were assessed by accounting for all expenses incurred in each method. Completely Randomized Block Design (CRBD) was used to allocate 600-1200 eggs into each of the experimental units.

Statistical analysis

The results of hatching rates were analysed by two-way analysis of variance (ANOVA). Multiple comparison analysis was used to assess any heterogeneity. Multiple Range Tests in Stat graphics Version 2.1 (Statistical Graphics Corporation 1994, Maryland, USA) that was based on the Fisher's least significance procedure as described by Zar (1984) was used to discriminate among means and offered more than two rankings. The tests were at 95% significance level, ($p<0.05$).

For each of the substrates data, a two-way ANOVA was carried out to assess for differences among the replicates. The two-way ANOVA was carried out to assess for differences between the hatching rates of eggs from the different female spawners.

The samples were pooled to represent only the substrates data classification. The resulting data was subjected to a one-way ANOVA.

Results

The experiments were carried out in the same environment with similar ambient conditions (Table 1).

Table 1: The mean parameters values

Parameter	Mean value	Unit
Dissolved Oxygen (DO)	5.9±0.2	Mg\l
Temperature	22.9±1.1	^{o}C
PH	7.0±0.5	No
Conductivity	649.2± 10.2	µS

Artificial substrates

From the analyses of the data, it can be noted that there was a statistically significant difference in the means of the hatching rates of all the artificial test substrates. The mean percentage rates of hatching ranged from 0.0 to 9.0 for plastic, 2.0 to 21.0 for kakaban, 5.0 to 9.0 for papyrus, 0.0 to 28.0 for sisal mats and from 0.0 to 31.0 for concrete slab (Table 2). The general hatching rates of the eggs on this category of the substrates was low with the highest being 31.0 for the concrete slab (control). The mean rates were 4.0, 11.8, 12.2, 18.6 and 18.6 for plastic, kakaban, papyrus, sisal mats and concrete slabs respectively. There was a statistically significant difference between the means of the four data samples at 95.0% confidence level.

Multiple range tests based on the Fisher's least significance difference procedure as described by Zar, (1984) was used to discriminate among the means. The tests revealed that all the means were statistically different from each other. However two pairs, the concrete and sisal and that of kakaban and papyrus, distinctively portrayed insignificant differences. Three homogenous groups of means were identified while ranking the substrates performance.

The plastic performed below 8% and was ranked lowest, the kakaban and papyrus performed between 8% and 16% while the sisal and concrete performed between 15 % and 25 % and was ranked highest as can be seen in Table 2.

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Table 2: The percentage hatching rates of artificial test substrates.

Substrate number	Sisal	Plastic	Kakaban	Papyrus	Concrete
1	15	6	21	13	25
2	14	1	9	17	21
3	27	5	16	17	31
4	0	0	16	6	3
5	7	1	5	5	0
6	2	0	19	5	1
7	25	2	10	12	10
8	20	3	13	10	12
9	15	4	12	7	17
10	20	5	2	15	9
11	18	2	4	11	14
12	17	2	11	9	16
13	15	2	10	8	17
14	17	6	12	11	22
15	22	3	20	10	26
16	24	5	2	12	20
17	25	1	10	13	29
18	17	2	14	16	30
19	24	7	5	18	25
20	20	3	12	15	24
21	25	2	10	12	10
22	27	5	16	18	31
23	17	6	15	12	15
24	21	5	16	10	19
25	19	3	6	13	16
26	22	7	16	14	21
27	21	5	6	14	17
28	22	7	4	13	16
29	28	6	17	19	31
30	18	8	21	15	28
31	16	5	13	9	19
32	17	9	15	12	22

The plastic performed below 8% and was ranked lowest, the kakaban and papyrus performed between 8% and 16% while the sisal and concrete performed between 15 % and 25 % and was ranked highest as can be seen in Table 2.

Natural substrates

The rates of hatching on the natural substrates ranged from 33.0% to 82.0% for *Pistia*, 0.0% to 33.0% for *C. dermasum* (pond weed), 34.0% to 74.0% for the green grass leaves (*Commelina Sp*.), and from 28.0% to 68.0% for the *E. crassipes* (water hyacinth) roots.

The general hatching rates of the eggs on this category of the substrates was noted to be significantly high with the highest being 82% for the *Pistia* roots (Table 3).

The mean rates were 66.2%, 13.0%, 54.0%, and 49.7% for *Pistia*, *C. dermasum*, green grass leaves (*Commelina Sp*.) and the *E. crassipes* roots respectively. The p-value of the F-test was less than 0.05 and therefore there was a statistically significant difference between the means of the four data samples at 95.0% confidence level.

The multiple range tests revealed that all the means were significantly different from each other except for one pair, the green grass leaves and water hyacinth roots which portrayed an insignificant difference. Three homogenous groups of means were identified with *C. dermasum* performing between 15% and 18%, the green grass leaves (*Commelina Sp.*) and *E. crassipes* roots rates falling between 43% and 60% while the *Pistia* rates ranking the highest at between 62% and 76%.

From the on going, it can be noted that the artificial substrates performed very poorly compared to the natural substrates. The best of the natural substrates were the *pistia* roots. The hatching rates from the *C. dermasum* substrate were quite low, this was thought to be due to the fast decaying and settling to the bottom characteristics of the substrate. Other substrates performed better probably because of their free-floating ability hence allowing better aeration of the eggs.

The costs of the substrates

There was significant cost difference incurred during the use of the artificial substrates and that of the natural substrates. The costs of using the artificial substrate was higher than that of the natural ones as can be seen in Tables 4 and 5. The costs in the use of artificial materials were more of direct in contrast with the indirect costs in the use of the natural materials. The indirect costs emanated from the opportunity costs for doing other activities or the man-hours used while collecting the substrate materials.

Table 3: The percentage (%) hatching rates of natural test substrates

Substrate number	Water cabbage	Pond weed	Grass	Water hyacinth
1	45	33	51	52
2	33	5	36	39
3	70	16	38	47
4	53	0	57	68
5	49	1	37	28
6	71	1	35	41
7	64	16	50	50
8	48	19	57	49
9	57	11	37	37
10	62	18	58	49
11	70	15	71	41
12	75	10	57	46
13	81	11	45	51
14	60	10	74	58
15	72	9	66	44
16	68	10	62	51
17	75	13	60	43
18	79	11	58	55
19	72	9	52	48
20	75	22	58	50
21	80	15	34	49
22	71	16	54	50
23	63	11	45	42
24	76	19	59	65
25	72	9	60	52
26	73	12	73	68
27	81	19	54	63
28	74	13	67	65
29	70	11	58	49
30	49	19	57	48
31	63	18	59	42
32	65	16	50	50

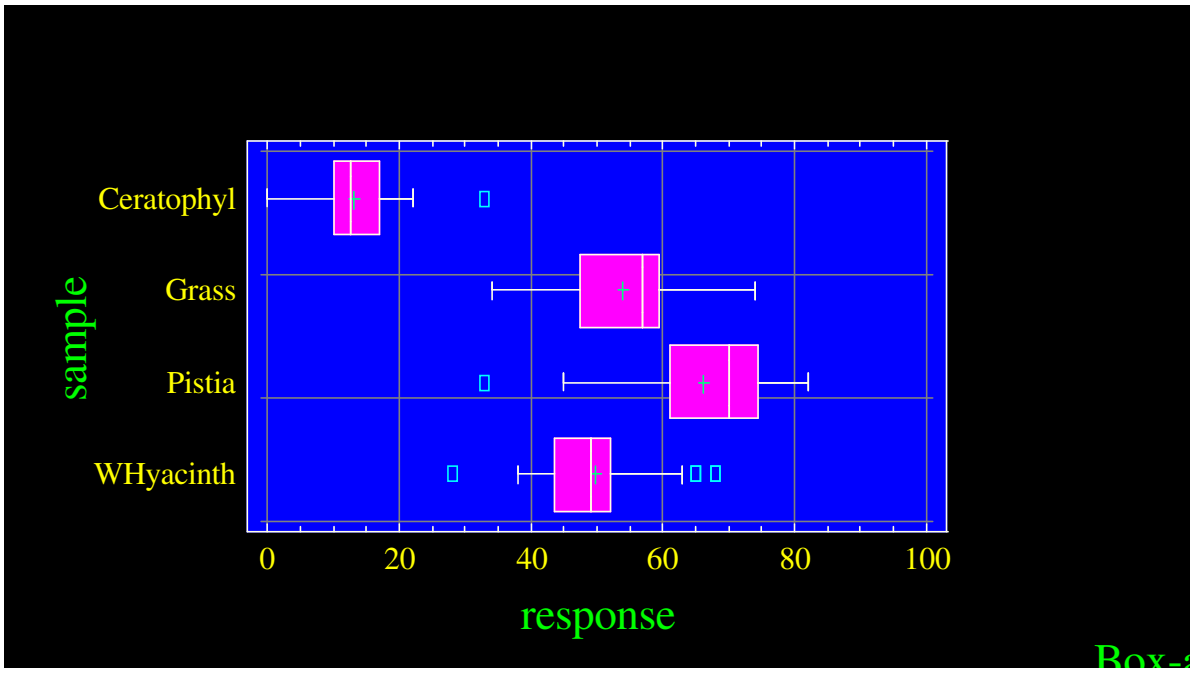

Figure 1. The percentage ranking of performance of the natural substrates.

Table 4: Costs of the artificial substrates

Substrate material	**Surface area**	**Costs (Kshs)**
Kakaban mat	1350 cm^2	10
Sisal mat	1350 cm^2	8
Plastic mat	1350 cm^2	10
Concrete slab	1350 cm^2	20

Table 5: Costs of the natural substrate

Substrate material	**Number of fibrous strands/leaves**	**Costs (Kshs)**
Water cabbage	200	2
Water hyacinth	200	2
Pondweed	200	2
Grass	200	1

Discussion

The *Clarias gariepinus* hatching protocols practiced in the experiments described above are easy to apply by small-scale fish farmers. They are particularly appropriate in the rural areas where there is no electricity and where most of the small-scale farmers are based. The costs are also minimal hence not a burden to the farmers the majority of whom are very poor.

This experiment showed that the hatching rates of the natural substrates especially those of the *Pistia* whose average hatching rate ranged between 62% and 76% were quite high when compared to the 25% hatching rates in Western Kenya as indicated by Obuya *et al.,* (1995).

The fecundity of *C. gariepinus* is high, using the Hogendoorn (1980) method of estimating fecundity, *Total no. of eggs = 66.6 x female body weight (g)* the estimated fecundity of the local catfish ranged from 15,000 to 50,000 eggs. Based on the small-scale fish farming levels in Kenya where most farmers have 1- 5 ponds of 50 m^2 each, a farmer using only one female to address his annual catfish seeds requirements may have an over supply if he uses the appropriate hatching and nursing procedures.

Fertilized eggs are usually incubated in stagnant or running water. A general principle of egg incubation is that water is renewed in order to provide oxygen and that after hatching the larvae are separated from the remaining egg-shells and dead eggs. This is important in order to avoid fungal infection of the hatchlings and consequent larval mortalities. Several incubation techniques have been used as outlined by de Graaf, (1996). One method is whereby the eggs are spread out on the bottom of a concrete basin. This

method works well but it has the disadvantages that dead eggs and eggshells are not separated from the hatchlings.

The eggs can also be spread out on a screen (mesh size 1 mm), which is then placed on the bottom of a concrete basin. This method works well as the hatchlings will pass through the screen and the dead eggs and shell remain on the screen. By removing the screen from the basin, separation of the hatchling and the dead eggs is readily achieved.
Our experiments show that the eggs can be allowed to stick to the roots of floating substrates such as water hyacinth (*Eichhornia crassipes*), Nile cabbage or water lettuce (*Pistia stratiotes*), or any other such as the green grass leaves that floats inside a concrete basin or hapa. The cost of the natural substrates are low and the hatchlings are easily separated from the dead eggs as long as the distance between the roots of the plants and the hapa bottom is kept at between 15-20 cm. The fertilized eggs usually develop normally if the incubation conditions mainly the oxygen, temperature and cleanness are provided.
In actual practice, there is always some mortally. We believe that the hatching rates are usually low due to low dissolved oxygen level and high turbidity of the water brought about by the use of a particular incubation substrate. Moreover, collection techniques of the hatchlings from the ponds or the large concrete tank beds increase mortality due to stress, is time consuming and laborious. Hence, low cost hatching protocols that can be practiced with ease by farmers in simple rural based hatcheries need to be developed.

The use of screened substrates, or fibrous materials placed in small holding container units may enhance oxygenation of eggs during incubation and make collection of hatchlings easier, faster and less stressing resulting to lower mortality rates.
According to Pillay (1990), about 3-5 % of the eggs die a few hours after incubation and another 5-10 % die near completion of development or during hatching. Higher mortalities are not normal and can be caused by several factors including a prolonged latency period, high stressing of female spawners during latency period, incubation of more than one layer of eggs per incubation substrate, poor incubation conditions or high concentration of fungicide. We believe that the hatching rates have hitherto been low due to low dissolved oxygen and high turbidity of the water that brought about by the use of a particular substrate. Moreover, collection techniques of the hatchling contribute greatly to the survival of the hatchlings. Less stressing techniques will have higher survival rates

Acknowledgement

The authors gratefully acknowledge the Lake Victoria Environment Management Project (LVEMP-Kenya) for sponsoring the research work, The Government of Kenya-Fisheries Department, Moi University Fisheries Department and the Lake Basin Development Authority (LBDA) for offering research facilities.

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