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INFLUENCE OF FEED TYPE AND AGE OF BROODSTOCKS ON EGGS QUALITY OF HETEROBRANCHUS lONGIFILIS

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Abstracts

This study was conducted to investigate the influence of feed type at different age of broodstock on the egg quality of Heterobranchus longifilis. Six (6) month old, 1½ year old and 2½ year old Heterobranchus longifilis males and females of equal numbers (150males and 150females) were subjected to commercial and Farm made feed treatments with 42 crude protein level for one year. This produced broodstocks which were 1½ year old, 2½ year old and 3½ year old. This were used for evaluation of egg quality of Heterobranchus longifilis. The statistical analysis revealed that, Both feed type and age of broodstock showed significant(P< 0.0001) effects on Egg weight, number of egg/ gram ovary weight , absolute and relative fecundity, including GSI. Above parameters also increased significantly (p<0.0001) with the increase in the age of broodstock. Broodstock fed commercial feed produced 378.32g of eggs, 503.46 number of egg/gram ovary weight, 188024 and 74.56 absolute and relative fecundity respectively and 21.18 Gonadosomatic index (G.S.I) while broodstock fed farm made feed produced 367.96g of eggs, 551.25 as the highest number of egg /gram ovary weight, 188024 and 73.63 absolute and relative fecundity with 16.86 G.S.I. Three and a half (3½) year old broodstock had 391.38g weight of egg, 499.75 number of egg/ gram ovary weight, 195581 absolute fecundity, 76.57 relative fecundity, and 20.23 gonadosomatic index while the youngest broodstock (1½ old) produced 357.82g of eggs, 513.56 number of eggs /gram ovary weight, 183728 and 71.94 absolute and relative fecundity respectively with G.S.I value of 17.50. Interaction (P<0.0001) of feed type and age of parent broodstock were observed on all the egg quality accessed in this study.

INTRODUCTION

Constant demand for fish in the global nutrition with alarming increase in the world population requires intensification of aquaculture due to decline in captured fisheries. The African catfish (H longifilis) belonging to the family Clariidae is generally acceptable for culture as a species of significant importance in aquaculture industry in Nigeria. This is due to it fast growth rate, good flesh quality , high stocking density and ability to withstand poor water quality. (Hogendoorn, 1981, Fang, et al. 1986, Ayinla, and Akande, 1988) **. With this species aquaculture could produce nearly unlimited fish supply of superior quality but challenged with poor survival of larvae at the hatcheries management levels which could be attributed to egg or semen quality of the species. Fish feed technology is one of the least developed sector of aquaculture in Africa and other developing countries of the world (** FAO,2003). **. Feed accounts for at least 60% of the total cost of fish production which determines the profitability of fish farming enterprise (** Jamu, and Ayinla, 2003)

However the successful story of aquaculture in Nigeria is being punctuated by high cost of imported fish feed such as Coppens, Vital, Podder feeds etc. An attempt to overcome this feed problem, initiated the ideas of farm made feeds of equal crude protein level that can competes with Coppens commercial feed.

Age remains a natural parameter for assessment of lifetime of individual and a biological prerequisite for determination of sexual maturity of living organism of which fish is included. It has been observed that H longifilis , unlike Clarias gariepinus , requires longer period to attain sexual maturity like 1 year and above for male and 1½ for female which to some extent influenced production and breeding exercise of the species in artificial environment and finally result in low supply of fingerling for pond stocking. The use of good quality gametes from captive fish broodstock is of great importance for ensuring the production of viable larvae (Kjorsvick *et al* 1990).

The knowledge of egg quality of culturable species of fish is crucial for effective management and breeding exercise for production of fish.

According to Reznickick *et a*l (2000) . fish fecundity increased with age of breeders while Ridha and Cruz (1989) reported that 1 year old Nile tilapia, Oreochromis niloticus broodstock had higher fecundity than in 2 and 5 years individual. However , total fecundity and egg diameter increased with age Springate *et al* (1984) and Bromage and Cumaranatunga (1988)

The number of ovules in each gram of body weight significantly increased with increased in the female broodstock age , Rahbar *et al* (2012)

Many authors had investigated the effect of egg quality of different species of fish but no information had been reported on the influence of broodstock age on egg quality of Heterobranchus longifilis which is the focus of this study as well as the relationship between the body weight and egg quality of broodstock at different age

MATERIAL AND METHODS

STUDY AREA

The research was conducted at the Fish Farm Complex of Akwa Ibom State University Obio Akpa campus which is located between latitude $5^0$17'N and $7^0$27'N, Longitude $7^0$27'E and $7^0$58'E with an annual rainfall ranging from 3500mm-5000mm and average monthly temperature of 25^0C. Akwa Ibom State is a coastal state lying between latitude $4^0$28'N and $5^0$3'N and between longitude $7^0$27'E and $8^0$20'E with a relative humidity `between 60-70%. It is in the tropical rainforest zone of Nigeria. (Otoh, 2016)

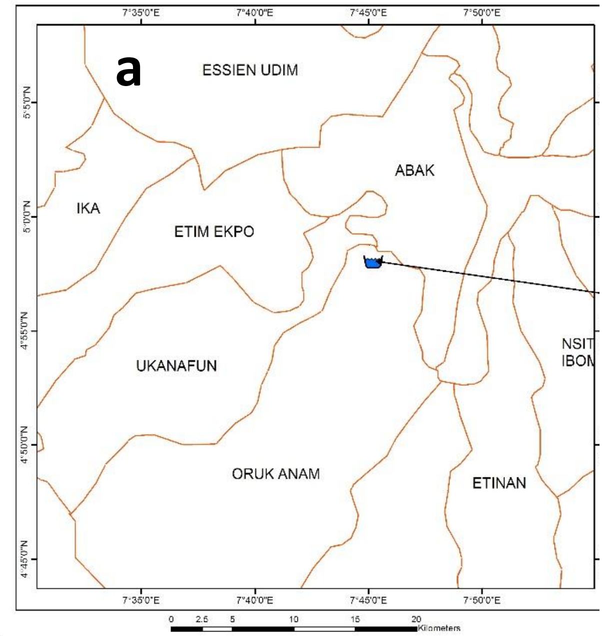
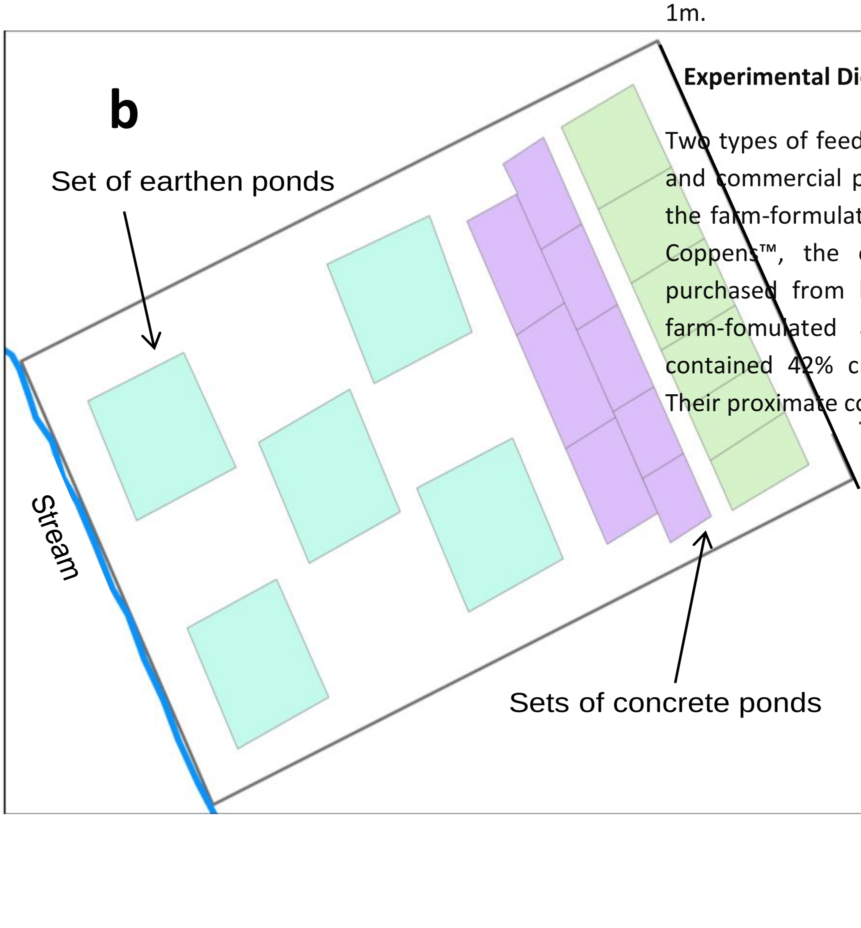


Fig..1 A cross section of Akwa Ibom State showing location of Fish Farm in Obio Akpa Campus of Akwa Ibom State University (a) and pond layout (b (Otoh, 2016)

Aquisition and Care of Broodstock

The initial experimental broodstock (n = 300) of three ages: ½ year old, 1½ years old and 2½ year old (n = 100 each) of farm-raised *Heterobranchus longifilis* were selected from a stock raised in earthen ponds of Akwa Ibom State University Fish Farm, Obio Akpa and divided into two sets, each containing 50 samples (25 males and 25 females) of *Heterobranchus longifilis and* stocked at 2 fish/m^2 in six (6) concrete ponds, each of dimension 5m x 5m x 1m.



Experimental Diets

Two types of feed used were: farm-formulated feed and commercial pelleted feed. The composition of the farm-formulated feed is shown in Table .1 and Coppens™, the commercial pelleted feed, were purchased from local fish feed dealers. Both the farm-fomulated and commercial pelleted feeds contained 42% crude protein of pellet size 6mm Their proximate compositions are shown in Table 2.

TABLE 1

Composition of farm formulated feeds

S/N	Ingredient	Composition of Feed (kg)
1.	Maize	13
2.	Soya beans meal	24
3.	Wheat offal	14
4.	Groundnut cake	24
5.	Fish meal	24
6.	Lysine	0.1
7.	Methionine	0.1
8.	Mineral Premix	0.1
9.	Vitamin C	0.35
10.	Bone meal	0.05
11.	Salt	0.1
12.	Enzymes	0. 1
13.	Palm oil	0.1
	Total	**100kg**

TABLE 2

Proximate composition of experimental diets

Ingredient	Farm Feed	Coppens™ Feed
Crude Protein	41.65	41.99
Lipid	12.35	13.00
Ash	11.70	12.05
Fibre	12.24	13.03
NFE	9.46	8.54
ME kJ/g	1327.293	1342.764

NFE = Nitrogen free extract

ME = Metabolizable Energy

Feeding of Broodstock

The broodstock were fed 3% of their body weight .They were fed twice daily in split doses at 8.00am and 5.00pm. The broodstock were fed in two separate groups (n = 150) with Farm-formulated feed and Coppens™ pelleted feed (Commercial Feed, CF; for 12 consecutive months in order to attain the desired age of the broodstock, BS: 18-months, 30-months and 42-months, i.e. 1½, 2½ and 3½ years, respectively. Water parameters such as dissolved oxygen, pH and temperature were considered.

Evaluation of Egg Quality

48 samples of female *Heterobranchus longifilis* broodstock were selected following the method of (Aluko and Ali, 2001) for determination of fecundity and egg parameters. Eight samples from each treatment were separately transferred to the hatchery, where each length and weight was taken, sacrificed and dissected to remove the ovary for measurement without hormonal inducement. Length and weight of female *Heterobranchus longifilis* were considered using meter rule and Digital weighing balance and the following were carried out;

(a)Absolute and relative fecundity, weight of egg, Egg diameter and gonadosomatic index of *Heterobranchus longifilis* broodstock

Fecundity and weight of egg were determined by weighing method (Bozkurt *et al.,* 2006) and egg size was measured using vernier calliper (at 0.02mm sensitivity).

Relative fecundity was calculated as the number of egg per gram weight of the fish. Relative fecundity (no of eggs/g weight of female) = Total egg number/total body weight, Gonadosomatic index (GS1) = Gonad weight /whole fish weight [x 100] (Ekanem *et al*., 2013).

(b) Number of egg per gram ovary weight: Extraction and preparation of eggs

Eggs from each gravid fish were removed by cutting-open the abdomen with a pair of scissors. Eggs were washed in

distilled water and weighed on an electronic weighing balance to the nearest 0.1g. The eggs were fixed in Gilson

fluid in sample bottles for 48 hours before estimation.. Ekanem *et al* (2013)

Statistical Analysis.

All data were subjected to Analysis of valiance (ANOVA) using PROCMIXED statement (SAS, 2001). Replicates were treated as random effect while feed type and age of *Heterobranchus longifilis* were treated as fixed effects in determining the expected mean square and appropriate F test in the analysis of variance. Standard Error of Difference (S.E.D) at 5% probability level was calculated and used for comparing treatment means.

The experimental design was factorial analysis two way ANOVA, 2X3X4 factorial

Three different hypotheses tested were effect of the following;

Feed; (a) feed type fed parent broodstock has significance. effect on egg quality of H. longifilis

(b) Feed type fed parent brodstock has no significant effect on egg quality of H . longifilis.

Age; (a) Age of parent broodstock has significant effect on egg quality of H longifilis

(b) Age of parent broodstock has no significant effect on egg quality of H longifilis

Interaction; (a) A significant interaction of feed type and age of parent broodstock exist on egg quality of H. longifilis (b) A significant interaction of feed type and age of parent broodstock exist on egg quality of H. longifilis.

RESULTS

The results of influence of feed type and age of broodstock on egg quality of H. longifilis is presented in Table 1. . Feed type showed significant (p<0.0001) difference on egg weight , and number of eggs /gram ovary weight of *Heterobranchus longifilis* as shown in table 1 . Broodstock fed commercial feed showed significant (p<0.0001) increase in egg weight by 10.36 % above the egg weight produced by broodstock fed farm made feed while broodstock fed farm made feed produced 511 eggs / gram ovary weight significantly (p<0.0001) higher than 503 eggs produced by broodstock fed commercial feed . Egg weight increased with the age of broodstock while number of eggs per gram ovary weight decreased with the age of broodstock. The oldest broodstock 3 ½ years old had 391.38 g while the youngest bbroostock 1 ½ year old had 357.79g of eggs . The youngest broodstock 1½ year old produced the highest number of eggs per gram ovary weight 513.56 while the oldest broodstock 3½ years old produced the least number of egg per gram ovary weight 499 .75

A significant (P<0.0001) interaction of feed type and age on egg weight and number of egg /gram ovary weight was observed among broodstock of the same age fed different feed type.

Table 1. Influence of feed type and age on egg weight and number of *Heterobrancus longifilis* brood stock

Age	Egg weight (g)			Egg number		
	Local feed	Commercial feed	Mean	Local feed	Commercial feed	Mean
18 months	350.41	365.23	357.82	518.25	508.88	513.56
30 months	365.23	375.21	370.22	512.25	505.25	508.75
42 months	388.25	394.51	391.38	503.25	496.25	499.75
Mean	367.96	378.32		511.25	503.46	
S.E.D Feed type (F)		0.0576			0.1080	
S.E.D Age (A)		0.0706			0.1714	
S.E.D F x A		0.0998			0.2165	

Table 2. revealed the Influence of feed type and age of broodstock on absolute fecundity, relative fecundity and GSI of *Heterobrancus longifilis* Broodstock fed commercial feed had absolute fecundity of 190,400eggs, relative fecundity 0f 74.56 and G.S.I value of 21.16 which were significantly (p<0001) higher than 18804 absolute fecundity, 73.63 relative fecundity and 16.86 GSI value produced by broodstock fed farm made feed.

Absolute and relative fecundity along with GSI value of the broodstock significantly (p<0.0001) increased with age of broodstock. The oldest broodstock 3½ year old had absolute fecundity value of 195581, relative fecundity of 76.53 and GSI value of 20.23 while the youngest broodstock 1½ year old had 18378 absolute fecundity 71.19 relative fecundity and GSI value of 17.50. A significant (P<0.0001) interactions on absolute fecundity, relative fecundity and GSI were observed among broodstock of different age classes as well as broodstock of the same age fed different feed type.

Table 2. Influence of feed type and age on absolute fecundity, relative fecundity and GSI of *Heterobrancus longifilis* brood stock

Age	Absolute fecundity			Relative fecundity			GSI	
	Local feed	Commercial feed	Mean	Local feed	Commercial feed	Mean	Local feed	C fe
18 months	181601	185855	183728	71.11	72.78	71.94	17.51	
30 months	187087	189573	188330	73.30	74.23	73.76	16.40	
42 months	195386	195777	195581	76.47	76.66	76.57	16.66	
Mean	188024	190402		73.63	74.56		16.86	
S.E.D Feed type (F)		40.35			0.043			
S.E.D Age (A)		60.66			0.073			
S.E.D F x A		69.89			0.074			

Table 3 revealed that feed type had no significant ($p< 0.0233$) effect on egg diameter and H. S .I of H longifilis whereas egg diameter and H.S.I increase with the age of broodstock .Three and a half 3 ½ years old broodstock had the highest value of 1.42mm egg diameter and 0.84 H.S.I while the youngest broostock 1 ½ year old had 1.16 egg diameter and 0.67 value of H.S.I.

Table 3. Influence of feed type and age on egg diameter and HIS of *Heterobrancus longifilis* brood stock

Age	Egg diameter (cm)			HIS		
	Local feed	Commercial feed	Mean	Local feed	Commercial feed	Mean
18 months	1.11	1.21	1.16	0.64	0.69	0.67

30 months	1.24	1.27	2.26	0.67	0.75	0.71
42 months	1.41	1.43	1.42	0.75	0.92	0.84
Mean	1.25	1.30	1.61	0.69	0.79	
S.E.D Feed type (F)						
S.E.D Age (A)						
S.E.D F x A						

DISCUSSION

The knowledge of egg quality of any culturable species of fish is crucial for successful breeding, production and multiplication of the product. while nutrition is necessary for the growth of somatic cell which determine the viability of reproductive gametes but at what age of the broodstock will the farm made feed, produce the best eggs quality that can compete with the one produced by broodstock fed coppens commercial feed. Broodstock fed commercial feed showed significant (p<0.0001) increase in egg weight by 10.36 % above the egg weight produced by broodstock fed farm made feed.

This result disagreed with that of Ekanem, *et al*.,(2013) on egg weight . This could be attributed to differences in the rate of response to feed among the broodstock . Commercial feed had attractive odor and float for a longer period than farm made feed. The oldest broodstock 3½ year old had 391.38g of egg weight which is significantly (p<0.0001) higher than 370.22 produced by 2½ year old broodstock and 357.82g produced by 1½ year old broodstock. This result could be possible since the weight of egg depends on the body weight of fish. Moreover, broodstock of the same size with different age shows variations in the weight of eggs which could be attribute to differences in the body cavity to accumulate eggs. A significant (P<0.0001) interaction of feed type and age on egg weight was observed among broodstock of the same age fed different feed type.

A significant different in the number of egg per gram body weight was observed among broodstock of *Heterobranchus longifilis* as a result of feed type, could be attributed to the size of eggs which is nutritionally influenced. Broodstock fed farm made feed produced 511 eggs per gram ovary weight which is significantly (p<0.0001) higher than 503 mean number of egg produced by broodstock fed commercial feed. This result is in agreement with Ekanem *et al.,* (2013) who reported that fish with same size had different ovary weight and number of eggs in their ovaries which is also supported by Musa and Bhuiyan ,(2007). Similar observation was made by Springate *et al.,* (1985) who reported that availability of feed affect egg size of female broodstock.

Variation in the number of eggs per gram ovary weight as a result of age difference could be attribute to egg size which is smaller in the younger broodstock than in the older ones. The youngest broodstock 1½ year old produced the highest number of eggs per gram ovary weight of 513.56 eggs while the oldest broodstock 3½ years old produced the least number of egg per gram ovary weight of 499.76 eggs. Similar result was reported by Lahnsteiner, (2000) and supported by Rahbar *et al.,* (2011). A significant interaction of feed type and age on number of egg per gram ovary weight was observed among broodstock of the same age fed different feed types.

The broodstock fed commercial feed recorded significant ($p<0.0001$) increase in mean absolute and relative fecundity (190,402 eggs, and 74.56 egg/gram body weight above their counterparts fed farm made feeds (188,024 eggs, 73.63 egg/gram body weight respectively. These results disagree with Ekanem *et al*., (2013) who reported of non significant effect on the above parameters using Unical feed. The difference could be attributed to differences in the rate of response to feed among the broodstock . The oldest broodstock, 3½ years-old, recorded 195,581 eggs, and 76.57 egg/gram body weight which were significantly ($p<0.0001$) higher than those of youngest broodstock (1½ year -old): 183728 eggs, and 71.94 egg/gram body weight respectively. This is in agreement with the findings of Rahbar *et al.,* (2011), Pitman (1979) and Shamspour *et al.* (2009) but deviated in relative fecundity which was reported to decrease with age (Rahbar *et al.,* 2011). The effect of age was accentuated by the uniformity in the size of broodstock irrespective of age. Though older broodstocks had equal body size with the younger ones, their body cavities (spaces) provided more space for more eggs than the younger broodstock, hence, relative fecundity equally increased with age. A significant ($p<0.0001$) interaction of feed type and age on absolute and relative fecundity was observed among broodstock of the same age class fed different feed type and broodstock of different age class fed either of the feed type. Gonadosomatic index (G.S.I) of the broodstock also showed significant ($p <0.0001$) difference as a result of feed type and age of broostock . Commercial feed and oldest broodstock produced higher value of GSI than farm made feed which indicated that although Feed type were iso nitrogenous, commercial feed attracted broodstock (due to odor and floating capacity) more than farm made feed. It is further suggested that broodstock age probably influences the efficiencies in metabolism and digestibility of experimental feeds

In addition, since the broodstock received iso-nitrogenous feed, it is probably that the commercial feed provided higher concentration of nutrients in easily digestible and available forms for egg formation, egg yolk composition, yolk formation and deposition (vitellogenesis), resulting in egg size. Sundararaj, (1981) recommened the development of special diets that are rich in lipids, calcium, protein and other components of yolk material, like Coppens, for broodstock as such enhance vitellogenin synthesis through faster deposition of lipids and proteins in tissues and fat bodies. Shim *et al.,* (1989) reported that the quantity and composition of dietary protein are known to affect fish fecundity. In the present study, fecundity of *Heterobranchus. longifilis* broodstock fed two experimental diets were significantly different ($P<0.05$). Both diets supported fast gonadal development in catfish as observed in this study. In a similar development, Ekanem *et al.,* (2013) observed no significant difference ($p>0.05$) in *Clarias gariepinus* also fed two experimental diets, one of which was Coppens. They also observed that *Clarias gariepinus* with same size had different ovary weight and number of eggs in their ovaries. Fecundity of *Clarias gariepinus* varied with increase in length and weight of the fish. In the present study, fishes of the same sizes at different ages differed in fecundity and number of eggs per gram ovary weight. The number of eggs per gram ovary weight depends on the egg size which in turn depends on age of *Heterobranchus longifilis* broodstock. Coppens is an imported commercial floating feed that has an inviting fishy odor and is well received by different farmed fish species compared to farm made feeds which sink. Fish fed Coppens feed, responded more aggressively to the feed compared to farm made feeds as observed in this study and also reported by Ekanem *et al.,* (2012) which is probably because of the differences in composition of the two diets. Ekanem *et al.,* (2013) however observed similar responses to feed by catfish fed iso-nitrogenous commercial (Coppens) and farm made feeds. This presents the challenge of how to produce farm made feed that can compete favourably with coppens commercial feed. It is further suggested that broodstock age probably influences the efficiencies in metabolism and digestibility of experimental feeds in attaining maximum egg weight. Egg weight increased with age of broodstock while number of eggs (fecundity) per gram ovary weight decreased significantly with age. The oldest broodstock (3½ year -old) had 391.38g egg weight and 499.75

eggs/gram ovary weight while the youngest broodstock (1½) years-old had 357.82g egg weight and 513.56 eggs per gram ovary weight of fish. Rahbar *et al.,* (2011) had similar result in female Caspian brown trout (*Salmo trutta caspious*, Kessler 1877). This is possible because the quantity of egg produced by the older broodstock with heavier body weight is larger than the quantity produced by the younger broodstock with the heavy body weight. Moreover, the youngest broodstock (1½ year old) had the highest number of egg/gram ovary weight because, the smaller the size of egg, the greater the number required to (make up) attain a gram. The aquacultural implication of this observation are enormous . Thus, it is possible to get large quantities of yolky eggs of catfish by maintaining a limited number of older *Heterobranchus longifilis* broodstock for routine induced spawning, four to five times, from each female during their spawning seas

Broodstock fed commercial feed had gonadosomatic index of 21.16 which is significantly ($p< 0.0001$) higher than 16.86 produced by broodstock fed farm made feed. This result disagrees with Ekanem *et al.,* (2013) who compared Unical and coppens feed in his study. Gonadosomatic index also increased significantly ($p<0.0001$) with the age of broodstock. The oldest broodstock 3½ year old had the highest G.S.I. value of 20.28 while the youngest broodstock 1½ year old had the least gonadosomatic index value of 17.50. A significant ($p<0.0001$) interaction of feed type and age on gonadosomatic index was observed among broodstock of the same age class fed different feed type except in the youngest broodstock 1½ years old which showed no interaction. The influence of age on egg diameter reported in this study is similar to the report of Lahnsteiner (2000) and Rahbar *et al.,* (2011), who observed that older and heavier female broodstock produced larger eggs than younger and smaller fishes while Rahbar *et al.,* (2011), reported that 6 year old broodstock had higher egg parameters than 4 and 5 years-old broodstock

CONCLUSION;

The result of this study revealed that both feed types actually stimulated the growth of fish but commercial feed performed better than the farm made feed in terms of egg quality. From the analysis, egg quality produced by farm made feed was up to 80% of the performances of commercial feed which is enough to sustain any commercial farm . The younger broodstock, 2½ years old produced better egg quality than 1½ year old and close to that of 3½ years old broodstock, therefore considering the long period of sexual maturity of H. longifilis and economics of feeding, farm made feed and 2½ years old broodstock are recommended for the production of good egg quality needed for production of H. longifilis. More studies should be carried out on production method of farm made feed to compete favourably with commercial feed like coppens.

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