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Morphometric comparison among three wild Nigerian strains of *Heterobranchus longifilis* (Valenciennes, 1840) and their fingerling intraspecific hybrids.

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ABSTRACT: Morphometric characterization was carried out in intraspecific hybrids of three wild strains of *Heterobranchus longifilis* obtained from three geographical locations in Nigeria – rainforest, Onitsha strain, guinea savanna, Kainji strain and montanne vegetation, Jos strain with the aim of identifying the strains and their hybrids. The adipose: dorsal fin relationship show that almost equidistant relationship (0.90) in the Kainji strain whereas there were longer dorsal fin in Onitsha (0.84) and Jos strains (0.77). Moreover, easily observable gaps between posterior dorsal and anterior adipose were identification marks for Onitsha and Jos strains and absent in the Kainji strain. The Onitsha-Jos hybrid complex had the widest gap (0.33 cm – 0.35 cm). A range of 50.00 – 66.70% retained this gap. The hybrid group with the least gap was the Jos-Kainji crosses (0.17 cm – 0.24 cm). Seventy-five percent of the offspring of the hybrid cross between female Jos strain and male Kainji retained this gap, whereas 100% of the cross-Kainji female x Jos male had this gap. Anal ray counts tend to decrease with increasing longitude but does not follow any pattern with increasing latitude. In addition, anal ray count was lowest in the strain collected from the montanne vegetation (1200m above mean sea level).

Key Words: Morphometric comparison; *Heterobranchus longifilis*; Fish morphology.

Introduction

Body morphology is believed to be under conflicting selectional pressures in fishes (Riddel and Legget, 1981; Taylor and Mcphail, 1985; Swain and Holtby, 1989). Differences in morphology between geographic (coastal versus interior) and ecological (stream versus lake rearing) types of Coho Salmon (*Onchorynchus kisutch*) have been reported by Taylor and Mcphail (1985) and by Swain and Holtby, (1989). According to these authors, Coho Salmon from interior or lake rearing populations are more treated with shallower bodies and smaller median fins than those from coastal or stream rearing population. In addition, Swain, Riddell and Murray (1991) have reported wild population of Coho Salmon having greater head dimensions, larger median fins and deeper bodies than did hatchery-reared Coho Salmon. In the American catfish, headwater catfish, *Ictalurus lupus* and the Channel catfish, *I. punctatus* have been reported to show a south to north cline of decreasing anal fin ray counts (Kelsch, 1995). Taylor and

Mcphail (1985) concluded that the differences between interior and coastal populations are known to have a genetic basis.

The genus *Heterobranchus* is popular in African aquaculture. It is similar in many respects to *Clarias* but can readily be differentiated from *Clarias* by the presence of rayed dorsal fin followed by an adipose tissue. They have four pairs of barbells on their flattened, strongly depressed head. The adipose tissue is normally blackish near to the caudal end, which make it different from other catfishes. *H. longifilis* has the adipose tissue clearly not so high as the rayed dorsal fin, the length of the adipose at its base generally equals about 0.8 times that of the rayed dorsal. The pectoral fin is 0.4 to 0.5 times as long as the head that depressed and has the upper surface granulated. Dorsal fin has 29 to 34 rays while anal fin has 48 rays with rounded caudal fin (Reed *et al*., 1967; Teugels, 1990).

H. longifilis is morphologically almost similar to *H. bidorsalis* but hatchery operators hardly distinguish between them. Morphological variations exist within this species across geographical zones in Nigeria, particularly with respect to the adipose and dorsal fins ratio. Barlow (1961) reported that meristic variables such as anal ray count normally increase with decreasing temperature and altitude. A single morphological variable is not sufficient to distinguish one wild strain from another.

This study was carried out to investigate morphometric differences among intraspecific hybrids of three wild strains of *Heterobranchus longifilis.*

Materials and Methods

Experimental Site

This experiment was carried out at the Fish Genetic Improvement Laboratory of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria.

Sources of Breeders

The breeders used for this experiment were collected from three different geographical locations in the country. The Plateau terrain around Jos, the savanna belt around Kainji Basin (Local) and the rain forest belt around Onitsha. These fishes were stocked separately in the laboratory concrete tanks for more than a year for fattening and were fed with 40% crude protein from NIFFR prepared fish feed.

Breeders Selection

Breeders were collected from the tank using drag net. The swollen stomach of the female and its reddish vent as well as extrusion of eggs upon a slight pressure on the abdomen indicates readiness for use while the redness of the genital papillae and its pointed end indicates sexual maturity of the male. The weight of the male as well as that of the female were taken and recorded. Kainji strain was selected on the basis of dorsal and adipose fins (Plate 1a). Onitsha and Jos strains were selected on the basis of gaps between the posterior dorsal and anterior adipose fins (Plate 1b and 1c respectively).

Hormone Injection

These specimens were separated into sexes and species. The males and females used were kept separately inside the four plastic bowls containing well aerated water and covered. The synthetic hormone Ovaprim was used in injecting the fish. The males and females of each species were weighed and injected with Ovaprim. The hormone was administered transmuscularly to the fish at a single dose of 0.5 ml/kg of fish. Immediately after injection, breeders were returned into various plastic bowls labeled alphabetically and containing well aerated water for a period of 15 hours as a latency period. This is the period that the fish will be ready for stripping.

Milt Collection

After the latency period of fifteen hours, milt was collected by sacrificing the male. This was done because unlike the female, it is not possible to strip milt from the male because of the location of the testis. Testes were cleaned with tissue paper to remove blood. They were then kept inside a Petri-dish and covered with another Petri-dish until used, not more than 10 minutes after the removal of the testes.

Stripping of the Female Spawner

Fifteen hours after inducement, the female breeders were stripped of their eggs by a gentle application of pressure on the abdominal region. Before stripping, the body was properly cleansed with a towel to avoid contact of eggs with water. The eggs were collected into a Petri-dish.

Artificial Fertilization of the Eggs

Testes were cut open with clean, sharp razor blade to release the milt for the fertilization of eggs. The milt collected was diluted with physiological saline (0.9% NaCl) solution in a ratio of 1:5 and was used in fertilizing the eggs using a clean, dry feather to avoid contamination of eggs.

Experimental Crosses

The following crosses were carried out in duplicate:

A. Parental Group

H. longifilis (Kainji) x *H. longifilis* (Kainji).

H. longifilis (Jos) x *H. longifilis* (Jos).

H. longifilis (Onitsha) x *H. longifilis* (Onitsha).

B. Intraspecific Hybridization Group

H. longifilis (Kainji) x *H. longifilis* (Kainji).

H. longifilis (Jos) x *H. longifilis* (Kainji).

H. longifilis (Kainji) x *H. longifilis* (Onitsha).

H. longifilis (Onitsha) x *H. longifilis* (Kainji).

H. longifilis (Jos) x *H. longifilis* (Onitsha).

H. longifilis (Onitsha) x *H. longifilis* (Jos).

Incubation and Hatching of Eggs

Eighteen well aerated aquaria tanks with dimensions 60 x 30 x 30 cm^3 were filled with water up to one-third level. Kakabans or egg collectors were placed in each aquarium for the eggs to attach. The temperature range was between 25^{o}C and 26^{o}C in the aquaria with pH 7.1.

Feeding of the Larvae

One hundred post hatchlings from each treatment were put in different aquaria. The pooled weight measurements of the hatchlings were done and length measurement of about five hatchlings was done for each treatment. On the fourth day after hatching, feeding of the larvae commenced and was done twice a

day, everyday with zooplankton *Moina micrura* harvested from the fish genetic outdoor concrete tanks. Zooplankton was filtered and fed *ad libitum* to the post hatchlings for a period of fourteen days indoors.

Outdoor Rearing

All the nine mating groups were duplicated. the fry were put in eighteen 2 x 2 x 1 m^3 fertilized concrete tanks. The fry were fed with 40% C.P. artificial fish diet in tanks rich in zooplankton for a period of three weeks.

Morphological Characteristics

The specimens were separated into males and females. Each fish was placed on the measuring board and thread was used to take measurements of some parts while the following measurements were carried out: (i) Total length (TL), (ii) Standard length (SL), (iii) Head length (HL), (iv) Pre-anal distance, (v) Pre-pelvic distance, (vi) Dorsal fin length, (vii) Anal fin length, (viii) Distance between the occipital process and dorsal fin origin, (ix) Dorsal fin depth (height), (x) Distance between dorsal and caudal fins, (xi) Adipose fin length, (xii) Adipose fin depth, (xiii) Pectoral spine length, (xiv) Pectoral fin length, (xv) Pelvic fin length, (xvi) Body depth at widest point, (xvii) Caudal peduncle depth, (xviii) Pre-dorsal distance, (xix) Distance between dorsal fin and adipose tissue and rays/spine count for instance, dorsal fin rays, anal fin rays, caudal fin ray, pectoral fin ray/spine and pelvic fin rays/spine.

Statistical Analysis

The results of the morphometric characterization were subjected to statistical analysis and graphical presentation.

Results and Discussion

Table 1 shows the characteristic ratios of different parts of the body of the three wild strains. The biggest profile index value was that of the Onitsha strain (8.41) followed by Kainji strain (6.65) and that of Jos strain was the smallest (5.42). The tail index of the Kainji strain was longest (12.29) and that of Onitsha strain was least (11.31). The trend of the head index shows that Onitsha strain has the biggest head with a value of 4.10 and the smallest head index was that of Jos strain with a value of 3.86. The head index of Kainji strain was very close to that of Onitsha strain.

Table 1: Characteristic body indices of three strains of *H. longifilis.*

	Index (%)		
	H. longifilis (Onitsha)	*H. longifilis* (Kainji)	*H. longifilis* (Jos)
1. Profile Index, L/Tm	49.31/5.86 = 8.41	34.28/5.15 = 6.65	38.13/7.03 = 5.42
2. Caudal Index, L/fh	49.31/4.36 = 11.31	34.28/2.79 = 12.29	38.13/3.35 = 11.38
3. Head Index, L/Fh	49.31/12.02 = 4.10	34.28/8.46 = 4.05	38.13/9.87 = 3.86

L = Total length; Tm = Height of body; fh = Length of tail; Fh = Length of head.

The dorsal fin length:adipose fin length ratio at fingerling and wild adult stages was analyzed (Table 2, Fig. 1). The ratio of dorsal:adipose lengths was highest in the Kainji strain (0.93 and 0.90 for fingerlings and adult, respectively) indicating that the adipose fin length was 93% of the dorsal fin at the fingerling stage and the proportion reduced to 90% in adult stage. There was no noticeable gap between posterior

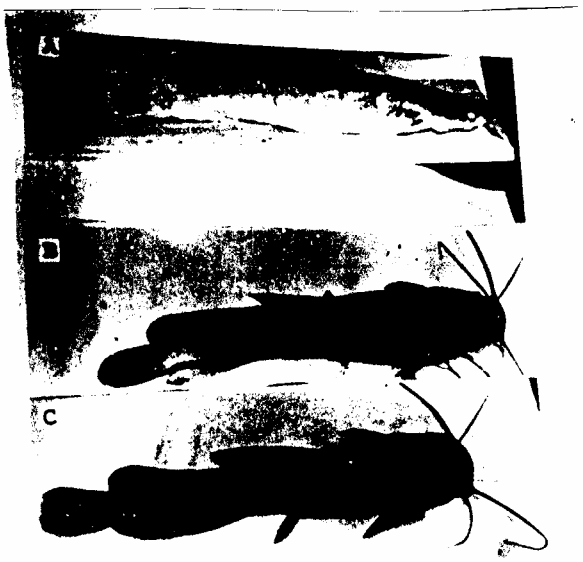

Plate 1: Morphological appearance of *H. longifilis* strains. (A = Kainji strain; B = Onitsha strain; C = Jos strain).

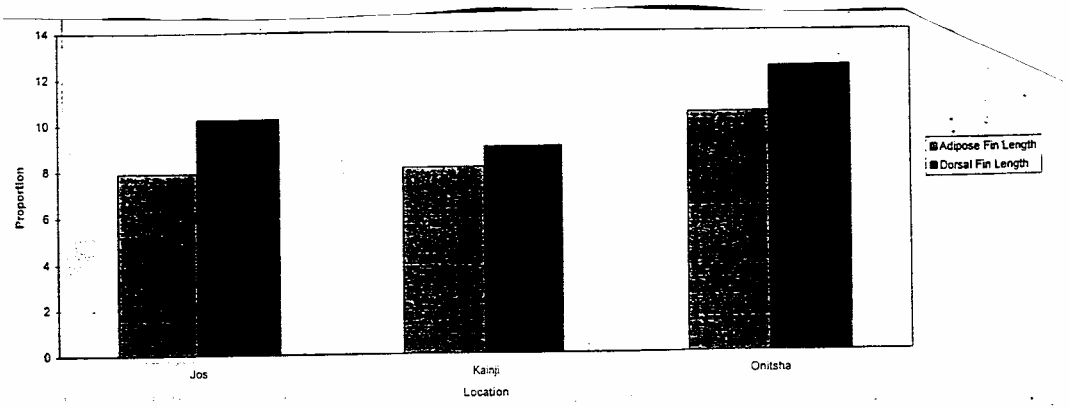

Fig. 1: Proportion of dorsal to adipose fin length in collections of *H. longifilis* from three locations in Nigeria.

dorsal and anterior adipose fins at the fingerling stage whereas at the adult stage the difference between posterior end of dorsal fin and anterior beginning of adipose fin was 0.12 cm. At a glance, the adipose fin and dorsal fin lengths appear equidistant. This is an important distinguishing trait of *H. longifilis*. The strain with the nearest adipose:dorsal fin ratio to that of Kainji strain was the collection from Onitsha (0.91 and 0.84 in fingerlings and wild adult, respectively). The Onitsha strain was characterized by shorter adipose fin length (84% of the dorsal fin) leaving a narrow gap (mean of 0.20 cm in the fingerling stage and 2.87 cm in the adult) between the posterior end of the dorsal fin and anterior beginning of the adipose fin. All specimens observed had this gap at both fingerlings and adult. Slightly shorter adipose fin (81% and 77% of dorsal fin in fingerlings and adult stages respectively) was recorded in the collection from Jos, leaving a wider gap (0.28 cm and 3.15 cm in fingerling and adult stages respectively in between the posterior dorsal fin and anterior adipose fin. The relationship between dorsal fin and adipose fin in the Jos strain is very important in that this strain to the untrained eyes looks like a hybrid cross between *Clarias* and *Heterobranchus* species.

Table 2: Proportion of adipose to dorsal fin length in the fingerlings of the parental and hybrids of the three strains.

Strain/Hybrid	Mean adipose fin length (cm)	Mean dorsal fin length (cm)	Proportion of adipose fin to dorsal fin	Gap between dorsal fin and adipose fin (cm)	Percentage offspring with gap.
Onitsha strain (f x m)	1.47	1.60	0.91	0.20	100.00
Kainji strain (f x m)	1.63	1.75	0.93	0.00	0.00
Jos strain (f x m)	1.53	1.78	0.81	0.28	100.00
Jos (f) x Onitsha (m)	1.46	1.56	0.94	0.33	50.00
Onitsha (f) x Jos (m)	1.80	2.00	0.90	0.35	66.70
Jos (f) x Kainji (m)	1.35	1.50	0.90	0.17	75.00
Kainji (f) x Jos (m)	1.38	1.54	0.89	0.24	100.00
Kainji (f) x Onitsha (m)	1.04	1.55	0.67	0.18	100.00
Onitsha (f) x Kainji (m)	1.40	1.45	0.96	0.30	50.00

Among the hybrids at the fingerling stage, the gap between the posterior dorsal fin and anterior adipose was either retained or closed completely depending on the nature of the cross (Table 2). The Onitsha-Jos hybrid complex had the widest gap (0.33 cm – 0.35 cm). A range of 50.00 – 66.70% retained this gap. The hybrid group with the least gap was the Jos-Kainji cross (0.17 – 0.24 cm). Seventy-five percent of offspring of the hybrid cross between female Jos strain and male Kainji strain retained this gap whereas 100% of the cross Kainji female x Jos male had this gap. Table 2 further shows the inheritance in this gap. In hybrid crosses involving female Kainji strain, 100% of the offspring retain this gap. Taylor and Mcphail (1985) have demonstrated that population-specific traits in juvenile Coho Salmon may be maintained in adults. This indicates that the gap retaining genes exhibited dominance in Onitsha and Jos males. The gene determining gap was therefore recessive in female Kainji strain. In the male of the Kainji strain, the gap determining genes were not completely dominant in that only 50 – 70% of offspring retained the gap.

Median anal ray count was 39 (range 28 – 46) in the Onitsha strain, 44 (35 – 48) in Kainji strain and 34 (32 – 37) in the Jos strain. Anal ray counts for the three strains (Kainji: 9.53^{o} N, 4.29^{o} E; Onitsha: 6.09^{o} N, 6.48^{o} E and Jos: 9.55^{o} N, 8.54^{o} E) tended to decrease with increasing longitude. However, this did not follow any pattern with increasing latitude. From lower latitude Onitsha it increased from 39 to 44 in Kainji and reduced to 34 in Jos with the highest latitude (Table 3). The range of anal ray counts was lowest in the Jos strain (32 – 37) which was located in altitude of 1200 metres above mean sea level. This indicates that altitude probably plays a vital role in determining the number of anal fin rays.

Table 3: Fin forms of the three wild strains of *H. longifilis.*

Description of fin	Onitsha strain	Kainji strain	Jos strain
Dorsal fin rays	25 – 39 (30)	30 – 33 (30)	26 – 30 (28)
Caudal fin rays	18 – 23 (20)	20 – 23 (21)	18 – 22 (20)
Anal fin rays	28 – 46 (38)	35 – 48 (42)	32 – 37 (34)
Pelvic fin rays	5 – 7 (6)	5 – 6 (6)	5 – 8 (6)
Pectoral fin rays	1,7 – 1,10 (1,8)	1,4 – 1,6 (1,5)	1,5 – 1,9 (1,7)

Table 3 also shows the meristic counts of the fin rays of the three wild strains. The Jos strain was observed to have the least number of dorsal fin rays (modal value of 28) while the remaining two strains had modal values of 30 dorsal fin rays each. In addition, the range of dorsal fin rays in the Onitsha strain appeared wider than the range of the other two strains. The values of dorsal fin rays as low as 25 and 26 were recorded in Onitsha and Jos strains respectively. An extreme high value of 39 dorsal fin rays was also observed in the Onitsha strain. The range of caudal fin rays appeared wider in Onitsha and Jos strains (18 – 23 and 18 – 22, respectively) than in the Kainji strain (20 – 23) (Table 3). Even though the Kainji strain had a narrow range of caudal fin rays, the highest modal value of caudal fin rays (21) was recorded for this strain, thereby indicating a slightly wider caudal fin in the Kainji strain than in the other two strains.

The picture of the anal fin rays in the Jos strain was completely different from the other two strains (Table 3). Only 42.9% of the samples of the Jos strain had anal fin rays counts in the range of minimum and fin ray counts observewd for Kainji and Onitsha strains, indicating that the length of the anal fin was smaller in the Jos strain than in the remaining two strains. The highest modal values of the anal fin ray was observed in the Kainji strain (42) and least observed in the Jos strain (34).

The number of rays in the pelvic fin was the same in the three strains, each having a modal value of 6 (Table 3). The widest range (5 – 8) was observed in the Jos strain while the narrowest range (5 – 6) was recorded in the Kainji strain. The pectoral hard fin rays was the same (1) in all three strains except in one sample of Jos strain where two hard rays were observed (Table 3). The highest soft pectoral fin rays modal value was recorded for the Onitsha strain (8) and least in the Kainji strain (5). The range of dorsal fin rays observed for the three strains: Onitsha (25 – 39), Kainji (30 – 33) and Jos (26 – 30) agree with the range described for *H. longifilis* (26 – 35) by Teugels *et al*. (1990). About 90% of samples had less than the minimum value of 26 and maximum value of 35 in the Onitsha strain. However, about 57% of samples counted for the anal fin rays of the Kainji strain (35 – 48) fall within the range (42 – 52) described by Teugels *et al*. (1990), the range observed for the Jos strain was slightly out of the range described by Teugels *et al*. (1990).

Anal ray counts for the three strains decreased with increasing longitude. Barlow (1961) and Kelsch (1995) have reported that meristic variables such as anal ray count normally increase with decreasing temperature of development and correspondingly with latitude. From the Onitsha strain to the Kainji strain tne anal ray counts increased with latitude in agreement with the report of Barlow (1961). However, the low counts of the anal fin reported for the Jos strain could have arisen from an interplay of latitude and altitude. It is highly probable that altitude played a vital role in decreasing the anal fin rays count in the Jos strains to the extent that the anal ray count did not fall into the range described for *H. longifilis* by Teugels *et al.* (1990). Median anal ray counts differed among the three strains collected from three geographical locations. Even though there was an overlap in the range of anal fin counts for the three strains, the Jos strain maintained a consistent distinguishing low count anal fin rays.

Apart from the dorsal fin length:adipose fin length relationship, no other morphological variable examined in the present study could completely distinguish the three strains. Both Onitsha and Jos strains could easily be distinguished from the Kainji strain on the basis of the ratio of adipose dorsal fin length. The near equidistant relationship between adipose and dorsal fin lengths in the Kainji strain agrees with the report of Teugels *et al*. (1990). This report, however, is not in agreement with the results of the present

findings on the ratio of adipose and dorsal fin lengths of collections from Onitsha and Jos. Jos strain could also be distinguished from Onitsha strain by the presence of a wider gap between posterior dorsal and anterior adipose fins coupled with the small size of Jos collection.

A lot of research efforts should be focused on this line of research. This will help to produce improved strains of *Heterobranchus* by cross-breeding and further assist in overcoming the problem of inbreeding depression in our hatcheries brought about by long, continuous, use of the same breeders without replacement.

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