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Size Differentiation of Fingerlings of Two African Catfishes: *Clarias gariepinus* and *Heterobranchus longifilis* and Their Cross Breeds

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Abstract: Size heterogeneity in the African catfish is a major problem in successful mass production of fry in the hatchery. This paper outlines the genetic aspects of size heterogeneity in pure line and crossbred African catfish. Hatchery propagation of *C. gariepinus* (Cl) and *H. longifilis* (Ht) as well as the reciprocal cross to obtain Cl X Cl, Ht X Ht, Ht X Cl and Cl X Ht. Triplicate batches of 100, 15-day old hatchlings were selected and stocked in plastic tanks with static water culture system and constant aeration. The term growth shooters was used to describe individuals with total length that is approximately greater than the sum of the current week's mean length and the difference between the current week's mean length and the preceding week's mean length. Other parameters estimated include the ratio of growth shooters, their percentage occurrence, survival rate of the fish stocked and skewness (Sk) of length. Temporal variation in growth shooter to non-growth shooter ratio was only significant in the second week of culture ($p<0.05$). Body length skewness was high for Ht x Cl (Sk=1.450) at the start of the trial while others were moderate. Final body length was approximately symmetric in Cl x Cl (Sk=0.230) and Cl x Ht (Sk=–0.510) with a moderate skew for the Ht x Ht (Sk=0.750) and Ht x Cl (Sk=–0.840) crosses. There is therefore a maternal influence on body length heterogeneity in *H. longifilis*.

Keywords: Growth shooters, size heterogeneity, length, hatchery.

INTRODUCTION

Growth variation has been observed in both species of the African catfish under study. de Graaf & Janssen (1996) distinguished two groups of the African catfish *C. gariepinus* after four to five weeks of rearing. The management of the African catfish under aquaculture involves sorting of individuals that are larger than the others or deviate from homogeneity of size usually in weight and length. However, length is the factor used by farmers to determine the presence of larger size fish within the cohort. The consequence of heterogenous size stocking is the depletion of the population as a result of cannibalism. At the point of exogenous feeding, there would be pressure from larger fish to cannibalize on smaller fish (Baras & Fortuné dAlmeida, 2001) but when size heterogeneity is low, cannibalism would be delayed and the duration of this delay is a function of the extent of growth heterogeneity. This is supported by (Sahoo et al., 2004) who reported that larger fry tends to have better developed organs for capture of feed particles, better consumption ability, strong musculature for swimming, more developed organs for digestion and absorption of feed hence higher growth.

The African catfish's *C. gariepinus* and *H. longifilis* undergo allometric growth such that larger fish grow faster than small sized fish (Ayo-Olalusi, 2014). Size heterogeneity within a batch of spawned fry and fingerlings can have a tremendous effect on final growth performance (Martins et al., 2005) through high rate of cannibalism (Baras & Fortuné dAlmeida, 2001). However, size variation in the African catfishes, *C. gariepinus* and *H. longifilis* is not the sole cause of cannibalism since this phenomenon is also determined by stocking density (Coulibaly et al., 2007; & Ewa-Oboho & Enyenihi, 1999) and food (Ewa-Oboho & Enyenihi, 1999). According to Melard et al. (1996) the enormity of cannibalism reaches a climax in the early weeks or months of culture when the differences in growth of individuals would attain a peak. (Atsé et al., 2008) reported that cannibalism occurred in the first two months of feeding *H. longifilis* fingerlings. Size differences in *C. gariepinus* is not a direct consequence of social hierarchies where the larger fish dominate the smaller fish but the feeding behaviour exhibited by the heavier fish may give them advantage when feed is limited (Martins et al., 2005). Since stocking density is a critical facto that determines size variation in juvenile African catfish *H. longifilis* (Ewa-Oboho & Enyenihi, 1999), the possibility of gene and environment interaction cannot be ruled out. Genetic-environment interaction has been reported for growth in crosses of the channel catfish and the blue catfish (Abass et al., 2017) hence it is possible to understand the genetics of size heterogeneity in the African catfishes as a stepping stone into exploiting it for greater yield.

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This experiment is designed to elucidate size differences among fingerlings of two African catfishes and their crossbreeds within eight weeks of rearing in plastic aquaria under hatchery conditions.

MATERIALS AND METHODS

Hatchery propagation of *C. gariepinus* and *H. longifilis* as well as the reciprocal cross breeds was carried out. The crosses include Cl X Cl (*C. gariepinus* X *C. gariepinus*), Ht X Ht (*H. longifilis* X *H. longifilis*), Ht X Cl (*H. longifilis* X *C. gariepinus*) and Cl X Ht (*C. gariepinus* X *H. longifilis*). Sex combination is in the order ♀ × ♂. Oocyte maturation and ovulation was induced by a single intramuscular injection of Ovaprim at a dose of 0.5 ml kg^{-1}.

Triplicate batches of eggs from each cross were fertilized and incubated in 60L plastic aquaria with flow through water system using rubber type mosquito mesh netting as substrate. Hatchlings were transferred to concrete tanks of 2.4m X 1.2m X 1.0m to be reared for 15 days. Triplicate batches of 100, 15-day old hatchlings were selected and stocked in plastic tanks with constant aeration. These were fed with coppers catfish feed *ad libitum* for 56 days. Total length of fry was determined every week using a millimeter ruler. For sampling, all fish were gently caught using a fine mesh mosquito size net and then allowed to rest in the net while immersed in water. Length of sample fish was taken individually. Sorting of growth shooters was carried out every week. Fish was termed a shooter if its total length becomes approximately greater than the sum of the current weeks mean length and difference between the current weeks mean length and the preceding week's mean length. This can be represented as:

$$C_T = \mu_2 + (\mu_2 - \mu_1)$$

Where: C_T = Shooter cutoff length
μ_1 = Previous week's mean length
μ_2 = Current Week's mean length
Shooters were sorted out without replacement.

The ratio of non-shooting growth progeny's length to shooting progeny length was derived as:

$$[(L_1/L_1):(L_2/L_1)]$$

Where L_1 = Mean length of non-growth shooters
L_2 = Mean length of growth shooters

Ratio was first determined in decimal form as:

$$Decimal\ Ratio = {}^{L_1}/_{L_2}$$

The decimal values were then harnessed for data analysis.

The percentage of growth shooters per week was determined using the equation:

$$\%\ Growth\ shooters = [(S_1/(V_2 - S_2))] \times 100$$

Where: S_1 = Current No. of growth shooters
S_2 = Previous week's No. of growth shooters
V_2 = No. of surviving fish in the preceding week

The percentage survival of progeny in each cross was determined using the formula:

$$\%\ Survival = [S_T + (N_1 - S_F)/N_0] \times 100$$

Where: S_T = Total Number of growth shooters
S_F = No. of shooters at Final sampling
N_1 = Final No. of fish
N_0 = Initial No. stocked

Data Analysis

Data was analysed using R version 3.4.3 (R Core Team, 2017). Descriptive statistics for length and length ratios were obtained using Rmisc package in R (Hope, 2013) and reshape2 (Wickham, 2007). Differences in weekly length ratios and percentage occurrence of growth shooters across the treatments were determined using one-way ANOVA in R (R Core Team, 2017) via agricolae and emmeans packages (de-Mendiburu, 2017; & Lenth, 2017). Mean separation was done using the Tukey HSD method implemented in multcomp package (Hothorn et al., 2008) and viewed using multcomp View (Graves et al., 2015). Graphical comparison of shooter length and shooter percentages was done using the package ggplot2 (Wickham, 2016).

RESULTS

The coefficient of skewness (Table 1) for total length measured which is a function of growth variation was greater than zero at the inception of the experiment with a highly skewed collection of progeny in the cross Ht × Cl and moderate skewness for progeny in the other three crosses. On the other hand, there was negative and moderate skewness of final length in progeny from two crosses (Ht × Cl and Cl × Ht) while the cross Ht × Ht had a positive but moderate skewness. The cross Cl × Cl had an approximately symmetric positive skewness. Body length skewness ranged from –0.840 (Ht × Cl) to 0.750 (Ht × Ht). Mean initial body lengths ranged from12.35mm in the cross Ht × Ht to 19.19mm in the cross Cl × Ht. The mean final lengths were in the range of 33.70mm (Ht × Ht) to 39.68mm (Cl × Cl).

Table 1. Body length distribution of progeny from crosses of two African catfishes: *Clarias gariepinus* and *Heterobranchus longifilis.*

Cross	No.	*Survival Rate (%)	Body Length (mm)							
			Initial Length				Final Length			
			Range	Mean	S.E.	SK	Range	Mean	S.E.	SK
Cl×Cl	300	69.3	10 – 28	17.63	0.16	0.600^{M}	33–48	39.68	0.39	0.230^{A}
Ht×Cl	300	60.7	9 – 37	14.92	0.28	1.450^{H}	26–46	38.24	0.67	$–0.840^{M}$

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Cl×Ht	300	51.3	11 – 29	19.19	0.15	0.690^{M}	26–48	39.12	0.77	-0.510^{A}
Ht×Ht	300	55.7	8 – 18	12.35	0.11	0.600^{M}	26–45	33.70	0.52	0.750^{M}

S.E. = Standard Error; SK = Skewness; M = Moderately skewed; H = Highly skewed; A = Approximate symmetry
*Survival rate includes sorted out growth shooters.

Body length distribution of growth shooter progeny (Figure 1) from three out of the four crosses: Ht × Ht (0.569), Ht × Cl (0.544) and Cl × Cl (0.797) was moderately skewed at the point of sorting (Week 1). However, the length distribution of growth shooters from the cross Cl × Ht was approximately symmetric (0.413). Also, growth shooting progeny from three crosses: Ht × Ht, Cl × Ht and Cl × Cl at some point had negative skewness for length while the cross Ht × Cl maintained a positive skew throughout the sorting period. At the end of the experiment, progeny from the crosses Cl × Ht (0.493), Cl × Cl (0.394) and Ht × CL (0.363) exhibited approximate symmetry (skewness between −½ and +½). The final length distribution for progeny of the cross Ht × Ht (0.648) exhibited a moderate skew (skew between +½ and +1).

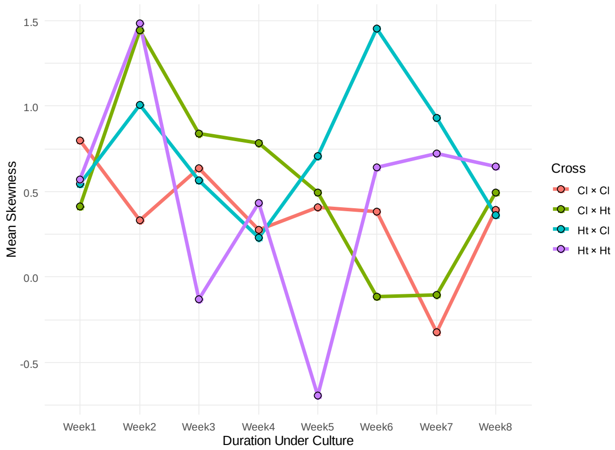

Figure 1: Body length skewness of growth shooters from crosses of two African catfishes: *Clarias gariepinus* and *Heterobranchus longifilis*

The percentage of growth shooters in each cross (Figure 2) shows that the cross Ht × Cl consistently had lower percentages of growth shooters over time while the cross Cl × Ht and Ht × Ht maintained close percentages through the duration of the experiment. The peaks in percentage of growth shooters includes 18.2% at week 8 for Cl × Cl, 20.7% in week 4 for Cl × Ht, 15.3% in week 7 for Ht × Cl and 19.8% in week 8 for Ht × Ht. There was a significant difference in the percentage of growth shooters in week 4 (p=0.016) with progeny from the cross Ht × Cl having the least percentage occurrence of growth shooters (8.31%) and those from the cross Cl × Ht had the highest occurrence of 20.65%. There was however no significant difference in the percentage occurrence of growth shooters in the other weeks (p>0.05).

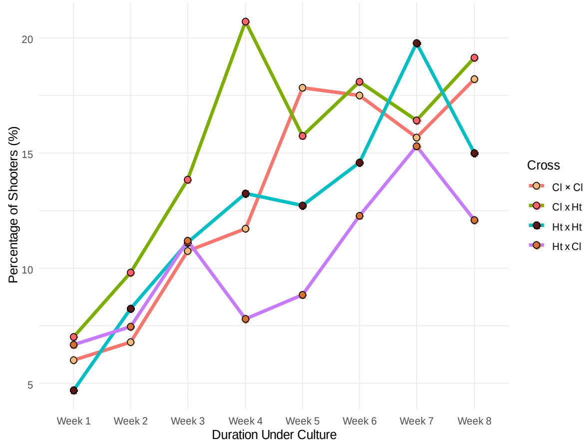

Figure 2: Percentage of growth shooters in crosses of two African catfishes: *Clarias gariepinus* and *Heterobranchus longifilis*.

As expected, the mean length of growth shooters in all crosses (Figure 3 to 6) was consistently higher than the mean length of the population with the cutoff length (C_T) also being higher than the mean length of growth shooters. Cut off length increased with time for each cross with the progeny from the cross: Cl × Ht exhibiting the highest final cut off length (45.48mm) while the progeny of the cross Ht × Ht exhibited the lowest cut off length (40.42mm).

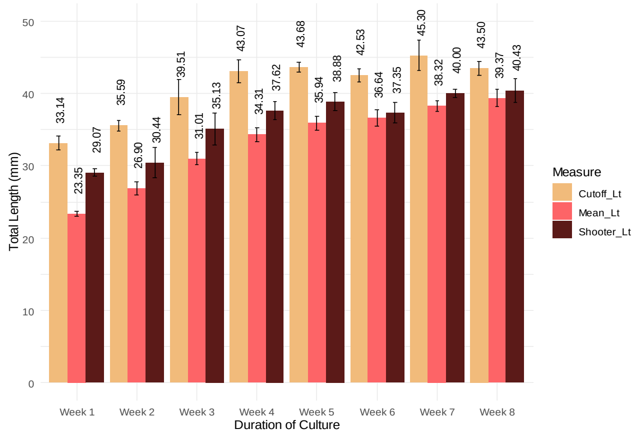

Figure 3: Temporal pattern of population length and length of growth shooters from progeny of the cross: Cl × Cl

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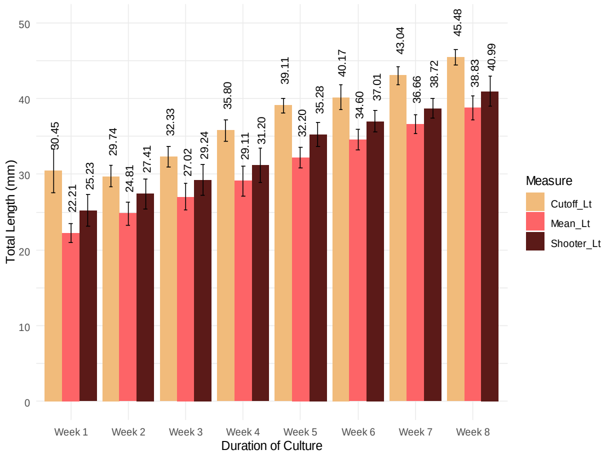

Figure 4: Temporal pattern of population length and length of growth shooters from progeny of the cross: Cl x Ht

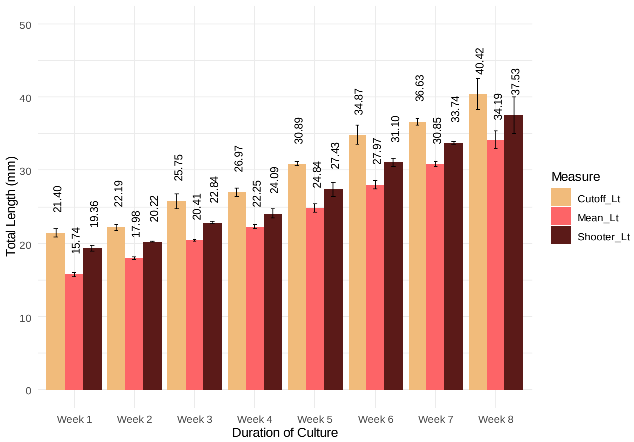

Figure 5: Temporal pattern of population length and length of growth shooters from progeny of the cross: Ht x Ht

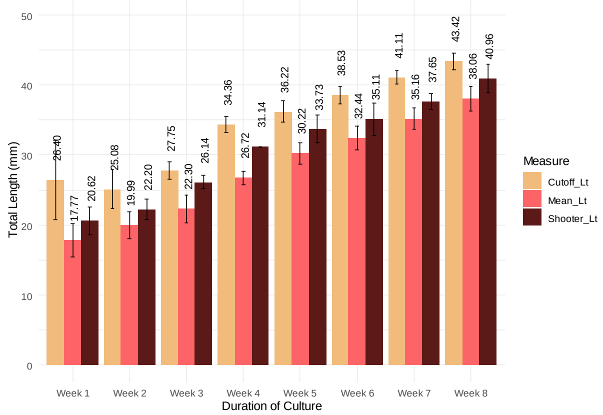

Figure 6: Temporal pattern of population length and length of growth shooters from progeny of the cross: Ht x Cl

The ratio of length of growth shooters to non-growth shooters (Table 2) shows a general reduction in ratio over time. There was a significant difference ($p<0.05$) between the ratios for the progeny from the crosses in week 2 with growth shooters of the cross Ht × Cl having the highest length ratio (1:1.30) against non-growth shooters while progeny of the cross Cl × Ht had the least (1:1.18). overall ratios were similar for the four crosses with the pure line crosses Ht × Ht and Cl × Cl sharing the same ratio (1:1.22).

Table 2. Temporal record of length ratio of non-growth shooters to growth shooters in progeny of crosses between two African catfishes: *Clarias gariepinus* and *Heterobranchus longifilis*.

Cross	Week.1	Week.2	Week.3	Week.4	Week.5	Week.6	Week.7	Week.8	Overall
Cl × Cl	1:1.42	1:1.28^{a}	1:1.25	1:1.25	1:1.20	1:1.16	1:1.16	1:1.10	1:1.22
Cl × Ht	1:1.38	1:1.18^{b}	1:1.19	1:1.22	1:1.21	1:1.16	1:1.16	1:1.15	1:1.20
Ht × Cl	1:1.64	1:1.30^{ab}	1:1.27	1:1.27	1:1.20	1:1.18	1:1.16	1:1.13	1:1.24
Ht × Ht	1:1.34	1:1.23^{ab}	1:1.27	1:1.22	1:1.24	1:1.24	1:1.18	1:1.16	1:1.22
p-value	0.871	0.017	0.744	0.792	0.800	0.156	0.993	0.150	–

Most water quality parameters (Table 3) were within recommended ranges. The dissolved oxygen levels for water used to rear progeny from each cross was $\geq 5.0 mg.l^{-1}$ ($\geq 80\%$ saturation) and is within range recommended for tropical freshwater fish (Mallya, 2007) while pH was within the range of 6.0 to 9.0 as recommended (Riffel et al., 2012), total alkalinity was $>20 mg.l^{-1}$ as recommended by Wurts, (2002). Biological oxygen demand (BOD) is below the $5 mg.l^{-1}$ threshold as recommended by Das (1997) while temperature was below the recommended range of 25-32°C recommended by Das (1997). This is due to the fact that this experiment was conducted during the cold season.

Table 3. Water quality parameters in tanks used to rear progeny of crosses between *C. gariepinus* and *H. longifilis*

Cross	pH	DO ($mg.l^{-1}$)	Temp (°C)	BOD ($mg.l^{-1}$)	Alkalinity ($mg.l^{-1}$)
Cl × Cl	8.18 ± 0.08	6.97 ± 0.14	23.62 ± 0.21	3.26 ± 0.13	23.15 ± 0.14
Cl × Ht	8.01 ± 0.11	6.96 ± 0.10	23.66 ± 0.22	3.16 ± 0.15	22.67 ± 0.30
Ht × Cl	8.12 ± 0.05	6.93 ± 0.12	23.63 ± 0.22	3.09 ± 0.07	22.87 ± 0.22
Ht × Ht	8.11 ± 0.09	6.87 ± 0.07	23.62 ± 0.24	3.21 ± 0.13	22.73 ± 0.46

DISCUSSION

Size variation in larvae and fry of the two African catfishes cannot be precluded (Ewa-Oboho & Enyenihi, 1999). Under culture conditions, the African catfish has been known to evince reduced feed intake, aggression and cannibalism as a result of size variation within the cohort (Martins et al., 2005). The skewness for total length in the current data indicates that a highly positively skewed population (a population with more individuals with higher values of length) can be curtailed with frequent sorting of growth shooters. This explains the approximate symmetry for total length at the end of the culture period most of the crosses. The importance of uniformity in size during culture is linked to cannibalism and access to food (Martins et al., 2005). However, the presence or absence of light can determine feeding behaviour as well (Sallehudin et al., 2017; & Solomon & Okomoda, 2012). The size of prey a cannibalistic fish can ingest is roughly 78% of the body length of the conspecific predator (Ribeiro & Qin, 2013). The current study used a threshold that involves both the current and previous week's mean total length of the cohort in order to determine the growth shooters that need to be sorted out. The specific thresholds in terms of ratio as determined for the crossbreeds of these two African catfishes based on the much simpler method in this study suggests that non-growth shooter to growth shooter ratio for Ht x Cl was 1:1.24 as against 1:1.4 proposed by Umanah (2019).

In the current study, size heterogeneity within the cohort of crosses was already present at the start of the experiment (15 days post-hatching). This corresponds to studies by Hecht & Appelbaum (1988); & Baras & Fortuné dAlmeida (2001). The increase in cut-off length for growth shooters on a weekly basis suggests the presence of size heterogeneity on a temporal scale. Moreover, the percentage of growth shooters undulates through the period under culture indicating that ratios can vary and the use of sorting can reduce the presence of growth shooters and avoid cannibalism or intraspecific competition (Naumowicz et al., 2017). Size heterogeneity thresholds observed in this study were within the range of 1:1.10 and 1:1.28 as reported by Hecht & Appelbaum (1988) as precaution against type I cannibalism. Although the focus of this research was not cannibalism, it is important to identify the potential cannibals within a cohort especially for crossbreeds since information about them is scarce.

The data on length distribution among the growth shooters also suggest some form of hierarchy in size with negative skew suggesting presence of smaller fish within the giants and this leveled off with 75% of the crosses exhibiting approximate symmetry and 25% exhibiting moderate skewness. The implication of size heterogeneity within growth shooters is that a rigid ratio may not be ideal for sorting them out. The current approach that uses both the antecedent and successive means of body length to estimate ratio of growth shooters to non-growth shooters therefore presents a more reliable way to identify growth shooters. Inconsistency in growth within cohorts is the outcome of size hierarchy within the population (Stefánsson et al., 2000). Conspecific competition and cannibalism will be high in a culture facility with size heterogeneity. This has been shown for various fish species including *Clarias gariepinus* (Baras & Fortuné dAlmeida, 2001), Eurasian perch and European seabass (Kestemont et al., 2003) and Atlantic salmon (Adams et al., 2000).

When there is a high incidence of size heterogeneity that corresponds to asymmetric negative skewness at the beginning of fry or larval culture, some conspecific siblings may take advantage of their larger size and begin acts of cannibalism even immediately after yolk sac absorption (Baras & Fortuné dAlmeida, 2001). The genetics of body length skewness as observed in the current study shows that the maternal *H. longifilis* has an affinity for offspring that violate size homogeneity. This can be seen from the combination of parents and initial and final body length skewness. The pure line *H. longifilis* and *C. gariepinus* crosses had a moderate skew at the start while the initial skew for the crossbreed with maternal *H. longifilis* and paternal *C. gariepinus* had a high skew. At the end of the trials, crosses that involved the maternal *C. gariepinus* leveled off size heterogeneity with an approximately asymmetric skew. However, there was a moderate skew in the final body length for the cross Ht x Ht just as it was at the start of the trials and the cross Ht x Cl reduced size heterogeneity and recorded a moderate skew. The plausible reason behind this could be the sorting out that was carried out. The catfish *H. longifilis* is known to be an omnivorous species that can adopt a piscivorous feeding habit (Ajah, 2010; & Fagbenro, 1992). Observations of behaviour in the tanks indicated behaviour of dormancy in some individuals that remain at the bottom as a result of loss of fitness and inability to compete with siblings that have size advantage.

CONCLUSIONS

Number of growth shooters increased with time as their size also increased. The current method of determining size deviant fish that can become aggressive and cannibalistic is a step forward from the rigid ratio system that has been in use. It ensures stepwise determination of growth shooters using data at each sampling. In addition, there is a maternal influence on size heterogeneity in *H. longifilis*.

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