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Dietary Tryptophan Supplementation Made No Difference on the Cannibalism, Survival and Growth of *Heterobranchus longifilis* fingerlings

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ABSTRACT: *Heterobranchus longifilis* is a popular cultured and food fish but its cannibalism greatly reduces harvest thus necessitating proactive control measures. Tryptophan is believed to raise the serotogenic activity in the brain at certain levels leading to reduced aggression and cannibalism in certain species of fish which possibilities this research tried to explore in *Heterobranchus longifilis*. Fingerlings (0.58 ± 0.123g mean weight and 3. 8 ± 0.52cm mean length) were stocked in 18 round plastic tanks, each 45 litres containing 40 litres of water, at 45 fish per tank. Tryptophan was incorporated at 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 % in commercial Catfish feed by spraying each tryptophan - 70% alcohol solution to separate feed samples to form six different experimental feeds. The dried feeds were fed to the fish daily at 4 % body weight for 8 weeks. The experiment used a complete randomized design with 6 feed treatments and 3 replications. Water quality, growth, mortality and feed utilization data noted and at the end, their associated indices were determined and subjected to the analysis of variance for significant differences (P= 0.05). The results revealed that tryptophan supplementation at the levels used in the diets of *Heterobranchus longifilis* did not have any significant effects on all the parameters examined excepting mean weight gain. This was discussed in the context of growth and cannibalism.

KEYWORDS: *Heterobrancus longifilis*, Typtophan, Growth, Cannibalism, Mortalities, Survival

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1.0 INTRODUCTION

High incidence of cannibalism in the culture of the African catfish necessitates proactive efforts to address it. *Heterobranchus longifilis* is one of the most important catfishes for aquaculture enterprises largely in the Sub-Saharan Africa and to some extent in many other parts of the world. The many desirable qualities possessed by this fish which include hardiness, fast growth rate, excellent meat quality, high acceptability by consumers and premium market value enhance its high popularity among fish farmers. The main constraint of the culture of the African catfish, *Heterobranchus longifilis* remains the high mortality rate due mainly to cannibalism (Baras *et al*, 2014; Umanah and Nlewadim 2019) consequent on its vigorous carnivorous tendencies. The cultural methods such as grading and sorting used in controlling cannibalism in many predatory fishes as well as in *H. longifilis* are very labour intensive, expensive and prone to inducing fish mortalities (Naumowicz *et al*, 2017). It becomes imperative to explore other approaches such as nutritional interventions with dietary tryptophan supplementation.

The amino acid L-tryptophan is essential to fish as a precursor of serotonin, a neurotransmitter that functions in reducing stress and aggression (de Pedro *et al.* 1998; Höglund *et al.* 2005; Schjolden *et al.* 2006.). Serotonin acts through the HPI axis, which controls osmo-regulatory, immunological and behavioral responses. It has been shown that little supplementation of the feed with Tryptophan causes reduced stress in various cultivated fish species and improves their immune response (Hseu. *et al., 2003*). Exogenous tryptophan is believed to decrease cannibalism and increase survival rate in several fish species (Kumar *et al*, 2017). It has not yet been recorded that tryptophan (TRP) has been supplemented in the diets of *Heterobranchus longifilis* or any other African catfishes for the purpose of mitigating cannibalism. This research effort was therefore focused on establishing the effectiveness and application rate of TRP in the diet for the control of cannibalism in *Heterobranchus longifilis* with a view to improving its survival rate under culture conditions.

2.0 MATERIALS AND METHODS

2.1 Preparation of Experimental Feed

Different sizes (0.5 to 4.5 mm) of catfish feed (Aller qua) were used as the fish increased in weight. Six diets were separately prepared at tryptophan (Xi'an Sonwu Biotech Co. Ltd, China.) supplementary levels of 0%, 0.5%, 1%, 1.5%, 2 % and 2.5% as treatments. The required amount of tryptophan (TRP) was dissolved in hot water containing 70% alcohol and sprinkled on the particular feed according to Król and Zakes (2016). All the five TRP supplemented diets (TRPD) were prepared this way. The control feed (0% TRP) was sprinkled with 70% alcohol-water solution devoid of tryptophan to prevent taste difference. The feeds were then dried in an oven set at 37^{o} C for one hour after which each feed type was kept in a separate labelled dark polythelene bag inside a freezer at 4^{o}C till required for feeding.

2.2 Experimental Design and Procedures

Eighteen round plastic tanks of 45litres each containing 40 litres of water were used for the experiment. 810 *Heterobranchus longifilis* fingerlings (mean weight:0.58±0.123g; length 3.8±0.52 cm) were distributed into six groups of three tanks at 45 fish per tank**.** Each set of three tanks constituted three replicates of a feed treatment and was assigned a particular feed. A completely randomized design was used. The fish were picked randomly and ignoring variation in size in order to propagate heterogeneity within the replicates in the various treatments. The weight of each fish was determined individually. Feeding started 24 hours after stocking at 4 % body weight per day in two rations till the end. The unconsumed feed and faecal matters were siphoned each morning before feeding and the tanks cleaned while mortalities were noted and removed, if any. Dead fish were carefully observed for mutilation of body parts. Daily water pH, dissolved oxygen and temperature were measured using appropriate test meters at 8:00 and 16:00hours. The fish were reared for 8 weeks.

2.3 Data Collection and Analysis

2.3.1 Growth Performance, Mortality Indices and Feed Conversion Ratio

Total length and weight were recorded on the first day and thereafter fortnightly throughout the experimental period. At the end of the experiment, the number, length and weight of surviving fish, and number of missing fish were noted. Growth performance indices (Mean weight gain, g; Specific growth rate, % day^{-1}); Feed conversion ratio; cannibalism (%); normal mortality (%): total mortality (%) survival rate (%); coefficient of variation (%) for the initial and final lenght of fish, and heterogeneity of the fish at the end of the experiment were determined (Król and Zake, 2016). All percentage data were transformed into angular arc sine before Analysis of variance and back transformed after (McDonald, 2014).

2.4 Statistical analysis

Data obtained were subjected to one - way analysis of variance (ANOVA) for significant difference and Fisher's Least Significant Difference for means separation using a computer Statistical Package for Social Sciences (SPSS) Version 25, IBM Inc, USA. Graphical presentation was achieved with Microsoft Excel (2016), Microsoft Inc., USA.

3.0 RESULTS

The results of the experiment were as follows.

3.1 Water Quality parameters

The observed mean daily water quality indices were: dissolved oxygen (DO) = 10.12 mgl^{-1}; pH = 7.77 and temperature 27.5 ^{0}C.

3.2 Mean Growth Parameters and Food Conversion Ratio

Mean growth performances and feed utilization of *Heterobranchus longifilis* fed the experimental diet were as presented in Table 1.

Table 1: Mean Growth Indices and Feed Conversion Ratio of *Heterobranchus longifilis* fed with varying levels of Tryptophan Supplemented Diets for 8 weeks

TRPD	MIW	MFW	MWG	SGR	FCR
0	0.45±0.02	10.91±0.48	10.46±0.46 ^{c}	4.46±0.15^{a}	0.65±0.03 ^{a}
0.5	0.74±0.05	13.94±0.00	13.20±0.05 ^{ab}	4.28±0.31 ^{a}	0.69±0.07 ^{a}
1.0	0.60±0.03	14.38±0.20	13.79±0.17 ^{a}	4.21±0.08 ^{a}	0.70±0.02 ^{a}
1.5	0.53±0.01	13.93±1.23	13.40±1.24 ^{a}	4.66±0.06 ^{a}	0.61±0.01 ^{a}
2.0	0.51±0.01	11.90±0.28	11.38±0.27 ^{bc}	5.04±0.45 ^{a}	0.56±0.07 ^{a}
2.5	0.62±0.01	11.37±0.56	10.75±0.56 ^{c}	4.11±0.11^{a}	0.72±0.03 ^{a}
P-value	0.000228	0.004322	0.005363	0.139851^{a}	0.143367^{a}
Significant			Yes	No	No

Means in the same column with different superscripts are significantly different (P<0.05). TRPD = TRP supplemented diet; MIW = Mean initial weight; MFW = Mean final weight, MWG = Mean weight gain; SGR = Specific growth rate; FCR = Feed conversion ratio; ± Standard error of the mean.

The results indicate that the fish fed on 1.0 % TRPD had the highest weight gain (MWG) which was not significantly different (P < 0.05) from fish on 0.5 and 1.5 % TRPD but different from 0.0 % TRPD (control) with lowest MWG, 2.0 and 2.5 % TRPD. However, 0.5 % and 2.0% TRPD gave non-significant results while 2.5% TRPD and 2.0% TRPD were also not significantly different (P > 0.05). SGR and FCR had similar quality trend without any significant superiority (P > 0.05) in all treatments. The growth trend of the experimental fish over the period of 8 weeks is shown in Figure1. The fish exhibited slow growth in the early weeks till week 4 when increased growth was observed which got faster from the 6th week.

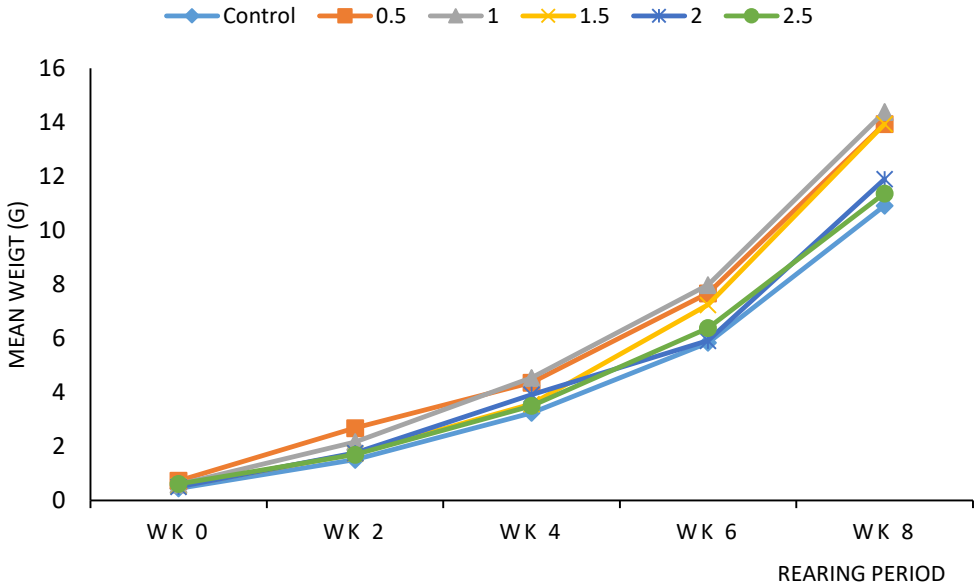

Figure 1: Bi - weekly mean cumulative weight gain of *Heterobranchus longifilis* fed with varying levels of Tryptophan supplemented diets for 8 weeks

3.3 Size Variation, Mortality and Survival Indices

Size variation indices (initial coefficient of variation – CV1, final coefficient of variation – CV2, and Heterogeneity); Mortality indices (Normal Mortality Rate – NDR, Cannibalism Rate – CR and Total Mortality Rate - MR) and survival rate (SR) obtained in the experiment were recorded in the following Table 2.

Table 2: Mean Size Variation, Mortality and Survival Indices of *Heterobranchus longifilis* fed on varying levels of tryptophan supplemented diets for 8 weeks

TRPD	CV1	CV2	Heterogeneity	**NDR (%)**	**CR (%)**	**MR (%)**	**SR (%)**
0	16.02±2.08 [a]	9.25±1.35 [a]	0.58±0.02 [a]	10.84±0.17 [a]	8.03±0.21 [a]	18.97±0.24 [a]	81.03±0.24 [a]
0.5	20.58±2.10 [a]	12.93±1.33 [a]	0.63±0.04 [a]	10.08±0.18 [a]	16.4±1.44 [a]	26.73±0.77 [a]	73.28±0.77 [a]
1.0	21.73±2.79 [a]	12.66±2.05 [a]	0.58±0.05 [a]	10.19±0.11 [a]	14.42±2.51 [a]	25.14±0.81 [a]	74.86±0.81 [a]
1.5	29.81±5.26 [a]	15.12±1.77 [a]	0.54±0.10 [a]	7.9±0.14 [a]	19.36±1.61 [a]	27.48±0.76 [a]	72.52±0.76 [a]
2.0	33.06±9.31 [a]	10.92±0.54 [a]	0.38±0.09 [a]	7.17±0.14 [a]	11.05±0.13 [a]	18.4740.04 [a]	81.53±0.04 [a]
2.5	28.22±5.69 [a]	14.45±1.15 [a]	0.54±0.07 [a]	5.55±0.21 [a]	9.46±0.32 [a]	15.27±0.21 [a]	84.73±0.21 [a]
P-value	0.2495	0.1123	0.2476	0.507699	0.36359	0.560654	0.560654
Significant	No	No	No	No	No	No	No

Means in the same column with the same superscripts are not significantly different *(P>0.05).*

The results revealed that the mean values of all the parameters were not significantly different though, there were nominal differences in the values of each parameter along the treatment lines. The specific mean mortality indices are depicted in Figure 2 while mean bi-weekly total mortality trend is shown in Figure 3.

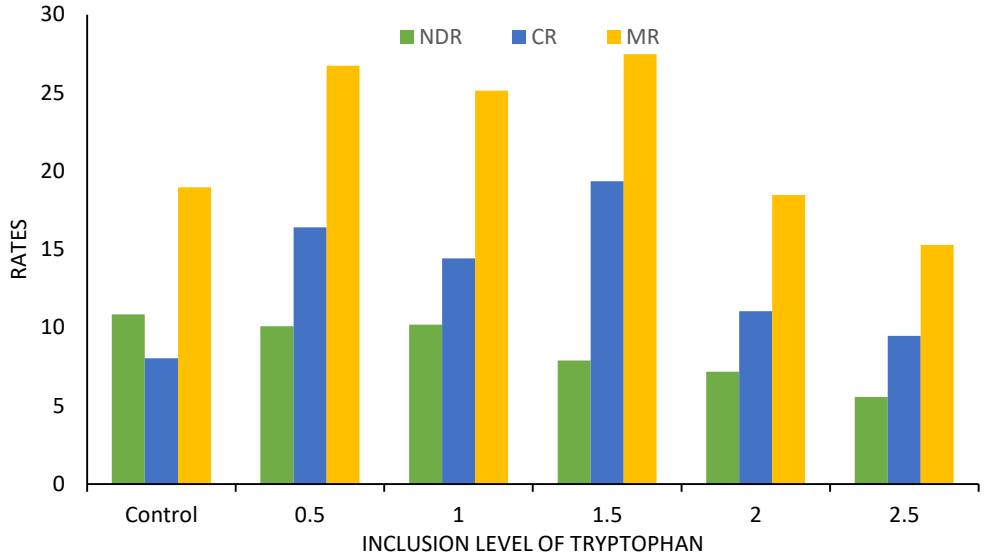

Figure 2: Mean Cannibalism Rate (CR), Normal Mortality Rate (NDR) and Total Mortality Rate (MR) of *Heterobranchus longifilis* fed with Varying Levels of Tryptophan Supplemented Diets for 8 weeks

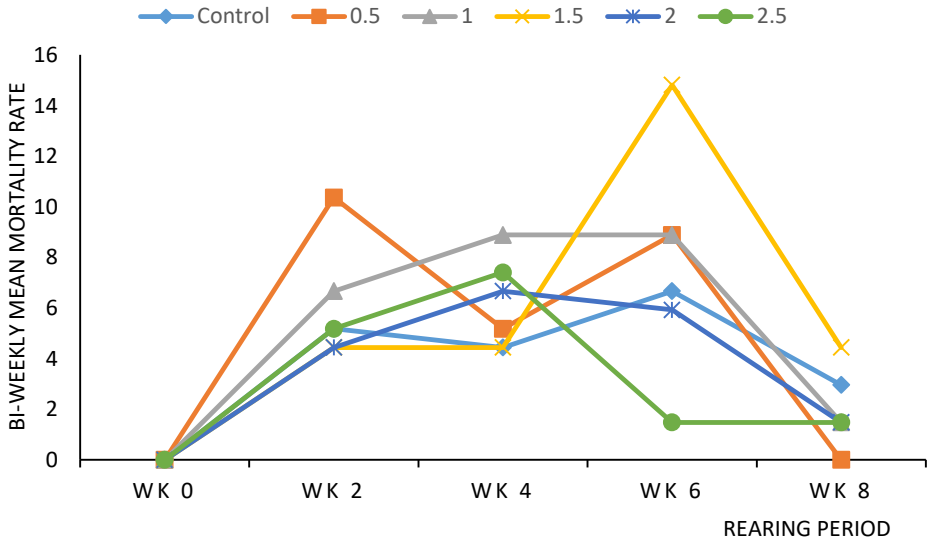

Figure 3: Mean Bi - weekly Total Mortality Rate of *Heterobranchus longifilis* fed with Varying Levels of Tryptophan Supplemented Diets for 8 weeks

Highest peaks of mean total mortality were recorded among fish on 0.5 % TRPD and 1.5 % TRPD on the second and sixth weeks of the experiment respectively. Mean total mortality rose steadily till the end of week 2 and from there, different treatments produced their different peaks and by the sixth week the rate of mortality started declining.

4. 0 DISCUSION

4.1 Effects of Tryptophan Supplemented Diets on Cannibalism and Survival of *Heterobranchus longifilis* fry/fingerlings

Tryptophan is believed to be precursor of Serotonin (5-Hydroxytryptamine, 5-HT) widely acclaimed to suppress aggressive behaviour and consequently reduce cannibalism in vertebrates including fishes by raising serotonergic activity in the brain (Winberg *et al*, 2001; Hseu *et al*, 2003). Dietary supplementation with tryptophan (TRP) is considered a viable means of supplying TRP to cultured fish in order to achieve high brain serotonergic activity with the view to suppressing aggression, and its attendant cannibalism (de Pedro *et al*. 1998, Winberg et al. 2001, Lepage *et al*. 2002; Kumar, et al 2017) for improved survival of the cultured stocks. This has been demonstrated in many species of fish and crustaceans including rainbow trout, *Oncorhynchus mykess* (Winberg *et al*, 2001); the spotted grouper, *Epinephelus coides* (Hseu, *et al*, 2003); Atlantic salmon, *Salmon salar* (Cubitt *et al*, 2008), Pabda, *Ompok bimaculatus* (Bloch) (Biswas *et al*, 2018) and *Macrobrachium rosenbergii*, de Mann (Suharyanto and Himawan, 2015).

However, in the current study on the African catfish non – significant results were obtained in both cannibalism snd survival rates. Król *et al* (2014) observed similarly among the larvae of the European catfish (*Silurus glanis*). This was possibly linked to the dark conditions under which the experiment was conducted. Accordingly, dark conditions favour the synthesis of melatonin from its precursor, 5 – HT, thereby depleting the concentration of the latter with the consequence of lowering the 5- HT activity in the brain. This tends to limit inhibition of aggressive and agonistic behaviour thus allowing cannibalistic activities. This probably could be the case in the current experiment which took place in a reduced light environment. Low 5 – HT activity in the brain of *Heterobranchus longifilis* could undermine the possible effectiveness of dietary TRP supplementation in a fish known to be more active at night (Baras *et al* 1998: Anselme *et al*, 2008) thus the insignificant cannibalism inhibition in the fish under the current study.

Although insignificant, the trend of the nominal results could be viewed in the perspective of size variations in the experimental fish and serotonergic activity. Size heterogeneity has been shown to impacts greatly on cannibalism especially, among predatory species (Baras and Jobling, 2002). The striking result in this work was the higher rates of cannibalism among the fish on TRPDs (0.5 - 2.5%) compared to those on the control diet (0.0 % TRP). Both the initial and final coefficients of variation (CV1; CV2) were lowest in the fish on the 0.0 % TRPD in which the much smaller individuals had been removed through cannibalistic and non- cannibalistic mortalities earlier in the culture period thus lowering CV2. The highest CV1 occurred in fish on 2.0 % TRPD followed by1.5 and 2.5 % TRPD in which cannibalism lasted till the sixth week reducing CV2 excepting 1.5 % which still recorded cannibalistic mortalities till the eight week. The potential cannibals though not segregated from other fish across the treatments, two of them in treatment 1.5 % TRP were the biggest, possibly still impacting higher CV2 and cannibalism. Also, a possible gradual rise in serotonergic activity along higher TRP concentration around 2 % and time gradients in the sixth week where and when cannibalism rates dropped with possible higher concentrations could be implicative. This however could have been more clearly established, had the experiment gone beyond 8 weeks. Hseu *et al* (2003) explained that the minimal percentage reduction in cannibalism between the control and TRPD groups of spotted grouper, *Epinephelus coides* was due to common practice of cannibalism by fish in all groups in the early days of the trial before TRP started taking effects on the behaviour of the grouper. Similarly, Suharyanto and Himawan (2015) observed the highest death of giant prawn (*Macrobrachium rosenbergii*) in the second week of feeding trial and believed that the role of TRP and glycine eaten in the diets was not yet optimum. Non – cannibalistic mortality got in this work is still moderate in catfish culture and might have arisen from handling stress and occasional ammonia and nitrite build up in a partially flow through system. Cannibalism still accounted for the greater part of total mortality in this study and is a common feature in the culture of predatory species (Król and Zake 2016; Kumar et al, 2017; Sangavi *et al*, 2019) even under optimal conditions (Rawat *et al*, 2018). The highest score of total mortality occurred between the second and the sixth weeks. It was noted that while total mortality declined sharply in all TRP treatments on or before the sixth week, it was rather slower in the control due to sporadic cannibalistic mortalities that subsisted. The impacts of total mortality as moderated by cannibalism on the survival rates of *H. longifilis* in this work was evident, though insignificant. Fish on 1.5 % TRPD experienced maximum total mortality as compared to the least mortality rate in 2.5 % TRPD with the highest survival rate. The improvement in survival rate of the African catfish between 1.5 % TRPD and 2.5 % TRPD could be the possible effect of TRP approaching a thresh hold with increasing concentration, but this seems vague considering the random nature of the results recorded for those on 0.0 – 1.0 % TRPD as moderated by the heterogeneous characteristics of the stocks in those treatments.

4.2 Effects of Tryptophan Supplemented Diets on Growth and Feed Conversion Ratio of *Heterobranchus longifilis* fingerlings

Generally, the weight gains of the *H. longifilis* were better from the fourth week of the experiment probably due to decreasing fish density. Incidentally, the significant weight gain did not reflect in the SGR and FCR of the fish implying that the incorporated TRP in the diets of this catfish had no effects on its growth rate and feed utilization (especially, given the random responses recorded across the treatments). This observation agreed with Król *et al* (2014) on *Silurus glanis* larvae, and Suharyanto and Himanan (2015) on *Macrobrachium rosenbergi*. However, Hseu (2003); Biwas *et al* (2019) and Sangavi *et al* (2019) had observed growth disparities between the control and TRP dietary supplemented fish with better growth rate in favour of the former. This could be that dietary TRP supplementation probably elevated the fish brain serotonin level and serotonergic activity which suppressed feed consumption resulting to lower growth (Pedro *et al*, 1998; Biwas *et al*, 2019).

With only the results of the current work and Król *et al* (2014), it is not certain whether dietary supplementation of TRP does not build enough 5-HTergic potential to affect the catfishes at the rates applied. The difference between the results of these two studies and others might be due to species difference and culture conditions. This was also the synthesis of Suharyanto and Himanan (2015) from the reports of Hseu (2003) that 0.5 % TRP significantly mitigated cannibalism in *Epinephelus coides* whereas 1 % was effective in tiger grouper, *Epinephelus fuscogluttatus* (Kamaruddin 2006) that the difference in the two results might be the implication of species and feed composition. Basic *et al* (201)3 and Hoseini *et al* (2019) had earlier submitted that the TRP requirements of fish vary with species, rearing environment and duration of exposure especially, since TRP is a functional amino acid being required for protein synthesis and other purposes (Wu 2009; Hoseini *et al*, 2019). *Heterobranchus longifilis* being a

distinct species, it is possible that the inclusion levels, experimental conditions and duration might have modulated the effects of TRP thus necessitating further researches.

4. CONCLUSION

Dietary tryptophan supplementation did not significantly affect feed conversion ratio and growth rate nor reduce cannibalism and improved the survival rate of *Heterobranchus longifilis* fingerlings in this study. This could be possibly due to the TRP concentration used, experimental conditions or the exposure duration. It is therefore recommended that

(a) further researches be carried out on the fish fed with dietary TRP at supplementation levels beyond those used in the current work under both high and low light intensities over a longer period.

(b) *Heterobranchus longifilis* culturists should continue to apply cultural approaches such as sorting and proper feeding to attempt to mitigate cannibalism in their culture stocks until alternative or complementary control measures are available.

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