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Research Article
Comparative Lethality of Agrolyser to Two African Catfish Species *Heterobranchus bidorsalis* and the Hybrid (*Heterobranchus bidorsalis*, ♂×*Clarias gariepinus*, ♀)

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Abstract
Background and Objective: Fish culture is associated with the application of fertilizers for nutrient enrichment of ponds. However, such practice sometimes is done without consideration of the concentrations that can be harmful or toxic to the fish. Also, the concentrations that may be tolerable to a particular fish species may be toxic to another. This study was therefore carried out comparatively to examine the lethality of agrolyser to two species of catfish reared in Nigeria. **Materials and Methods:** Fingerlings of the catfish hybrid, *Heterobranchus bidorsalis,* ♂× *Clarias gariepinus*, ♀ (total mean length, 7.0±1.0 cm; mean weight 3.0±0.7 g SD) and *Heterobranchus bidorsalis* (*H. bidorsalis*) (mean total length, 6.75±0.9 cm SD; mean weight, 2.94±0.34 g SD) were exposed to graded concentrations of the micronutrient fertilizer, agrolyser (1400, 1600, 1800, 2000 and 2200 mg L^{-1}) and a control (0.00 mg L^{-1}) in triplicates for 96 h to assess its effects on behavioral changes tail beat frequency (TBF), opercular beat frequency (OBF) and cumulative mortality. Data were analyzed by two-way ANOVA using DMRT. **Results:** The pattern of behavioral responses of both species to the agrochemical was similar. However, OBF and TBF in both species were inversely related to the duration and concentration. Within the species they differed ($p<0.05$) with duration and toxicant levels. Mortality of *H. bidorsalis* only differed ($p<0.05$) at the various levels of agrolyser. The mortality at the various exposure durations within the species differed at ($p<0.05$). OBF did not differ ($p>0.05$) between the species, but TBF did at ($p<0.01$). Mortality of the species differed at ($p<0.01$). The 96 h LC_{50} for the hybrid was 1353.66 mg L^{-1} and *H. bidorsalis*, 1688.58 mg L^{-1}. The MLT for the hybrid at 2200 mg L^{-1} was 40.76 h as against 50.91 h for *H. bidorsalis*. **Conclusion:** This study indicated that the *H. bidorsalis* can withstand agrolyser associated stress than the hybrid. However, the *clariids* are generally very tolerant to the micronutrient and hence, it can be safely applied in ponds to enhance the production of the catfishes.

Key words: Agrolyser, *Heterobranchus bidorsalis*, tail beat frequency, opercular beat frequency, comparative lethality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The need to increase production from agricultural fields to meet the ever-increasing world population has necessitated the heavy dependence on the use of fertilizers to increase yields from agricultural fields and fish farms. This has resulted in the contamination of several habitats particularly those in agricultural areas with fertilizers singly or by the interactive effects with abiotic and biotic factors[1,2]. Studies by several authors have shown that contamination of terrestrial and aquatic environments by agricultural fertilizers have negative effects on almost all life forms[3-5]. Investigations on fishes strongly suggest that agrochemicals or their effluent impair reproduction as shown by reduced gonadosomatic index and degenerative oocytes in *Channa punctatus*[6] and viability and hatchability of eggs in *Cyprinus carpio*[7].

The effect of agrochemical fertilizers on amphibians appeared to be the most extensively studied compared to the other organisms including fish[8-11]. Ortiz-Santaliestra *et al.*[8] observed that besides direct effects of ammonium nitrate fertilizers on embryonic and larval survival, it altered the breeding behaviours of dwarf newt, *Triturus boscai*. The authors noted that this may reduce reproductive success and ultimately affect population conditions, which could also be true for fish populations. Different fertilizers and their effluents have also been shown to impact on fish behaviours and caused mortality[12], haemtological[13,14], biochemical[15] and histopathological[16,17] changes in some organs in exposed fishes.

Sometimes, the responses in the aquatic environment can be very complex. In mecocosms[1], studied the roles chemical contamination by fertilizers, competition and predation played singly and in combination in aquatic community using five amphibians, a bull frog tadpole and blue gill sunfish, *Lepomis macrochirus*. Multiple factors affected the survival of the amphibians, *Bufo americanus* and *Ambyostoma maculatum* and lengthened the larval period of *Rana sphenocephala*. The blue gill had the largest impact on the community eliminating *B. americanus* and *A. maculatum* and reduced the abundance of *R. sphenocephala*. The fertilizer negatively influenced the time mass at metamorphosis for both anurans and *A. maculatum.* Results from this study, give insight into the possible complex nature of the responses of exposed organisms to fertilizers in the aquatic environment. Besides, abiotic factors have also been shown to modify the toxicity of fertilizers in fish, *Oreochromis niloticus*[18], Iberian newt[9] and *Bufo terrestris*[19].

Agrolyser, a micronutrient fertilizer is manufactured by Cybernetics Nigeria Limited, Lagos, Nigeria. It is a balanced nutrient formulation consisting of ten secondary nutrients (Cu, Mn, Fe, Na, Ca, S, B, Zn and Mo). Several regulatory authorities in the country have cleared, approved and recommended the product for use in crop fields and fish farms. In fish farms, the recommended rate of application is 6 mg L^{-1}. In field trials and laboratory studies, agrolyser enhanced dissolved oxygen levels and phytoplankton production[20]. Enhanced phytoplankton production is known to lead to increased fish yield in ponds. Previous studies indicated that the chemical was not toxic to two *clariid* species African catfish hybrid, *Heterobranchus bidorsalis* (♂)×*Clarias gariepinus*, (♀)[21] and *Heterobranchus bidorsalis*[22] at the recommended application, 6 mg L^{-1} although the fishes showed altered ventilatory rates and tail beat frequencies. Ventilatory rates and tail beat frequencies (behavioural toxicity) have been used to assess the potential hazards of a number of environmental toxicants to the *clariids* in the Niger Delta region[23-26] and have been proven reliable indicators of stress[21,22].

The present study, reported the findings of the assessment of the comparative lethality of agrolyser to two important cultured fish species, *Heterobranchus bidorslis* and hybrid (*Heterobranchus bidorsalis* (♂)×*Clarias gariepinus*, (♀)) under laboratory conditions.

MATERIALS AND METHODS

This study was carried out between January and March, 2015. Fingerlings of the hybrid catfish (total mean length, 7.0±1.0 cm; mean weight 3.0±0.7 g) and *Heterobranchus bidorsalis* (mean total length, 6.75±0.9 cm SD; mean weight, 2.94±0.34 cm SD) were obtained from the African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt. They were transported in plastic aquaria to the Fisheries Laboratory, The Rivers State University, Port Harcourt. The fishes were acclimated to laboratory conditions for 7 days in aerated rectangular glass aquaria ($30\times25\times25$ cm^3) with 20 L borehole water (characteristics: Temperature, 26.51±2.10°C; dissolved oxygen, 4.51±0.51 mg L^{-1}; pH, 6.49±0.21; alkalinity, 17.30±3.16 mg L^{-1} and hardness, 16.00±2.06 mg L^{-1}). A total of eighteen aquaria were used. Each aquarium held 10 fingerlings. The fishes were fed a commercial diet (35% crude protein) at one percent biomass once daily. The agrolyser was obtained from the Rivers State Agricultural Development Programme (ADP), Port Harcourt. The fishes were not fed 24 h before introduction into the toxicants and during the experimental period. The experiment was conducted following the procedure in APHA[27].

The fishes were exposed to graded concentrations of the micronutrient fertilizer (1400, 1600, 1800, 2000 and

2200 mg L^{-1}) and a control (0.00 mg L^{-1}) in triplicates. After introduction of the desired concentration of the fertilizer into each of the aquarium, it was properly mixed to avoid any hot spots. Ten fish was then randomly added to each of the aquaria containing 30 L solution of the fertilizer with aquarium net. The aquaria were not aerated during the trial. The solutions and water in control were renewed daily. The test lasted for 96 h. Behavioural changes (tail beat frequency, TBF; opercular beat frequency, OBF) and mortality were recorded at 12, 24, 48, 72 and 96 h. Dead fish was removed with an aquarium net to avoid contamination of the solution. A fish was considered dead when it failed to respond to a simple prodding with a glass rod. Other general behavioural changes were also recorded.

Statistical analysis: Data (OBF, TBF and mortality) obtained were analyzed with a two-way analysis of variance (ANOVA). Differences among means within the species were separated at 95% confidence intervals by Duncan's multiple range test (DMRT)[28]. Inter-specific differences were separated with t-test. The lethality, LCs (lethal concentrations, LC_{50} and LC_{95}) of the agrolyser to the fish and median lethal times, MLTs (duration from the time of exposure to the time at which 50 and 95%, respectively of exposed fish died) of the fish were determined by probit analysis, probit model[29].

RESULTS

The pattern of behavioural responses of both species to the agrochemical was similar relative to the exposure duration and concentrations. Although OBF and TBF in both species were inversely related to the exposure duration and concentration within the species, they differed ($p<0.05$) with duration and levels of the toxicant (Fig. 1-4). Mortality of *H. bidorsalis* at the various levels of agrolyser differed, $p\leq0.01$ (Fig. 5) but this was not the case with the hybrid (Fig. 6). The OBF did not differ ($p>0.05$) between the species, but TBF did ($p<0.01$). Cumulative mortality of each and also among the species under agrolyser exposure differed, $p\leq0.01$ (Fig. 6). OBF ($p\leq0.001$), TBF ($p\leq0.05$) and cumulative mortality ($p\leq0.05$) were correlated with species. At the various exposure duration the mortality within and also among the species differed, $p\leq0.01$ (Fig. 6).

The difference between the 24 h LC_{50} for the hybrid and the pure breed was 2109.30 mg L^{-1}. At the 96th h LC_{50}, the difference between the values was 334.92 mg L^{-1}. The 96 h LC_{95} for hybrid was 169.38 units less than that for *H. bidorsalis*

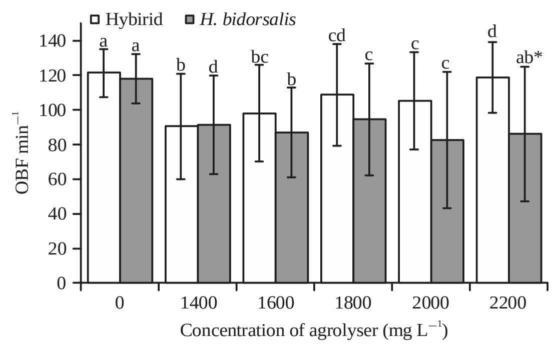

Fig. 1: Opercular beat frequency (OBF) of hybrid catfish and *H. bidorsalis* under lethal concentrations of agrolyser
Each data point, n = 3, bars with similar alphabets in the same species are not significantly different at p>0.05, Error bar = SD, *OBF for the species are significantly different at p<0.05

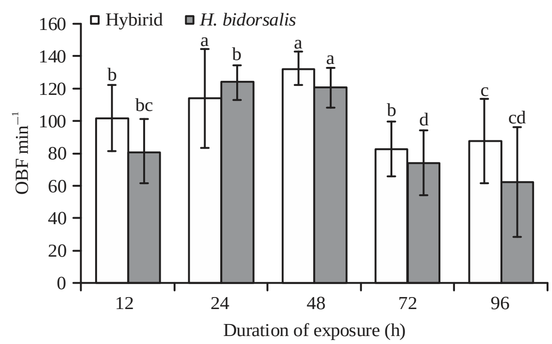

Fig. 2: Opercular beat frequency (OBF) of hybrid catfish and *H. bidorsalis* at the various time intervals under lethal concentrations of agrolyser
Each data point, n = 3, bars with similar alphabets in the same species are not significantly different at p>0.05, Error bar = SD

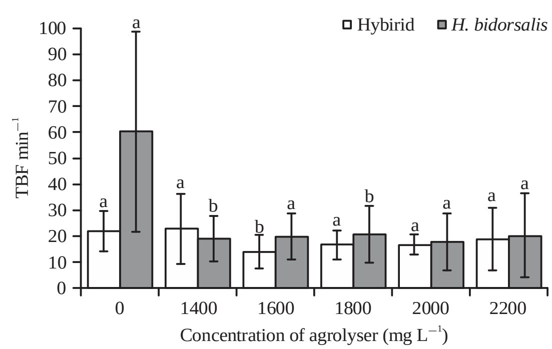

Fig. 3: Tail beat frequency (TBF) of hybrid catfish and *H. bidorsalis* under lethal concentrations of agrolyser
Each data point, n = 3, bars with similar alphabets in the same species are not significantly different at p>0.05, Error bar = SD

(Table 1). The MLT_{50} for the hybrid at 2200 mg L^{-1} was 10.15 h less as against the values recorded for *H. bidorsalis*. The difference in the MLT_{95} between the species was less than a

Table 1: Comparative 96 h lethal concentration (96 h $LC_{50,95}$) of agrolyser on hybrid and *H. bidorsalis* (probit) with associated 95% confidence (lower and upper limits)

	Lethal concentration, LC (mg L^{-1})			
	LC_{50} (CI 95%)		LC_{95} (CI 95%)	
Duration of exposure (h)	Hybrid	*H. bidorsalis*	Hybrid	*H. bidorsalis*
24	2388.41 (2129.85-3503.42)	4497.71 (3590.12-6012)	3718.25 (2959.07-7463.14)	7313.50 (6873.09-9201.63)
48	1874.59 (9152.06-2687.62)	3046.19 (2450.03-2511.77)	3223.08 (2536.32-13950.50)	4575.74 (3251.91-5582.45)
72	1588.21 (1520.06-2687.62)	1980.31 (1851.68-2195.38)	2554.43 (2259.72-4112.96)	2972.48 (2594.50-3995.10)
96	1353.66 (1560.76-1638.68)	1688.58 (1582.69-1776.50)	2357.29 (1991.91-2593.27)	2527.67 (2175.63-2654.56)

Table 2: Median lethal time to death of hybrid and *H. bidorsalis* exposed to acute concentrations of agrolyser (probit) with the associated 95% confidence (lower and upper) limit)

	Median lethal times, MLT (h)			
	MLT_{50} (95% CI)	MLT_{95} (95% CI)		
Exposure concentration (mg L^{-1})	Hybrid	*H. bidorsalis*	Hybrid	*H. bidorsalis*
1400	100.54 (84.94-133.97)	139.08 (104.24-297.62)	182.76 (144.84-278.06)	263.83 (181.41-667.75)
1600	61.30 (46.45-81.07)	105.41 (87.43-147.97)	127.71 (98.98-200.35)	195.66 (136.38-236.48)
1800	52.21 (41.23-63.67)	90.46 (60.37-123.13)	132.12 (108.82-179.30	167.60 (117.21-182.04)
2000	50.85 (37.53-64.62)	72.89 (49.76-80.43)	103.80 (79.99-170.92)	132.90 (98.65-154.65)
2200	40.76 (20.96-56.62)	50.91 (43.11-58.57)	103.30 (79.99-170.92)	104.72 (91.85-125.37)

Table 3: Prediction equations for the relationship between the independent variables concentration of agrolyser and duration of exposure) and the dependent variables (OBF min^{-1}, TBF min^{-1} and cumulative mortality (%))

Independent variable	Dependent variable	Species	Prediction equation	Curve type	R^2
Duration	OBF	*H. bidorsalis*	$y = 0.006x^2+1.64+42.225$	Polynomial	0.9341
	OBF	Hybrid	$y = 0.63+71.5$	Linear	0.9669
Concentration	OBF	*H. bidorsalis*	$y = 87.399 \text{ Ln}(x)-0.4$	Logarithmic	0.962
	OBF	Hybrid	$y = 0.373x = 87.981$	Linear	0.95
Duration	TBF	*H. bidorsalis*	$y = 0.0002x^3 \, 0.0266x^2+0.299x+54.694$	Polynomial	0.9439
	TBF	Hybrid	$y = 0.0025x^2-0.1x+14.75$	Polynomial	0.9123
Concentration	TBF	*H. bidorsalis*	$y = -0.3857x^2+60.857x-266.07$	Polynomial	0.9171
	TBF	Hybrid	$y = 0.0001x^2+6.0934x+12.57$	Polynomial	0.9586
Duration	Cumulative mortality	*H. bidorsalis*	$y = 0.0002x^3+0.0234z^2-0.5094x+7.6062$	Polynomial	0.9925
	Cumulative mortality	Hybrid	$y = 16.579\text{Ln}(x)-33.69$	Logarithmic	0.9838
Concentration	Cumulative mortality	*H. bidorsalis*	$y = 0.4567x-1.0095$	Linear	0.967
	Cumulative mortality	Hybrid	$y = 17.527\text{Ln}(x)-36.015$	Logarithmic	0.9792

y = Dependent variable, x = Independent variable, R^2 = Coefficient of determination

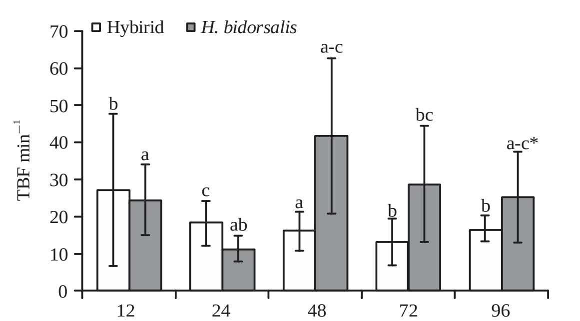

Fig. 4: Tail beat frequency (TBF) of hybrid catfish and *H. bidorsalis* at the various time intervals under lethal concentrations of agrolyser

Each data point, n = 3, bars with similar alphabets in the same species are not significantly different at p>0.05, Error bar = SD, *Cumulative mortality between the species are significantly different, DMRT, p<0.01

unit (Table 2). The prediction equations of the relationship between the independent and dependable variables and the respective R^2 values suggested that the species responded variably to the toxicant (Table 3).

DISCUSSION

The hybrid and *H. bidorsalis* in this study responded to the agrochemical by showing changes in the opercular and tail beat frequencies. The beat frequencies (OBF and TBF) decreased as time progressed and as concentration is increased. Fishes exhibit various adaptive responses to toxic chemicals in the environment to counter their negative impacts so as to maintain equilibrium in the organism[30]. These stress-associated responses in fishes have been classified and

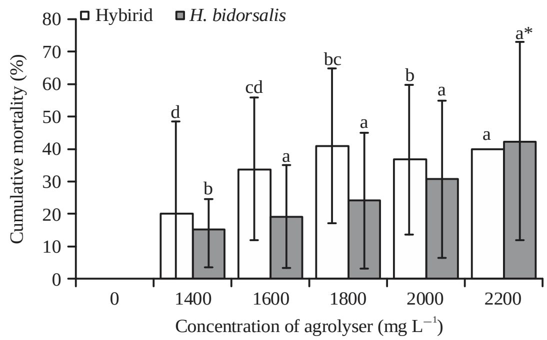

Fig. 5: Cumulative mortality of hybrid catfish and *H. bidorsalis* under lethal concentrations of agrolyser

Each data point, n = 3, bars with similar alphabets in the same species are not significantly different, p>0.05, Error bar = SD, *Cumulative mortality between the species are significantly different, DMRT, p<0.01

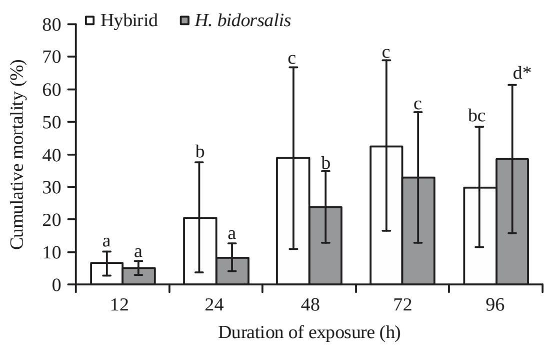

Fig. 6: Cumulative mortality of hybrid catfish and *H. bidorsalis* at the various duration under lethal concentrations of agrolyser

Each data point, n = 3, bars with similar alphabets in the same species are not significantly different, p>0.05, Error bar = SD, *Cumulative mortality for the species are significantly different, DMRT, p<0.01

interpreted in various ways[31,32]. The behaviours usually occur in phases: The active coping phase and the passive phase[31]. Physiological responses of fish to environmental stressors may involve initially the neuroendocrine responses such as the release of the catecholamines from the chromaffin tissues[33] and the stimulation of the hypothalamic-pituitary-interrenal axis which culminates in the release of corticosteroid hormones into circulation[34,35]. Furthermore, the authors noted that the final response included changes in the plasma levels and tissue ions, metabolic level among others, which relate to several aspects of physiological adjustment. These changes may explain the various concentration and duration-dependent behaviours manifested by the fishes under the effect of the toxicant in this study.

The pattern of the OBF and TBF of the exposed fish and death resulting from exposure of the fish to agrolyser concentrations, may be explained by the so-called classic stress response (physiological response). Under toxicant stress there is a strong surge of catecholamines, adrenaline and/or noradrenaline[36]. Elevated catecholamine mediates optimization of the cardio-vascular respiratory functions and a mobilization of energy reserves[36,37]. This results are an increased heart and ventilation rate, leading to higher and faster oxygen and metabolite delivery to the tissues to meet the increased demand. In response, the glycogen stored is mobilized[36]. The hypoxic internal condition created by the poison may have led to an increased energy supply during which the OBF and TBF increased gradually and peaked. But with continued depletion in available oxygen and energy store in the medium, the beats gradually declined. This was also reflected in the pattern of the general behavior of the exposed fishes.

Differences in the behavioural characteristics between the species under agrolyser exposure may be associated with the consistent physiological and neuroendocrine variations which may range from avoidance to aggression and eventually death[30,31]. Besides, the differences may be genetically-mediated because of differences in the brain monoaminergic functions[38] and the endocrine system[39]. The stressful behaviours exhibited by the fishes at the various concentrations of the fertilizer have similarly been reported in some *clariids* and *O. niloticus*[40], *Channa striatus* [41] and *Lebistes reticulates*[42] treated with various fertilizer effluents and *T. guineensis*[18] exposed to inorganic fertilizer. The authors attributed the behavioural changes exhibited by the exposed fishes to respiratory impairment and inhibition of general body metabolism by the active components of the fertilizer and wastewaters. The behaviours exhibited by the fishes under agrolyser exposure is definitely caused by similar reasons as those indicated by the authors in their studies[12,18,40,41].

The comparative LC_{50} values for the species suggested that the hybrid was more susceptible to the agrochemical than the pure breed. This indicated that tolerance to agrochemical like the fertilizer is not an advantage in the hybridization of this catfish species. However, the time to death (MLT_{50}) at 2200 mg L^{-1} for *H. bidorsalis* was about ten units higher than that of the hybrid, but the time to death of 95% of both species was the same. This indicated that although the fishes have differential tolerance to the fertilizer, nonetheless the time to death may not differ after all. Death of the exposed fishes may have resulted from the interference of the toxicant with normal functioning of the nervous system, which impaired the normal muscular activity and co-ordination[42]. Toxicant-induced death may also result from failure in gill functions[43]. Impaired gill function may result from direct damage to its architecture and/or increased production of mucus by mucus cells in reaction to irritation by the toxin[44]. Disruption of gill architecture and increased mucus production may reduce available surface for gaseous and ionic exchange. Impaired gill function under a hypoxic and anorexic internal environment may exacerbate the lethal effect of the agrochemical leading to death[45].

The 96 h LC_{50} values for the hybrid and *H. bidorsalis* were 1353.66 and 1688.58 mg L^{-1} compared to 6 mg L^{-1} the recommended rate. The *clariids* are very tolerant to the micronutrient fertilizer and hence it could be safely applied in ponds to boost yields from fish farms. However, this should take into consideration environmental factors which may modify the toxicity of the agrochemical.

CONCLUSION

The fish species showed same behavioural pattern towards the agrochemical at the different concentration levels. The *H. bidorsalis* showed more resistance to the toxicant than the hybrid catfish. The variables (TBF, OBF and mortality), showed a concentration and duration-dependent response. However, OBF and TBF showed an inverse relationship, while mortality showed a direct relationship with concentration and duration. Although, the concentration that produced the LC_{50} for each of the species were very low when compared to the recommended application rate, yet caution should be taken in applying this agrochemical/micronutrient in fish ponds. This is because of the possible bioaccumulation and toxicity of heavy metals in fish and man the final consumer.

SIGNIFICANCE STATEMENTS

This study has discovered that at very high concentrations, agrolyser can be injurious to the hybrid catfish and *Heterobranchus bidorsalis*. This study will help researchers to uncover the critical areas of application of agrolyser to different fish species and their hybrids. This will introduce new concept on fish farm fertilization that many researchers and fish farmers were not able to explore. Thus a new theory may be arrived that fish fertilizers affect breeds differently.

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