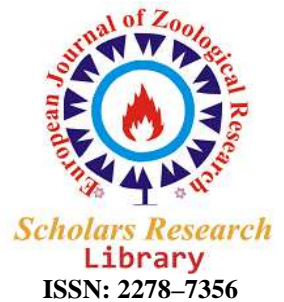




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### Environmental parameters for the production of juveniles African bonytongue, *Heterotis niloticus* (Cuvier, 1829) in rice-fish pond

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#### ABSTRACT

Rice and fish are for one, the staple food and the other, the main source of animal protein for Ivorian consumer. But the country is not self-sufficient in these two products. To ensure food security for its population, the Ivorian government emphasizes local production of rice and freshwater aquaculture. Rice-fish farming which is to combine or alternate rice and fish farming can help solve this problem. An experiment was conducted at the Fish Farming Research Station of Bouaké (Ivory Coast, West Africa) to study the environmental parameters of the juveniles *Heterotis niloticus* production in association with rice, with the aim of improving this farming practice in Ivory Coast. The experiment was carried from July to November 2013 with 440 larvae of *H. niloticus* and 19 200 15-day-old rice seedlings (*Oryza sativa* L., var. WITA 12) transplanted at 20 cm x 20 cm spacing. Water physical parameters were more or less equal in all treatment except dissolved oxygen with lowest values in rice-fish treatment which also displayed the highest temperature. The contents of mineral nutrients and growth rate and yield are higher in rice-fish treatment. This work shows that the production of juveniles *H. niloticus* is possible in rice-fish culture with a better comprehension of its ecological environment in order to improve its contribution to food security.

**Key words :** Ecological environment ; *Heterotis niloticus* ; juvenile production ; Rice-fish culture; Ivory Coast.

#### INTRODUCTION

Fish provides 22% of the protein intake in sub-Saharan Africa [1]. This share, however, can exceed 50% in countries where other sources of animal protein are scarce or expensive. In Côte d’Ivoire, fish is the main source of animal protein of the population with an average consumption of 13.2 kg / capita / year covered at 67% by imports [2]. The main reason is the upper limit of capture of fisheries production and the growing population. Since fishing products cannot meet the demand for fish, aquaculture will have to play a crucial role. Indeed, in 2001 the fisheries and aquaculture accounted for 3.1% of agricultural Gross Domestic Product (GDP) and 0.74% of total GDP [3]. Presently, Ivorian fish farming is based mostly on the breeding of *Oreochromis niloticus*. Unfortunately results obtained on fish ponds are very low. It is therefore necessary, on one hand, to intensify and integrate the actual techniques of *O. niloticus* breeding, and, on the other hand, to develop the domestication of species with high aquaculture potentialities. One of the species with remarkably high growth rate, air-breathing characteristic, omnivorous diet and good market potential is *Heterotis niloticus*, a species belonging to the Arapaimidae family [4]. Also, the food security in Côte d’Ivoire cannot be achieved without addressing the needs of rice. Rice is the staple food of Ivorians but the country is not self-sufficient. In 2006, imports of milled rice were 850 000 tones corresponding to 61.19% of the national consumption [5]. The inability of domestic production to meet the needs of local consumption leads to import large quantities of rice. According to, Côte d’Ivoire was in 2006 the seventh importer of rice in the world [6].

In this situation, rice-fish culture is an interesting alternative. According to [7], rice-fish farming performed in the right conditions has proven to be economically viable to rice or fish monoculture. Therefore, it is necessary to promote the expansion of this cultural practice which combining or alternating rice with fish farming in lowland ecology. This will greatly help to improve the production of rice and fish. Indeed, the shallows with an important agricultural intensification potential can contribute to food security and provide an alternative to land pressure and climate change. This rice-fish integration currently initiated in the central west Côte d'Ivoire is an important issue for the country, as these two components (rice and fish) are the major axes of the national food security policy. The success of this integrated system requires not only an appropriate infrastructure such as field depth, irrigation facilities and available fingerlings [8; 9], but also an elevated educational level of the farmers, who need skills in rice cultivation as well as fish management [10]. Systematic knowledge on the activity pattern of fish in rice fields is lacking to date, especially concerning juveniles of the very interesting African bonytongue, *Heterotis niloticus*. This is crucial for understanding their role in this ecosystem as well as their potential as biocontrol agents of rice pests. The present study was performed to evaluate various environmental effects of growth and yield in a rice-fish simultaneously under controlled conditions.

## MATERIALS AND METHODS

### *Experimental design*

The experiment was carried from July to November 2012 at the Fish Farming Research Station of the National Agricultural Research Center in Bouaké (Center of Côte d'Ivoire). The study was conducted in six randomly selected ponds in three treatments with two replicates. First, the seedlings in nursery were sown in a 400 m<sup>2</sup> pond. Then, 19 200 15-day-old rice seedlings (*Oryza sativa L.*, var. *WITA 12*) were transplanted into experimental ponds at 20 x 20 cm spacing and submerged progressively with water depending on the size of the plants. The water level was maintained constant at a 50-cm level using cistern valves. (Fig.1).



Fig 1: a) Rice Nursery; b) Transplanting Rice; c) pond gradually flooding; d) rice seedling

Fish with an average weight of  $3.18 \pm 1.37$  g were stocked one month after flooding at a density of 0,275 fish per m<sup>2</sup>, equivalent to 440 larvae (110 per pond) of *Heterotis niloticus* (Fig 2). There was no supplementary feeding.



Fig 2: *Heterotis niloticus* (CUVIER, 1829) (Arapaimidae, Osteoglossiformes)

The ponds were allotted to three treatments with two replicates each and arranged in a randomized complete block design. Three 1 x 1 m plots were delineated at the water inlet, middle and water outlet in each rice pond. The treatments were (1) rice (*Oryza sativa L.*, var. *WITA 12*) combined with fish (*Heterotis niloticus*) compared to (2) fish without rice and (3) rice only (Fig. 3).

### *Monitoring of the water ecology*

Water ecological parameters were regularly monitored throughout the rice growth period. Thus, Water temperature (°C), pH, conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), dissolved oxygen ( $\text{mg}\cdot\text{l}^{-1}$ ) and turbidity level (NTU) were measured fortnightly from 6:30 to 8:00 am in each pond using a multiparameter apparatus *WTW 840i* and an electronic turbidimeter *AQUALYTIC*. In addition, water samples were collected in polyethylene bottles and stored at a temperature below 4 °C. These were analyzed for dissolved inorganic nitrogen [nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ )] and orthophosphate ( $\text{PO}_4^{3-}$ ) according to the standard spectrophotometric method (AFNOR 93). For these ions, only two samples were analyzed (3rd and 5th fortnight).

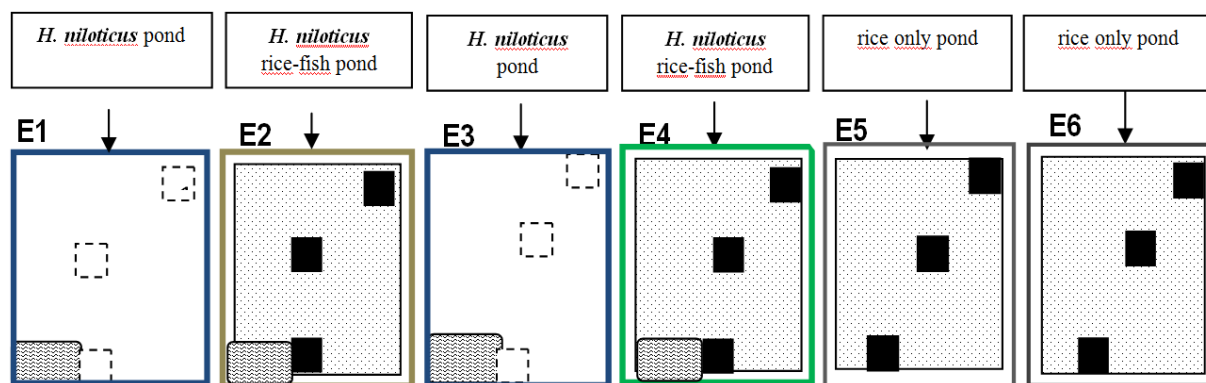


Fig 3:Diagrams of the experimental design

#### **Biotic parameters**

Biotic parameters were also sampled at fortnightly intervals and concerned fish, rice, phytoplankton, zooplankton and benthic macroinvertebrates. Plankton was collected by filtration of 30 liters of water in plankton net and identified under stereomicroscope using specialized literature [for phytoplankton; [11; 12; 13; 14] and [15; 16] for zooplankton]. Benthic macroinvertebrates were sampled using a long-handled scoop (20 cm diameter, 0.5 mm mesh size) for 10 minutes. The different samples were labeled and transported to the laboratory. [17; 18] were used to identify snails and [19; 20] for insects.

#### **Measurements of growth and yield**

Rice growth was monitored at 15, 30, 45, 60, and 75 Day After Flooding (DAF). This was done by measuring the plant height of all rice plants on a 1-m<sup>2</sup>-sub-plot, accompanied by counting the number of tillers. Rice was harvested 120 days after transplanting. The number of panicles per m<sup>2</sup> was first counted, followed by a separate harvest of rice grain and straw. The collected fractions were sun-dried and weighed. Rice Yield was calculated by dividing the dry biomass per hectare ponds.

$$\text{Rice Yield (kg/ha)} = \text{Production (kg)} / \text{surface (ha)}.$$

Fish were harvested by draining off the water at 124 DAF, which was 4 days prior to the rice harvest, measured (standard length) and weighed.

#### **Evaluation of the fish growth performance**

Average Weight Gain (AWG) and Specific Growth Rate (SGR) were calculated according to [21] as:

$$\text{AWG (g)} = M_f - M_i; \quad \text{SGR (\%/i)} = [\ln(M_f) - \ln(M_i)] \times 100 / D$$

where  $M_f$  is the final fish body average mass (g),  $M_i$  is the initial fish body average mass (g), and  $D$  is the duration of the experiment (days).

The condition factor  $K$  was computed as:  $K = W/L^3$

where  $W$  is the weight (g) and  $L$  is the standard length (cm)

The Survival Rate (SR) was also determined as:  $SR (\%) = (N_f - N_i) \times 100$

where  $N_f$  is the final number of fish and  $N_i$  the initial fish number

#### **Plankton and benthic macroinvertebrates.**

The assemblage's structure was studied through the species richness ( $S$ ) and the Shannon-Wiener diversity index ( $H'$ ).

#### **Statistics and graphics**

The analyses were carried out using the statistical software *XLSTAT 7.5.2 Excel* (Microsoft Office 2007) and *STATISTICA 7.1*. The Shapiro-Wilk test was used to test the normality of the parameters measured. For large samples (> 30), the comparison of means was made with the Student  $t$  test at significance level  $p = 0.05$ .

Non-parametric Kruskal-Wallis test (multiple comparisons) and Mann-Whitney *U* test (comparison of two samples) were performed to check differences between samples and results were presented by boxplots.

## RESULTS

### Water ecology

Some water physical parameters are shown in table 1. It is observed that there are great fluctuations in the Water temperature ( $26.6 \pm 0.44$  °C) which were higher in fish ponds compared to rice-fish ( $25.8 \pm 0.74$  °C). While pH values fluctuated between 6.1 to 7.3 and statistically significant differences occurred between the treatments. The highest pH was found in the fish only ponds, followed by the rice-fish and rice pond. Dissolved oxygen concentration showed great fluctuations ( $1.89 \pm 0.67$  to  $4.74 \pm 0.99$  mg.l<sup>-1</sup>) during the experiment. The lowest value in dissolved oxygen concentration was observed in the two rice-fish treatments, which however displayed the highest conductivity values (average  $288$   $\mu$ S.cm<sup>-1</sup>). There were no significant differences between turbidity over all treatments. There was no significant difference between treatments for turbidity (12.1 to 17.4 NTU).

**Table 1 Physical parameters of water in experimental ponds** *Alphabetical letters indicate a significant difference ( $p < 0.05$  ; Mann-Whitney two samples comparison); there is no significant difference between the box having the same alphabetical letter ( $p > 0.05$ ).*

Milieux	pH	DO (mg.l <sup>-1</sup> )	Cond ( $\mu$ S.cm <sup>-1</sup> )	Température (°C)	Turbidity(UTN)
Fish only	$7.1 \pm 0.2^a$	$4.74 \pm 0.99^a$	$247.5 \pm 11.2^a$	$26.6 \pm 0.44^a$	$22.25 \pm 2.9^a$
Rice-fish	$6.7 \pm 0.1^b$	$1.89 \pm 0.67^b$	$288.4 \pm 12.8^a$	$26.2 \pm 0.52^a$	$20.01 \pm 5.24^a$
Rice only	$6.6 \pm 0.1^b$	$3.00 \pm 0.26^b$	$238.3 \pm 15.2^a$	$25.8 \pm 0.74^a$	$18.3 \pm 4.46^a$

The concentrations of nutrient compounds from water in the different treatments are given in Table 2. The contents of dissolved inorganic nitrogen are higher in rice-fish treatment than in the other two treatments with respectively  $7.11 \pm 1.02$ ,  $41.91 \pm 6.71$  and  $171.1 \pm 23.9$   $\mu$ g.l<sup>-1</sup> for NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>2-</sup> and NH<sub>4</sub><sup>+</sup>. Rice only treatment displayed lowest content of orthophosphate ( $0.18 \pm 0.05$   $\mu$ g.l<sup>-1</sup>).

**Table 2: Chemical parameters of water in experimental ponds** *Alphabetical letters indicate a significant difference ( $p < 0.05$  ; Mann-Whitney two samples comparison); there is no significant difference between the treatments having the same alphabetical letter ( $p > 0.05$ ).*

	NO <sub>2</sub> ( $\mu$ g.l <sup>-1</sup> )	NO <sub>3</sub> <sup>2-</sup> ( $\mu$ g.l <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> ( $\mu$ g.l <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> ( $\mu$ g.l <sup>-1</sup> )
Fish only	$5.09 \pm 1.87^a$	$27.94 \pm 2.82^a$	$88.45 \pm 16.55^a$	$0.71 \pm 0.2^a$
Rice-fish	$7.11 \pm 1.02^b$	$41.91 \pm 6.71^b$	$171.1 \pm 23.9^b$	$0.64 \pm 0.12^a$
Rice only	$5.33 \pm 1.92^a$	$20.96 \pm 1.73^a$	$129 \pm 41.05^a$	$0.18 \pm 0.05^b$

### Benthic macroinvertebrates

Benthic macroinvertebrates collected during the experiment was mainly constituted by snails and insects (Table 3). Ponds generally had a low molluscan diversity with six species belonging to four families (Ampullariidae, Thiariidae, Lymnaeidae and Planorbidae). The number of species was higher in fish only ponds (6 taxa) than rice (5 taxa) and rice-fish treatment (4 taxa) but differences were not significant. The Shannon diversity index (*H'*) is greater in fish ponds than in others suggesting a better organization of the settlement. The aquatic insect recorded belongs to seven orders (Coleoptera, Diptera, Odonata, Ephemeroptera, Heteroptera, Megaloptera and Plécoptera). The number of taxa and the Shannon diversity index were higher in fish ponds but the differences were not significant.

**Table 3: Macroinvertebrates occurrences. Absent (-); rare ( $\pm$ ); common (+); very common (++)**. met: *melanoïdes tuberculata*, bip : *Biomphalaria pfeifferi*, pia: *Pila africana*, lyn : *Lymnaea natalensis*, bug : *Bulinus globosus*, buf : *Bulinus forskalii*, col : Coléoptères, dip : Diptères, odo : Odonates, eph : Epheméroptères, het : Hétéroptères, meg : Mégaloptères, ple : Plécoptères.

Treatments	Macroinvertebrates-occurrence distribution												
	meb	bip	pia	lyn	bug	buf	col	dip	odo	eph	het	meg	ple
Fish	++	++	++	++	$\pm$	-	-	-	++	+	++	+	-
Rice-fish	++	++	+	++	+	$\pm$	$\pm$	$\pm$	++	$\pm$	++	$\pm$	-
Rice	++	++	-	++	-	$\pm$	-	$\pm$	++	+	++	$\pm$	+

### Plankton

A total of 22 planktonic taxa (19 phytoplankton and 3 zooplankton) were recorded (Table 3). 19 Phytoplankton belonging to four phyla were identified: chlorophyte (7 taxa), Euglenophyte (4 taxa) Cyanobacteria (7 taxa) of Bacillariophytes (1 taxon). Zooplankton was composed of cladocerans, copepods and rotifers. There was no significant difference between the number of taxa in the different ponds but the fish ponds harbored more taxa than other treatments.

Table 4: Plankton occurrences of planktonic taxa: Absent (-); rare ( $\pm$ ); common (+); very common (++)

	Taxa	Fish	Rice-fish	Rice
Chlorophytes	<i>Micrasterias</i>	-	-	+
	<i>Pleurotaenium</i>	$\pm$	-	-
	<i>Cosmarium</i>	$\pm$	$\pm$	-
	<i>Pediastrum</i>	++	-	$\pm$
	<i>Scenedesmus</i>	++	++	+
	<i>Spirogyra</i>	-	$\pm$	$\pm$
	<i>Tetraedron</i>	$\pm$	$\pm$	$\pm$
Euglenophytes	<i>Phacus</i>	++	++	++
	<i>Euglena</i>	++	-	$\pm$
	<i>Strombomonas</i>	+	+	-
	<i>Trachelomonas</i>	+	+	$\pm$
Cyanobactère (cyanoschizophytes)	<i>Chrocooccus</i>	+	+	$\pm$
	<i>Coelospharium</i>	$\pm$	$\pm$	$\pm$
	<i>Cyanosarcina</i>	$\pm$	$\pm$	+
	<i>Merismopodia</i>	++	$\pm$	$\pm$
	<i>Microcystis</i>	+	+	+
	<i>Oxillatoria</i>	-	$\pm$	-
Bacillariophytes	<i>Pseudanabaena</i>	+	+	-
	Diatomées	++	++	++
Zooplankton	Cladocères	+	+	+
	Copépodes	++	+	++
	Rotifères	++	+	++

### Growth and yields

A graphic illustration of rice height development according to the position of the plots in the pond and the treatment is given in Fig. 4. Rice plants in the plots at the water outlet (near monk) exhibited the strongest growth regardless treatments, while the shortest are measured at the water inlet in fish ponds and at the middle in rice ponds. Mann-Whitney *U* test showed no significant differences between the tillers of plants among treatments.

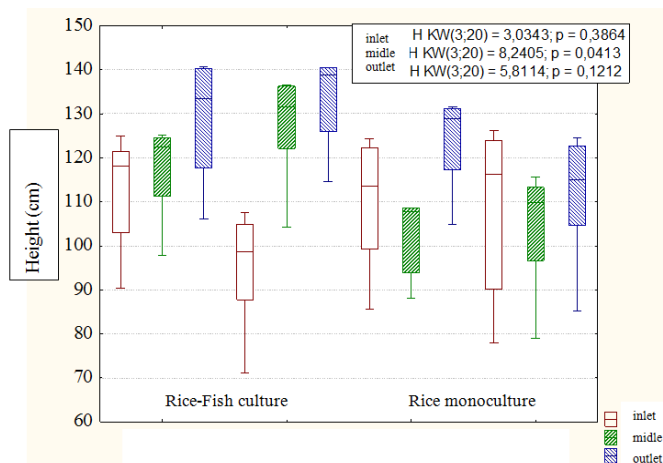


Fig 4: Boxplots representing variations in the height of the rice plants

Some rice yield variables are summarized in Table 5. Average grain yield was the highest in the rice-fish treatment (1884 kg.ha<sup>-1</sup>) and the lowest in the rice treatment (918 kg.ha<sup>-1</sup>), but the differences were not statistically significant. The survival rate of *Heterotis niloticus* larva is very low in the two treatments (fish and rice-fish) less than 50%. The Mann-Whitney test shows that this rate is significantly lower in rice-fish (38.18%) compared to fish only culture (47.27%). However we have noted no significant differences of Specific Growth Rate among treatments. The highest value (115.35 g.day<sup>-1</sup>) occurred in the rice-fish pond which displayed the lowest survival rate and the physiological conditions were similar in the both treatments. Overall, we note that the average weight gain of fish evolved inversely to survival rate of the treatments.

Table 5: Production parameters of rice and fish in different treatments

Production parameters	treatments	
	Fish monoculture	Rice-fish
Paddy yield (kg.ha <sup>-1</sup> )	918	1884
Survival (%)	47.27	38.18
Growth rate (g.day <sup>-1</sup> )	102.14	115.35
Condition factor	0,04 $\pm$ 0,002	0,05 $\pm$ 0,002

## DISCUSSION

Rice-fish ponds displayed highest temperature and lowest dissolved oxygen in accordance with the finding of [22; 23] who say that fish used in fish culture must be tolerant to high temperatures and low oxygen content. The main factors that affect fish and other animals in the rice fields are the water level, temperature, dissolved oxygen, acidity (measured as pH) and un-ionized ammonia ( $\text{NH}_3^+$ ). The hardy species that are selected for livestock management must withstand variations in characteristics such as dissolved oxygen critical parameters whose values are recorded in the early morning. A major source of dissolved oxygen in the water column oxygen is formed by the photosynthetic activity of aquatic plant biomass can lead to extreme mid afternoon saturation, though, during the night, the oxygen is consumed by respiration of plants. Thus, with the breath from the animals as well as bacteria and oxidation processes, anoxic conditions occur during the night and the period before the dawn [24]. The high acidity of ponds containing rice is due to the fact that respiration uses oxygen and produces carbon dioxide ( $\text{CO}_2$ ) which, when dissolved in water forms carbonic acid ( $\text{H}_2\text{CO}_3$ ), dissociates into bicarbonate ( $\text{HCO}_3^-$ ), and carbonate ( $\text{CO}_3^{2-}$ ). This results in the release of hydrogen ions ( $\text{H}^+$ ), increases the acidity of the water and to lower pH. Atmospheric  $\text{CO}_2$  through natural diffusion and agitation on surface water, and the decomposition of organic matter are other important sources of carbon dioxide. However, the removal of  $\text{CO}_2$  from the water due to the photosynthetic activity leads to increased hydroxyl ions ( $\text{OH}^-$ ) and increased the pH of the water.

The level of dissolved oxygen and pH of the water in a rice field are positively correlated to the extent that the concentration of dissolved oxygen is largely a result of the photosynthetic activity that consumes carbon reducing  $\text{CO}_2$  dissolved (and thereby the concentration of  $\text{H}^+$ ), actually increasing the content of both pH and dissolved oxygen. Conversely, the content of these two elements is reduced when breathing is at its highest rate [24].

Survival rates below 50% found support the conclusion of [25] which states that the loss of fry during the first four months are usually significant in *Heterotis niloticus*. This low rate could also be explained by the following factors: a massive presence of frogs and predatory birds in the ponds. Similar work done by [26] and FAO in Mali from 1987 to 1989 gave the same conclusion that frogs are large predatory fish [27]. This survival rate significantly lower pond fish culture could be due to the seed density too high. The average weight of fish culture ponds significantly higher confirmed work of [23] who said that rice-fish farming undoubtedly accelerates the growth phase of the fish to obtain yields and higher average weights. Also very small pond fish culture survival would have to remain to have more food fish. The yield of rice not significantly different treatment justifies the work of [23]. It concluded the first test does not give significantly higher performance and believes that with the accumulation over the years, fish feces, soil fertility is improved and allows, as has been found in Asia, a significant increase in rice yields. Furthermore, fish, feeding on aquatic larvae directly involved in biological control against pests. In addition, feces from fish act as organic fertilizer for rice. Also, the movement of fish in rice fields leads you there stirring movements that stir up the bottom and release the nutrients from the sediment [28]. Finally, the fact of maintaining the ponds water prevents the growth of weeds [23]. The presence of macro-invertebrates and plankton in different ponds is an important food source for fish. Indeed, studies by [29; 30] in Chad gave the following conclusions. *H. niloticus* is primarily a predator feeding to animals consuming the expense surface organic film (aquatic insect larvae, ostracods, molluscs, mainly). The percentage of occurrence of the prey is still high and the volume percentage is greater than 60%. If fish and shrimp seem to be accidental prey (5.0 and 10.0%), zooplankton and seeds are often used (25 and 75%), these two categories represent 26.0% of the ingested volume. Predation exerted on aquatic insect larvae concerns mainly chironomid larvae (Diptera) (17.0%), other insects are eaten Ephemeroptera and Trichoptera. Ingested molluscs have a very small size (from 1 to 8 mm). According to [31] *H. niloticus* is omnivorous and eats plants (36.0%), aquatic insects (22.0%), mud and other debris (17.2%), zooplankton (15.2 %) and benthic invertebrates (9.6%). Juveniles feed on zooplankton and the massive mortality of larvae during the first day post-hatching makes them difficult to farm [32].

## CONCLUSION

We concluded that it is possible to produce juveniles *Hétérotis niloticus* in rice-fish environment. This technique allows for better growth rate for fish and better rice yield but massive mortality of larvae during the first day post-hatching remains a major obstacle.

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