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Potential inert diets to supplement *Artemia* in larviculture of the giant Africa river prawn *Macrobrachium vollenhovenii* (Herklots, 1857) (Crustacea: Palaemonidae)

Abstract

The protocol of culture of *Macrobrachium vollenhovenii* the main indigenous candidate for freshwater prawn culture in Africa is still under study. Though just few information exists, the transition of larvae from stage V to stage VI has been reported as the critical rearing period in larviculture. This study was to evaluate the efficiency of two locally diets to supplement *Artemia* in the feeding scheme from stage V to post larvae in the larviculture of this species. The two experimental diets were differentiated by the main source of protein: fish silage (Diet 1) and shrimp meat (Diet 2). One batch of larvae was cultured till stage V. The experiment itself was conducted in triplicate with three treatments: feeding *Artemia* exclusively (TA, control); fed partial replacement of *Artemia* with inert diet 1 (T1) or fed partial replacement of *Artemia* with diet 2 (T2). Larval development in T2 was significantly faster than TA and T1. Survival rate was significantly higher in T2 (12.64±1.2%) than TA (6.57±0.29%) and T1 (6.77±0.17%). The total length of larvae in T2 was significantly higher than TA and T1. Though the highest survival obtained in this study is still low, it's however higher than those reported in other studies with this species. Also, the importance of finding alternatives to *Artemia* and cheaper diets remains very important.

Keywords: *Artemia*, inert diets, larval rearing, *Macrobrachium vollenhovenii*, survival

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Introduction

Prawns of the genus *Macrobrachium* are decapod crustaceans that have been identified globally in terms of their economic importance and aquaculture potentials.[1] Species of this genus are found in most inland waters such as ponds, lakes, rivers and irrigation ditches, as well as estuaries[2] and constitute a large proportion of macro-invertebrates of high economic value.[3] Among them, the Asia species *M. rosenbergii* has received more attention. It is the major commercial species and has been imported in many other areas of the world for culture purposes.[2]

In Africa, *M. vollenhovenii* is the largest freshwater prawn and the main target species for fisheries and freshwater prawn aquaculture of the continent. It is a very important food item and supports artisanal fisheries in many countries in West Africa sub-region.[4] Though many reports have shown a progressive decline in the wild stock of this species,[5,6] very few studies have been done on its culture aspects and no industrial farm of *M. vollenhovenii* exist. Willführ-Nast et al.,[7] examined rearing salinities and recommended salinity 16 part per thousand (ppt) for larviculture of this species. Dzidzornu[8] investigated hatchery and nursery operations for culture of *M. vollenhovenii* in Ghana. In Cameroon, Makombu et al.,[9] studied the larval development of six batches of larvae of this species and found in all the batches the 11 distinct larval stages as described for *M. rosenbergii*. However, the time of appearance of the first post larvae was very variable between batches (41-74 days) as well as the survival rate (3-9%) and high mortality of larvae in all the batches occurred in the transition from stage V to stage VI, which was also very long than the passage to others stages. For *M. rosenbergii* for which the larviculture is long established, larvae take 16 -22 days to metamorphose to post-larvae with a survival rate which is also variable but can be as high as 80%.[10] Studies on improvement of hatchery conditions of *M. vollenhovenii* are therefore urgently needed.

It is well known that the duration of the larval cycles and the survival rate depend on quantity and quality of food, water temperature and other water quality, light, genetics of the stock used and skill of the operator.[2] According to Girri et al.,[11] the availability of suitable diets that are readily consumed, efficiently digested and that provide the required nutrients to support good growth and health is the key factor for successful larviculture. Under culture conditions, *M. rosenbergii* feed mainly on live feed *Artemia* nauplii throughout the larval cycle.[12] In all the studies done on larviculture of *M. vollenhovenii,* larvae are fed *Artemia* nauplii exclusively. Athough *Artemia* nauplii is the most important feed for production of shellfish postlarvae and has proven successful for raising larvae of several *Macrobrachium* species, it seems to not cover all the nutritional requirements of *M. vollenhovenii,* particularly after stage V of development. Moreover, it is costly and for several authors, it is nutritionally inconsistent because of the lack of highly unsaturated fatty acids.[2,13–15] In addition, the nutritional quality and physical properties of *Artemia* nauplii are depending on the source and time of harvest of cysts.[16] According

to Murthy et al.,[17] dependence entirely on *Artemia* as feed not only makes hatchery operations expensive, but also unsustainable. Several attempts have therefore been made to replace *Artemia* with inert feed in the larviculture of *M. rosenbergii* and *M. amazonicum*.[17–22]

Such investigation has not yet been done for *M. vollenhovenii*. In the literature, the initial stage at which inert diets can be introduced to larvae of *M. rosenbergii* varies with authors: New and Singholka[23] recommended inert feed from stage III onwards, while Carvalho and Mathias[24] suggested the use of supplementary food from stages IV to V. Dinh[25] and Daniels et al.,[26] recommended diet supplementation from stages V to VI, Valenti et al.,[27] between stages VI and VII and Barros and Valenti[12] from stage VII onwards, while Shailender et al.,[28] suggested supplementation from larval stage VI.

For *M. vollenhovenii,* stage V to VI seems to be the critical periods of larval development[9,29] and larvae may need some specific requirements that can be covered by well formulated inert diet.

This work therefore seeks to investigate the effect of supplementation of *Artemia* nauplii with two locally compounded diets from stage V onwards of larvae of *M. vollenhovenii* on the growth, survival rate and first appearance of post larvae.

Materials and methods

Diet preparation

Two experimental semi-dried inert diets, differentiated with only the main source of protein, one with fish (*Bramabrama*) silage called Diet 1(silage-based diet) and another shrimp meat from *Penaeus monodon* called Diet 2 (shrimp-based diet) were prepared.

Preparation of fish silage

Silage was prepared according to Soltan and El-Laity.[30] The ingredients used in the preparation are presented in Table 1. The flesh and viscera (gonad, liver and intestine) of the fish were collected and minced to a fine paste by kitchen blender and all the other ingredients were added and mixed thoroughly. The resulting mixture was then transferred into 1.5 L plastic bottle and the bottle corked firmly. The content was incubated at room temperature for 15 days. pH was used for monitoring the fermentation process and every two days, 5g of mixture was swirled and mixed with 45 mL of distilled water for pH measurement. When pH stabilized, silage was filled with 30% soya beans and then dried in a closed electrified cupboard with 100W bulbs at a constant temperature of 40° C for 5 days.

Table 1 Ingredients for silage preparation

Ingredients	Quantity (g)
Brama fish	89
Molasses	5
Yoghurt	5
Potassium benzoate	1
Total	100

Shrimp meat preparation

Shrimp meat was prepared from the giant tiger shrimp locally called gambas (*Penaeus monodon*).The flesh was chopped into small pieces and steamed in an electric pot for 10min. After that, it was removed and dried for two days in a closed cupboard equipped with bulbs (100W) to produce heat (45° C) and then minced to a fine powder with kitchen blender.

Diet formulation and preparation

The various ingredients used for the preparation of the two inert diets are presented in Table 2.

Table 2 Ingredients for feed formulation

Ingredients	Quantity of ingredient (g)	
	Diet 1	Diet 2
Dried Silage	70	-
Shrimp powder	-	70
Egg	18	18
Powder milk	5.5	5.5
Yellow corn powder	4	4
Vitamin C	1	1
Vitamin and mineral premix	1	1
Cod liver oil	0.5	0.5
TOTAL	100	100

The ingredients were weighed using an electronic balance (model JA1203, precision. 0.0001g) and homogenized in a Moulinex blender until a smooth consistency was obtained. The diets were cooked in an electric pot for 15 min as in Murthy et al.,[17] cooled and stored at 4°C.

Proximate composition of fish silage, shrimp meat and experimental diets (Table 3) was determined according to the Association of Official Analytical Chemists (AOAC, 2003).

Brood stock collection and larval rearing till stage V

Gravid females of *M. vollenhovenii* used in this study were collected from Mabeta River, South West Region, Cameroon (N 03.98931°; E 009.28682°). They were transported in plastic bags to the laboratory of the Institute of Agricultural Research for Development (IRAD), Limbe-Batoke for experimentation.

When a change of colour of the eggs from orange to grey brown was observed in one of the largest females, she was removed and dried with paper tissue, weighed and sent to plastic hatching tank of 100L, with freshwater filled at 30% and salinity raised to 8ppt by addition of seawater to freshwater. The female was removed from the tank once the larvae had hatched and the salinity was then gradually increased to 16ppt before the newly hatched larvae were removed from the hatching tank and counted volumetrically.

The set up for larval rearing system from stage I to stage V consisted of a single recirculation system of a rectangular plastic culture tank of 500L connected to a sand filter (model SCD400) (30L) which linked to a biological filter container (model CBF-350) (80L) equipped with UV light. The biological filter was connected to a 100L reservoir where a submersible water pump (model HQB-3500) was installed. Larvae were gently collected and stocked in the larval rearing unit at the density of 50 larvae/L and salinity 16 ppt.

Feeding rates were based on the alternative hatchery feeding schedule derived from Correia et al.[31] Larvae were fed from one day after hatching to stage V with newly hatched *Artemia* nauplii (Great Salt Lake strain, Utah, US) exclusively three times daily at 08:00, 13:00 and 18:00 hours at the rate of five nauplii per ml of water.

Test of inert diets

When 90% of larvae were at stage V (day 10 after hatching), the actual experiment was set up. It consisted of three treatments with three replicates per treatments: treatment A (TA) where stage V larvae fed *Artemia* nauplii exclusively (control) at 5 nauplii/mL

of water three times daily; treatments one (T1) and two (T2) were larvae fed respectively silage based Diet 1 and Diet 2 at 8am only and *Artemia* nauplii in the afternoon and evening feeding from stage V to appearance of stage IX and from stage IX to post larvae, larvae fed inert diet two times (morning and afternoon) and *Artemia* nauplii only in the evening feeding at the rate of 5 nauplii per ml of water (Table 4).

Table 3 Proximate analysis (% of dry matter) of the experimental diets

Components	Silage	Shrimp meal	Artemia	Diet 1 (silage based feed)	Diet 2 (shrimp based feed)
Crude protein	54.93±0.88	52.14±1.02	49.33±1.19	44.48±0.28	44.30±0.39
Fat	11.23±0.21	9.32±0.34	18.77±0.55	9.69±0.33	6.36±0.26
Ash	5.80±0.18	5±0.24	7.67±0.37	6.25±0.21	5.07±0.11
Crude fiber				1.01±0.05	1.06±0.08

Table 4 Feeding schedule of *Macrobrachium vollenhovenii* during the experimental period

Stages	TA			T1			T2		
	8am	1pm	6pm	8am	1pm	6pm	8am	1pm	6pm
V-IX	*Artemia*	*Artemia*	*Artemia*	Diet 1	*Artemia*	*Artemia*	Diet 2	*Artemia*	*Artemia*
IX-PL	*Artemia*	*Artemia*	*Artemia*	Diet 1	Diet 1	*Artemia*	Diet 2	Diet 2	*Artemia*

The culture system consisted of three separate and identical recirculation systems of three blue 100L capacity culture tanks each. Culture tanks were filled with 50L of brackish water at 16 ppt salinity and stocked with stage V larvae at the density of 20 larvae/L.

The amount of formulated diets given to each tank was based on visual observation. Special care was taken not to overfeed. Acceptability of the diet was evaluated subjectively based on observations of the feeding behaviour of the larvae and microscopic observation of the diet in the larvae gut. Water was changed in the culture systems at a rate of 10% daily. This experiment ended when first larvae metamorphosed to post larvae in each treatment.

Water quality analysis

The water quality parameters that were monitored included water temperature, salinity, pH, dissolved oxygen, ammonia, nitrite and nitrate of the water in the culture system. Dissolved oxygen was measured using DO meter (model D.O 9100) and temperature measured using Digital aquarium thermometer, pH (PEN type pH meter). The salinity was measured using a portable refractometer while total ammonium concentrations and nitrite were determined by colorimetric tests.

Evaluation parameters

To assess larval development, Larval Stage Index (LSI) was determined every five-day following Maddox and Manzi.[32] For these 30 larvae were sampled from each rearing tank and the average larval stage determined. LSI was then calculated following the below formula.[32]

$$LSI = \sum Si/N$$

Where Si is the stage of the larvae (i = 1 to 11) and N is number of larvae estimated.

At the emergence of each new stage, 30 larvae were randomly selected and observed in the microscope for the determination of the larval stage in order to estimate the percentage transition to the next stage. Percentage transition was calculated by the formula:[29]

$$pt = \frac{Nc}{Nt} X100$$

Where pt= percentage transition, Nc= number of larvae at new stage, Nt = total of larvae sampled.

The total length (from the tip of the rostrum to the tip of the telson) of 10 larvae at the new stage of each tank were also measured using Vernier caliper (0.01mm).

The survival rate was estimated volumetrically after the transition from one stage to the next and at the end of the experimentation, final survival rate was evaluated following the below formula:

$$S = \frac{Nf}{Ni} X100$$

S = Larval survival rate expressed as percentage

Ni = initial number of larvae counted

Nf = final number of larvae countered

Statistical analysis

Data collected were organized in excel spreadsheet and analyzed with two methods: the descriptive and the inferential. The descriptive analysis consisted in calculating averages, standard deviations, percentages, as well as plotting graphs, chat and organizing tables. It was done on Excel 2013. The inferential analysis consisted entitled comparing the averages of treatments for each parameter studied at the level of 95% using ANOVA 1 on SPSS 20.0. When the results were significant, the post hoc Tukey's multiple range tests was used for multiple comparison to clarify the difference between the individual treatments.

Results

pH variation in the silage

The variation of the pH in the liquid silage samples is reported in Table 3. It can be seen from the result that the pH values declined from 5.95 to 4.0 within 10 days of fermentation and remained stable until day 14.

Acceptability of the inert diets

Larvae consumed the inert diets shyly at the beginning of the experiment but after seven days, nearly all larvae immediately consumed inert diet and their gastrointestinal tracts were observed to be full.

Larval development rate

Larval development rate in terms of larval stage index showed significant difference between treatments from day 30 onwards with T2 (shrimp-based feed) showing the highest value (7.57±0.06a)

(Table 6). There was however no significant difference between TA (control) and T1 on day 30 and day 45.

The sequences of appearance of stages from stage V onwards in the three treatments are illustrated by Figure 1. As shown in this figure, the transition between stages V to stage VI was longer than the passage to other stages, especially in TA and T1, where larvae took respectively 9 and 11 days to progress from stage V to stage VI. In T2, the transition from one stage to another was almost one week, except the transition from stage VIII to IX who took just three days. The post larvae first appeared in T2 (51 days), followed by TA (57 days) and T1 (61 days).

The Percentage transition of larvae to the day of emergence of new stage in the three treatments showed similar results between treatments except at the appearance of stage VIII where T2 was significantly different (P<0.05) from TA and T1 (Figure 2).

The length of larvae from stage VI to postlarvae showed significant difference between treatments from stage VII onwards (Figure 3) with larvae in T2 having the highest length while those from TA and T1 were very similar, except at stages VII and X.

The survival rate at the appearance of each larval stage till post larvae showed significant higher survival in T2, while TA and T1 were similar (Figure 4). It also appeared clearly that more than 50% of larvae died between the transitions from stage V to VI while the survival rate in all the treatments was more stable from stage VIII onwards.

The average values of water quality parameters measured during experimentation are presented in Table 5. For the three treatments, temperature, dissolved oxygen, pH and salinity were the same while the control treatment had the least amount of ammonia and nitrite, 0.02 ±0.01mg/L and 0.04 ±0.02 mg/L respectively. However, water-quality parameters of the three treatments were within recommended ranges for freshwater prawn hatchery.[2]

Table 5 pH of fermented product of *Brama brama*

	Fermentation period (days)							
	0	2	4	6	8	10	12	14
pH	5.95	5.03	4.76	4.65	4.14	4	4	4

Table 6 Larval stage index of *Macrobrachium vollenhovenii* larvae in the three treatments (TA, T1 and T2)

	TA	T1	T2
LSI Day 10	4.87±0.05a	4.87±0.05a	4.87±0.05a
LSI Day 15	5.0 a	5.0a	5.0a
LSI Day 20	5.53±0.06a	5.2±0.35a	5.8±0.01a
LSI Day 25	5.97±0.06a	5.8±0.10a	6.56±0.05a
LSI Day 30	6.9±0.10b	6.47±0.16b	7.57±0.06a
LSI Day 35	7.83±0.15c	7.43±0.15b	8.53±0.06a
LSI Day 40	8.26±0.06c	7.47±0.92b	8.93±0.06a
LSI Day 45	8.93±0.06b	8.90±0.0b	10.33±0.15a

Different letters within the row denote significant differences (P<0.05)

Table 7 Water quality parameters in the three treatments (TA, T1 and T2) during experimentation

Parameters	TA	T1	T2
Temperature (°C)	28.17 ± 0.42	28.21 ± 0.52	28.19 ± 0.39
D.O (mg/L)	6.20 ± 0.28	6,23± 0.23	6.19 ± 0.26
pH (range)	7.69 ±0.9	7.72 ±0.7	7.68 ±0.9
Salinity (ppt)	16	16	16
Ammonia (mg/L)	0.02 ±0.01	0.03 ±0.02	0.03 ±0.01
Nitrite (mg/L)	0.04 ±0.02	0.08 ±0.05	0.09 ±0.03

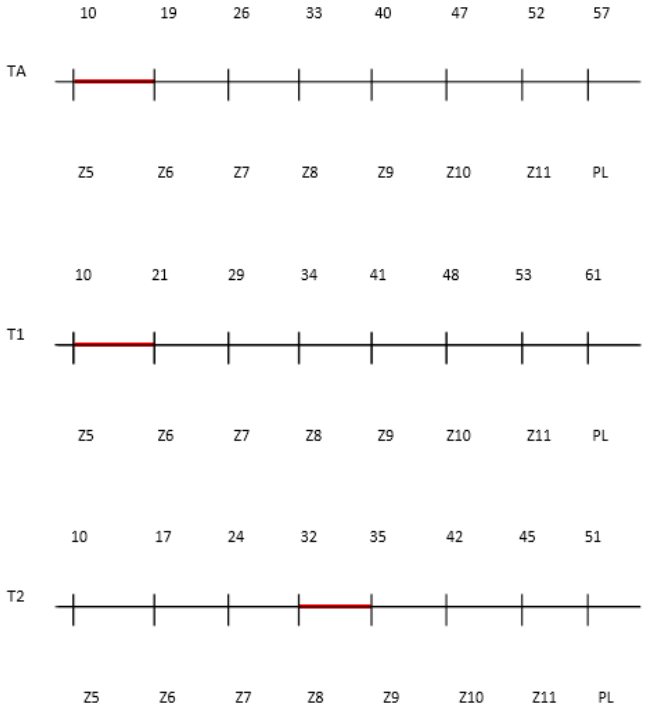

Figure 1 Sequence of appearance of different stage from stage V onwards in the three treatments. TA: control, T1: *Artemia* partially replace with silage-based feed, T2: *Artemia* partially replace with shrimp-based feed.

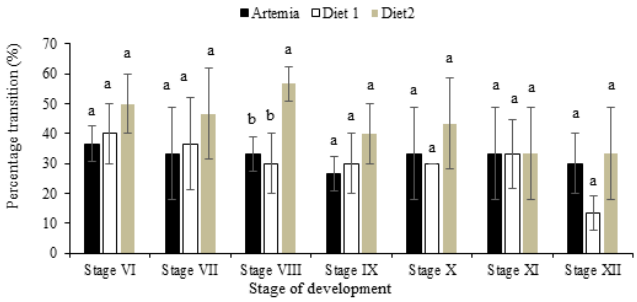

Figure 2 Percentage transition at each developmental stage in the different treatments.

Different letters between treatments in each developmental stage denote significant differences (P<0.05).

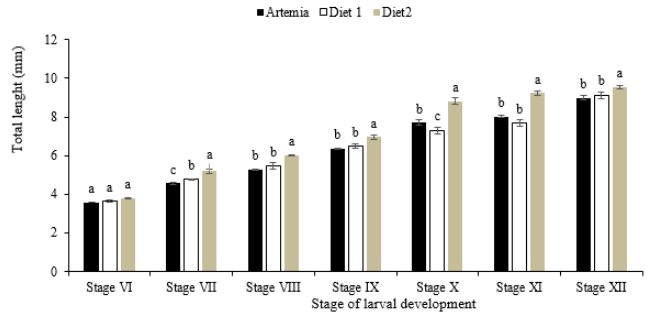

Figure 3 Length of larvae from stage VI to XII (post larvae) in the three treatments.

Different letters between treatments in each developmental stage in denote significant differences (P<0.05).

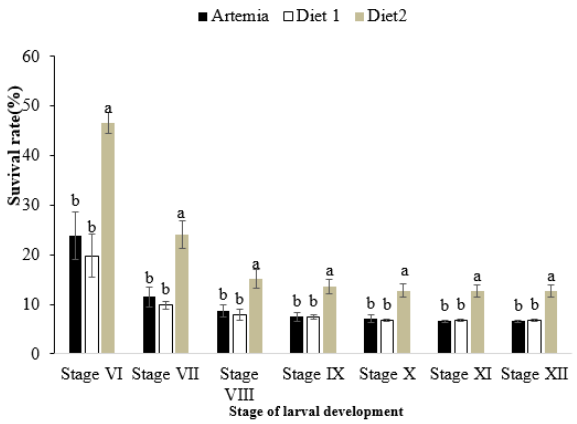

Figure 4 Survival rate from stage VI onwards in the different treatments.

Different letters between treatments in each developmental stage denote significant differences (P<0.05).

Discussion

Feed is the main factor controlling seed production of *Macrobrachium* and constitutes more than 60% of total investment in larval rearing.[33] Till today many hatcheries depend exclusively on *Artemia* as feed throughout the larval cycle, which is according to New,[2,34] a major constraint in the expansion of *Macrobrachium* hatcheries. In the present study, *Artemia* was partially replaced by two inert diets. The main proteins sources in the two diets were cooked to make the nutrients highly bioavailable and easily digestible for larvae. The proximate analysis of Diet 1 and Diet 2 showed no difference in the protein contain. This suggests that both diets had the same quantity of the main element responsible for growth.

Larvae of *M. vollenhovenii* accepted the two inert diets immediately but showed increased acceptance with time. The increase of acceptance may be explained by the changes in the behavior of the larvae. Indeed, according to Barros and Valenti,[35] morpho physiological characteristics of the larvae change during development and before stage VI, mechano reception seems to be the only mechanism used to detect food. Larvae at these stages seem to capture food by chance encounter.[36] The development of the digestive tract and increase of the enzyme activity from stage VI onwards[37,38] can also help to explain the increasing acceptance of inert diet with time, since digestion processes become thoroughly functional.

The results of larval development in term of larval stage index showed T2 (larvae fed partial replacement of *Artemia* with shrimp-based diet) the best while TA (control) and T1 (larvae fed partial replacement of *Artemia* with silage based diet) were more or less the same. This result can be attributed to shrimp meat which is known from a nutritional standpoint to have in addition of high amount of protein, a higher amount of unsaturated fatty acids (HUFA), astaxanthin, feed attractants and certain unknown growth factors.[39] Similar result was reported by Murthy et al.,[17] in larviculture of *M. amazonicum* with the diet containing shrimp meat.

Looking at the timing of appearance of new stage in the three treatments, it appears that larvae in the three treatments used more or less the same time to progress from one stage to another except the transition from stage V-VI and stage VII-VIII, where larvae in T2 took less time to progress, respectively 7 days and 3 days, while the progression from stage V-VI was more longer in T1 (11 days) followed by TA (9 days), with 7 days in both treatment to progress from stage VII-VIII. This suggests that adequate nutrition can reduce the time of progression of larvae from stage V-VI and VII-VIII in larviculture for *M. vollenhovenii*. According to New,[2] the time taken for a larval batch to metamorphose varies according to feeding and environmental conditions.[2] The larvae used in the three treatments were from the same batch and in the same environmental condition. This result suggest then that shrimp meat that was the only difference between T1 and T2, contains a growth factor that could help larvae to progress faster at those two challenges stages. Makombu et al.,[9] reported difficulty to progress from stage V-VI in six larval batches of *M. vollenhovenii* fed *Artemia* exclusively.

The results of the percentage transition of larvae from one stage to another in the three treatments showed no significant difference except the transition from stage VII-VIII where T2 had the highest percentage of stage VIII larvae the day of transition. This result suggests that shrimp based diet in addition of reducing the time span for the transition of larvae from one stage to another had also allow more larvae to progress from stage VII-VIII the first day of appearance of stage VIII.

The length measurement was highest for larvae in T2 from stage VI onwards while there was no significant difference on length measurement for larvae in TA and T1 except in stage VII and X, where TA was higher than T1. This result is in agreement with the findings of Murthy et al.,[17] in larviculture of *M. amazonicum* fed with feed containing high amount of shrimp meat.

Survival in the current study was highest in T2 from stage VI onwards while no significant difference was observed between TA and T1. It was also clear that most of larvae died between the transition from stage V-VI, with percentage mortality very high in TA and T1. This indicates the efficiency of shrimp-based diet and may suggest that *Artemia* alone did not cover the nutritional requirement of stage V larvae of *M. vollenhovenii*. Makombu et al.,[9] also reported high mortality of larvae at their progression to stage V to stage VI of the same species fed *Artemia* exclusively. Though the final survival recorded in T2 is still very low, the fact that TA and T1 had no significant difference in final survival rate suggests that shrimp meat in contrary to fish silage, may have a particular characteristic that contributed to better survival of larvae. We recall that from stage V-VIII only one third (33.33%) of *Artemia* ratio was replaced in T1 and T2 and this small amount made a difference a week after. For larviculture of *M. rosenbergii*, Murthy et al.,[17] suggested that feeding larvae with diet which contains shrimp meat in combination with *Artemia* nauplii showed higher survival than larvae fed *Artemia* exclusively. Many studies reported better survival of larvae of *M. rosenbergii*,[22,40,41] *M. amazonicum*[18,42] fed *Artemia* nauplii supplemented with inert diet.[43–45]

Conclusion

Looking at the general performances of larvae in this study, the three treatments can be distributed into two groups. The best group being T2 where larvae were fed partial replacement of *Artemia* with shrimp-based feed and the other group constituted by TA (*Artemia* nauplii fed exclusively, control) and T1 where larvae fed partial replacement of *Artemia* with silage-based feed. Highest survival recorded with T2 in this work is still very low but remain the best survival recorded with this species in larviculture. Also, the importance of finding alternatives to *Artemia* and cheaper diets remains very important in the feeding strategy of species like *M. vollenhovenii* where the protocol of culture is still in progress, although it is possible that something more radical is required to reduce larval cycle and improve the survival rate of *M.*

vollenhovenii. However, these inert diets should be tested on several batches of larvae before final conclusion can be drawn.

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Author Contributions

JM, JB, JN and JS conceived and coordinated the work. JM, CC, GN, RN MV, CB, ME, AD acquired data. JN, JS, CC, JB, GN, RN and JM analysed and interpreted the data. JM and CC drafted the manuscripts. All the authors contributed to revisions and edits of the manuscript.

Conflicts of interest

All authors declare no competing interests.

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