Embryonic development of *Atya gabonesis* (Giebel, 1875) from River Niger, Jebba, Jebba, Kwara State, Nigeria

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Received: August 2023 Accepted: November 2023

Abstract

Embryonic development of *Atya gabonensis* was investigated at River Niger in Jebba. One hundred (100) broodstock of both sexes of *A. gabonensis* were collected from River Niger, in March, 2020 from fishermen by picking them from under rocks and crevices. The specimens were transported to the laboratory for further studies. Identification to species level was by keys of Schneider. The sexes were determined by features of Anetekhai. The investigation revealed eleven stages of development: In stage I, no embryonic structure was visible; cleavage was evident in stage II; blastula was observed in stage III; in stage IV, gastrula was evident; formation of nauplius at different stages was observed from stage V to XI. The final stage revealed a well developed organism, distinctly segment with cephalothorax and abdomen. It is concluded that *A. gabonensis* completes its embryonic development in freshwater and could serve as good candidate for freshwater prawn culture. This results constitute the first database for embryonic development of *A. gabonensis.*

Keywords: *Atya gabonensis,* Aquaculture, Embryonic development, Freshwater, River Niger, Jebba

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Introduction

Prawns from the Atyidae and Palaemonidae families are important resource for the artisanal fishery (Fransen, 2014). Atya comprises of large number of the family Atyidea occurring in the tropic of West Africa and Americas (ITIS, 2022). Among the species of this genus, *Atya gabonensis* (Fig. 1), taxonomically classified by (ITIS, 2017), was reported to occur in abundance in Nigeria's main rivers and tributaries (Okayi *et al.*, 2015, Obetta and Obande, 2023). It was reported to be harvested by Ijaw fishermen, along the River Osse, in lengths of bamboo set on the river bottom to catch Chrvsichthys, but customarily released as the traditional "king" or protector of other shrimp species (Powell, 1982).

Figure 1: Photo of *Atya gabonensis* from River Niger, Jebba by Obetta.

Atya gabonensis has been reported to be amphidromous (Bauer, 2013), *i.e.*, the individual grows, mates and spawns in freshwater streams or rivers, but the planktonic larvae develop in brackish water estuaries or marine coastal waters. Upon the completion of larval development, the individual settles to the bottom as a postlarva and finds the mouth of a freshwater stream or river to migrate upstream where adults of the species are found (Bauer, 2013). They are generally found in rocky river beds, clean streams, and in places with fast water currents and high dissolved oxygen levels (Rocha and Bueno, 2004). They are specialized for filter feeding, using their brushes to obtain organic matter from suspended particles or to sweep microbial biofilms (Chace, 1972; Souza and Moulton, 2005) and trade (De Grave *et al*., 2008; Mrugala *et al*., 2019).

Because of their special morphology, shrimps of the genus Atya are widely exploited in the ornamental freshwater aquarium. Aquatic Arts (2017) reported that A. gabonensis is certainly among the most fascinating and unique aquarium shrimp available, and it is commonly called African Filter Shrimp, Viper Shrimp, Gabon Shrimp, and Cameroon Shrimp. It is characterized by a peculiarity of the external morphology of the first two pairs of pereiopods, which have brush-shaped ends with many setal t ufts (Hobbs and Hart, 1982; Bauer, 2004). It is reputed to have a superior flavor. Generally, shrimps and prawns are valued food organisms and have been considered one of the most important internationally traded fishery products, which generate substantial economic benefits, especially for many developing countries (FAO, 2008).

There are bright prospects and great potentials for prawn culture development in Nigeria (Anyanwu *et al*., 2012), due to the nation's favourable conditions. But prawn culture has not excelled in the country. The country, despite its natural endowements, can only boast of producing about 18,000 MT annually, based on secondary data collected from the Federal Department

of Fisheries. Aquaculture production in Nigeria increased from 25 718 tones in 2000 to 261 711 tones in 2020 with finfish having a share of 92.6% while shellfish production was 7.4% (FAO, 2022).

The inability of the country to thrive in shrimp and prawn production is partly due to paucity of information on the reproductive biology of culturable prawn species in Nigeria and inadequate information the farming technology. In order to take advantage of its great ecological and economic potentials, there is need to develop the cultivation of important freshwater prawns like *A. gabonensis*. Studies on the ecology, biology, behaviour and distribution of indigenous Nigerian shrimp and prawn species have been carried out by various researchers, to provide the scientific lead way for prawn culture in Nigeria (Abowei *et al*., 2006; Deekae and Abowei, 2010; Kingdom and Erondu, 2012; Lawal-Are and Owolabi, 2012; Opeh and Udo, 2014; Kingdom 2015, Obetta and Obande, 2023). Despite several studies, there was a significant knowledge gap in respect of developmental biology of *A. gabonensis*. Successful breeding of prawn species depends on the mastery of the reproduction process and the availability of its juveniles for a continuous supply of production farms (Vargas-Ceballos *et al.,* 2018). The developmental staging system presented here are novel and would serve as reference materials for the study of reproductive biology of *A. gabonensis.*

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Materials and methods

Location for sample collection

The samples of *A. gabonensis* were collected from River Niger, Jebba, located on latitude 9° 35` and 9° 50` N and longitude 9° 30` and 5° 00` E (Fig. 2). Apart from fishing and livestock activities, the river is also used for hydro-electrical, irrigational and navigational purposes.

Broodstock collection

A total of one hundred (100) broodstock of both sexes of *A. gabonensis* were collected from River Niger, Jebba in March, 2020, when the water level was low. The broodstock of mean total length of 8.37±0.10 cm for females and 8.66±0.07 cm for males were from the catches of fishermen who picked them from under rocks and crevices (Fig. 3A and 3B). The collected prawns were transported in iced box containing water to the laboratory for further studies. Identification to species level was carried out using the keys of Powell (1982). The sexes were determined using specific morphological features unique to male and female prawns, such as, the appendix masculina, reproductive

chamber, and nubs on the first abdominal segment as demonstrated by Anetekhai (1990).

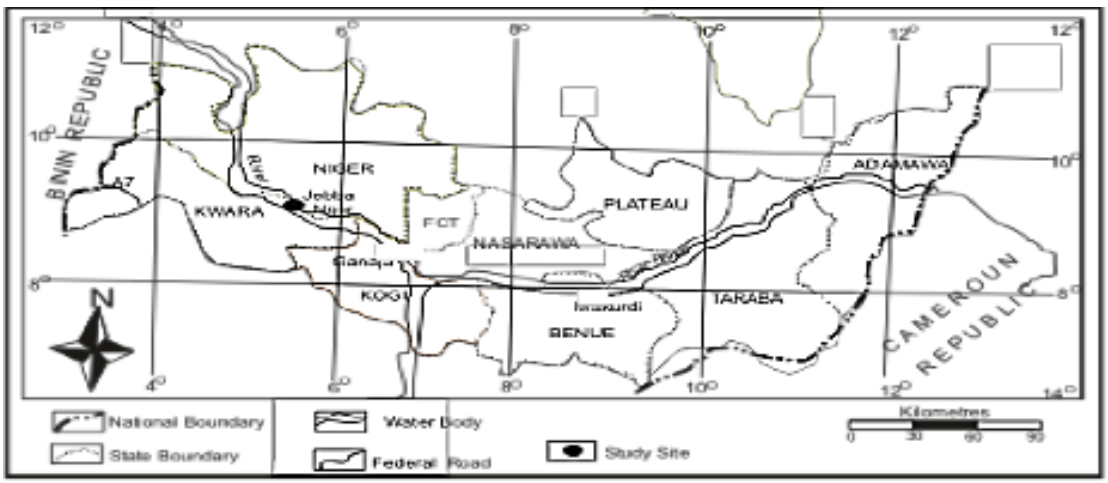

Figure 2: Map Showing Sample Collection Site at Jebba (Modified from Obaje, 2009).

Figure 3: Fishermen picking *A. gabonensis* from under the rocks, Niger River, Jebba

Broodstock management

Prior to the trial, the broodstock (Fig. 4A) was acclimated and kept in 1 m3 polyethylene tanks at a density of 50 individuals in 500 L and at a male-female sex ratio of 1:2 (Koussovi *et al*., 2021). Each tank was provided with two bubbles blowers connected to an aerator for continuous oxygenation. Artificial shelter was created for the prawns using bamboo stems and stones installed at the bottom of the tanks for hiding and spawning (Fig. 4B). The bamboo stems were 10 cm in diameter and 40 cm length with holes big enough to allow the entry and exit of the prawns. Prawns were fed on commercial flake fish food once a day. Uneaten food and other wastes were daily removed by siphoning. The water parameters such as temperature, pH,

dissolved oxygen, ammonia were monitored and kept at optimum levels. Hand net was used to check for ovigerous females every 24hrs. Ovigerous females were separated into a separate tank to prevent cannibalism, while the embryonic development was monitored. Continuous aeration was provided till hatching. The eggs incubation time was noticed as the time interval between fertilization and hatching (Willführ-Nast *et al*., 1993).

Figure 4: Broodstock of *A. gabonensis* (A) and Artificial shelter (B) created for breeding of the prawn from River Niger, Jebba. Photograph by Obetta.

Monitoring of embryonic development

A total of 5 ovigerous females were monitored in the above-mentioned conditions. The first sample was the newly spawned eggs, which appeared orange in colour under the pleopod (Fig. 5). Further samples were taken hourly and studied for embryonic development till hatching.

Figure 5: *A. gabonensis* with pleopod lifted to reveal the fertilised eggs.

Results

The process of embryonic development included cleavage, blastula formation, gastrula formation, segmentation, eye pigment development and larval formation. There are eleven notable developmental stages of embryo of *A. gabonensis* observed in this study. The following staging system is used to describe the embryonic development of *A.* gabonensis in this study.

Stages of embryonic development of A. gabonensis

Stage I (Newly spawned egg): This was the first egg sample collected. At this stage, fertilization has just taken place. The fertilized eggs were almost spherical in shape and included mainly a granulous mass of cells surrounded with a transparent chorion which was intimately attached to the vitelline membrane of the egg. The egg showed light orange colouration with no embryonic structures visible. Most of the egg was full of yolk (Fig. 6A).

Stage II (Cleavage): Series of cell divisions were observed at this point. First embryonic cells were formed here and the number of cells was increased. This was the beginning of embryonic development with a slight increase in eggs volume and change of egg colour as the light orange colour of the egg becomes darker (Figs. 6B and 6C).

Stage III (blastula): There was a short sudden change in the form of the developing embryo.Two regions were noticed in the egg at this stage: the first showed a gap formed at one end of the ovum below the perivitelline membrane. The gap was free of yolk, representing the abdominal part of the developing embryo; and a dark orange coloured part corresponding to its head region. The yolk-free part occupies about $1/6^{th}$ of the egg (Fig. 6D). Invagination of cells was observed in the yolk free part. Blastula is accompanied with the movement of cells to form two primary germ layers, the ectoderm and endoderm.

Stage IV: Gastrula: The yolk free part became increased by contracting the internal mass of the egg mostly in the peripheric part and occupied nearly $1/3^{th}$ of the total egg. Invagination was observed in the yolk free part (Fig. 6E). This stage facilitates the formation of mesoderm.

Stage V Nauplius: Organogenesis was observed at this stage. A broad black spot (eye pigment) appeared in the internal part of the Optical lobes. Cephalic region and naupliar appendages were recognized; The body was seen to be markedly curved (Fig. 6F).

Stage VI: Post-nauplius with a well-developed eye pigments: The optic region, previously set up, enlarged with clearer marked pigmentation (Fig. 6G). Both eye pigments were differentiated. They appeared in half moon shape one on either side. The eyes were thin and elongated with dark pigmentation that distinguished them from other embryonic structures. This stage ensured the development of further organs and as a result, there was a decreased content of the yolk.

Stage VII: Post-nauplius with a heartbeat: Heartbeats started with 81±16 beats/min on the average. The eye pigments became larger, bold and appeared dark brownish in colour. At the abdominal region, the caudal papilla clearly appeared with a rudimentary telson and folded in the direction of the optic region. The remaining content of the egg including mainly the vitellin reserve narrowed because of the development of embryonic structures (Fig. 6H).

Stage VIII: Post-nauplius with eyes visualization: It was observed here that eyes became more or less oval in shape, eyes became large and rounder allowing for visualization, yolk got reduced to is olated patches (Fig. 6I).

Stage IX: Post-nauplius with eyes condensation: The eyes were very big at this stage. Eyes diameter increased with an intensification of their colour. Some eyelashes were visible Above each eye. Abdominal segment that will comprise the abdominal region became evident (Fig. 6J)

Stage X:Pre-zoea: In the cephalothorax region, the antennules, antennae and mandibles were developed; The little yolk over the egg remained attached underneath the cephalothorax region; Contractile movements were observed in the embryo at various regions of differentiating organs; The egg mass was grayish (Fig. 6K).

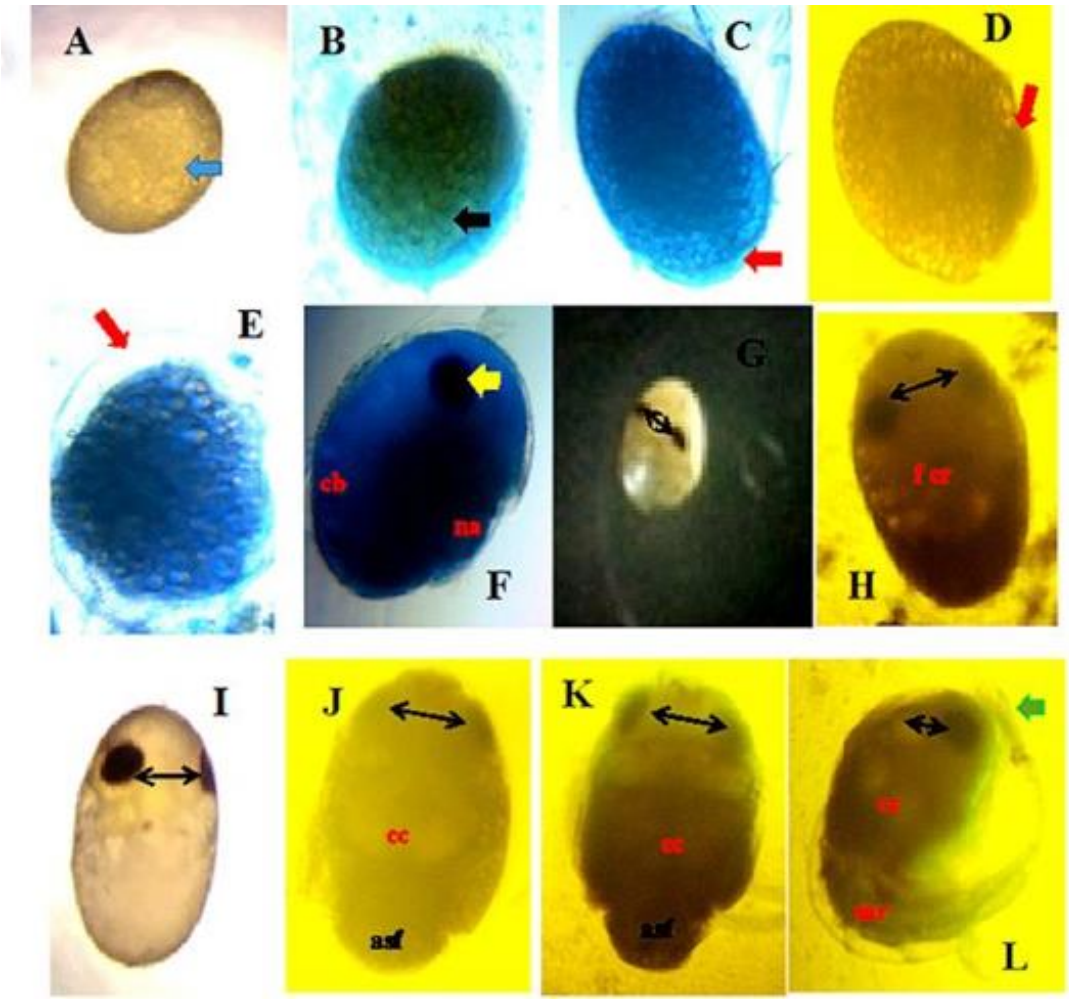

Figure 6: Different Stages of Embryonic Development of *A. gabonesis* (X10). A = Stage I, B= Stage II, C= Stage III, D= Stage IV, E= Stage V, F= Stage VI, G= Stage VII, H= Stage VIII, I= Stage IX, J= Stage X, K = Stage XI, L= Stage XII. Blue arrow= Egg filled with yolk, Black arrow= Blastomeres, Red arrow= Yolk free parts, yellow arrow= Single eye pigment, na=naupliar appendages, cb= Curved body, double blue lines= dark pigmented eyes, Black double arrow=Larger eye pigment, fcr= Formation of Cephalothorax region, asf= Abdominal segment formation, cc= Cephalothoraic carapace, sar= Segmented Abdominal region, green arrow= Telson.

Stage XI: Pre-hatching: Abdominal region was seen to be organized in 5 segments. The last one being the longest; The telson was seen to almost overlap the optical lobe at the posterior extremity of the body. Due to pronounced bending of the embryo; the cephalothoracic carapace was formed covering the heart, cephalothocic appendages and pattially the first abdominal segment. The egg mass appeared grayish. the chorion was seen detaching from the surface of the

embryo, allowing a greater mortitlity which led to emergence of free larvae. the zoae hatched out in 22 days at 26°C.

Discussion

The embryonic development in *A. gabonensis* was reported to include Twelve main stages, almost based on the same principles as most of Palaemonid (Sintondji *et al.*, 2020).

The number of individuals sampled in this study (N=100) is lower than recorded in a study by Almeida *et al*.(2010) (N=3,752) in Bahia, but higher than that reported by Herrera-Correal *et al*. (2013) (N=74) in São Paulo, both of which were carried out in Brazil. The difference in the number of individuals sampled may be related mainly to the methodology (sampling period and methods) and the geological characteristics of the river being sampling.

Embryogenesis in *A. gabonensis* started within 1 hour after the fertilization of ova and lasted for twenty-two days. Anderson (1973) reported that in three to twenty-seven hours after fertilization, some furrows started were established from the outside of eggs. Mousavi and Patil (2022) had the same finding during their study of the stages of embryonic development in the live-bearing fish, *Gambusia holbrooki*. He reported that eembryo genesis in *G. holbrooki*, starts approximately 1 to 3 h post first copulation, which served as an indicator for the fertilization of ova.

Eggs colour of *A. gabonensis* ranges from orange colour to gray and oval in shape before hatching of zoea. This corresponds with the findings of Obetta *et al*., 2022. Habashy *et al*. (2012) reported the embryonic development of *Macrobrachium rosenbergii* in which the eggs were slightly elliptical in shape, initially bright orange to yellow in colour which gradually changed to deep brown a few days before hatching. The fertilized eggs of *Macrobrachium idella Idella*, is opaque, greenish, and oval shape and, as development progresses, its greenish colour changes into light green, brownish-yellow and finally to dull whitish in colour when it is about to hatch, these colour changes were attributed to the absorption of the yellow yolk and development of dark pigment in the eyes (Vijayakumar,1992; Veera, 1994). In the present study, embryos turn grey prior to exclusion. Kanauija (2003) confirmed the same phenomenon in *M. gangeticum* in which the colour of eggs is green yellow and became grey corresponding to embryonic development.

Embryonic development of the eggs of *A. gabonensis* started with a fertilized egg containing yolk mass with an orange colouration. The embryonic development process included cleavage, blastula, gastrula, segmentation, formation of optic vesicle, eye/organogenesis pigmentation development and larvae (zoea) formation as described by Dinakaran *et al*. (2013).

The incubation period (22 days) of *A. gabonensis* in this study is in contrast with 40 days for *Cherax destructor* and 180 days for *H. americanus* (Jaconis *et al*., 2023). In *P. sanguinolentus*,

embryonic development lasted for 8-11 days (John, 2009), the embryonic developments of *M. idella idella* lasted for about 13-14 days (Dinakaran *et al*., 2013), the incubation time for *M. malcolmsonii* is 12-15 days and comparatively less duration of 12-13 days for in *M. gangeticum*, In giant freshwater prawn, *M. rosenbergii*, longer period for incubation and embryonic development was reported as 18- 25 days (Uno and Kwon,1969). Therefore, these variations in incubating period is attributed to the endogenous factors (like egg size and the amount of yolk) and also by exogenous factors like water temperature (Celada *et al*.,1991).

In the current investigation, the eggs of *A. gabonensis* hatched out in 22 days at 26°C. This is close to the number of days reported by Habashy *et al.* (2012) on *M. rosenbergii* that hatched out in 20 days at 28°C. Ogasawara (1984) reported that the eggs of *M. rosenbergii* hatched out in 25 days at 26°C, 20 days at 28°C, and in 17 days at 32°C. Crustaceans from warmer water environments typically have shorter embryonic development in the order of several days to weeks (Yamaguchi, 2001). Manush *et al*. (2006) found a direct linear relationship between rates of development of *M. rosenbergii* embryos with incubation temperature.

At the gastrulation stage subsequent movement of cell layers was observed at stage IV, after which beginning of eye formation was noticed in stage V. The greyish coloured eggs had two black eyes before hatching. Eye formation was visualized as a blackening of aggregated cells and in further stages the eye spots darkend, just as described by Abdur *et al*. (2013) in *M. rosenbergii*. In stage VIII, body segmentation started being separated with the formation of appendages. This took place at a later developmental stage. All these embryonic development stages are in line with the report of Abdur *et al*., (2013) in three macrobrachium species (*M. rosenbergii, M. malcolmsonii and M. lamarei*).

During the embryonic process, the eggs increased in size leading to their volume increase as embryos developed. This increase in eggs volume could be attributed to the osmotic absorption of water to ensure cells' mobility, the structural organization, and the biochemical composition of eggs (Kobayashi and Matsuura, 1995; Müller *et al*., 2003; Cuvin-Aralar, 2014). The development of the nauplius was much more focused on the abdominal segments formation and the eyes organization. At later stages, egg's content decreased and this can be due to metabolic processes using lipidic and proteinic reserves for the formation of some embryonic structures, especially the retina differentiation which led to a obvious pigmentation of eyes (Cuvin-Aralar, 2014). The progressive embryonic development observed in this study is similar to that in most of the decapods (Manush *et al*., 2006).

At the end of the embryonic development, the egg hatched releasing a zoea, similarly to the majority of decapods crustaceans (Yamaguchi, 2001; Sintondji, 2020).

Conclusion

The present study reports the results of the successful reproduction of *A. gabonensis* in hatchery conditions. These results are therefore useful for further surveys intended to develop techniques for the breeding of *A. gabonensis* in captivity. It is recommended that further studies be done to understand the larval development of this species in captivity, so as to have a stepping stone for the domestication of this prawn in Nigeria.

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