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# Scanning Electron Microscopy on Eggshell and Eclosion Process of *Tenagogonus fluviorum* (Fabricius) (Hemiptera: Gerridae)

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**Abstract:** The observation of scanning electron micrograph showed the architecture of the eggshell of *Tenagogonus fluviorum*. The egg chorion has a number of tubular projections arising in aeropylar region, to avoid water loss and to increase the surface area of respiration. Higher magnification of aeropylar region exhibits number of long filaments and chorion was porous without any reticulation. Eclosion process includes egg morphometry and hatching success in successive stages of development and success from egg to adult of *Tenagogonus fluviorum* (Fabricius). Low percentage of a dult emergence from fifth nymphal instar was observed.

**Key words:** Eggshell · Eclosion · Egg morphometry · Hatching success · *Tenagogonus fluviorum*

## INTRODUCTION

Eggs of aquatic insects have been widely studied by many biologists. Their investigations pertain to the chorionic structure and function [1], architecture of the eggshell [2], the behaviour of encumbered males in the process of brooding the eggs [3, 4], water loss during the development of egg [5], eggshell rupture [6] and chorionic structure and egg morphometry [7] and studies done by Cobben [2] showed the egg-laying pattern of some gerrids.

Oviposition method has a greater impact on the development of an egg and its eclosion success. In particular, aquatic insects lay eggs in a wide variety of places, those are characteristic for a particular species or genus. Specifically, water bugs glue the eggs to various objects [8] such as the back of males in belostomatidae [3, 9], on aquatic vegetation in nepidae [10] and on the substratum in naucoridae [11]. The number of eggs laid during oviposition also differs distinctly among aquatic insects [12]. The eggs may be laid singly as in *Ranatra* spp. [10], or laid and retained in masses with mucilage for adhesion on the margin of emergent vegetation as in *Lethocerus* [8]. In *Ranatra unicolor*, the eggs are glued on permanent stones or aquatic plants [13].

*Tenagogonus fluviorum* are generalized predators that feed on small invertebrates including mosquito larva. The estimation of number of expected generations and the capacity of population increase of *T. fluviorum* are worth while attempts in the present study. It is also interesting to know how far factors such as egg viability, egg morphometry, hatching success and life history may act as vital criteria in influencing the reproductive potential of this water strider.

## MATERIALS AND METHODS

Adults of *Tenagogonus fluviorum* were collected and reared in small pond present in the campus of Loyola College, Chennai, Tamilnadu, India. Fifth nymphal instar of *T. fluviorum* was collected from the rearing pond by sweeping nets and maintained in an aquarium (41 x 41 x 21 cm) containing 5 litres of aged tap water reaching to a height of 12 cm. After attaining sexual maturity live *Culex* mosquito larvae were provided as food for predator. Water was changed every 24 h to prevent accumulation of waste such as dead mosquito larvae and to maintain dissolved oxygen level of water. After moulting, male and female bugs were kept together in glass troughs containing 1 litre of water for mating and

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oviposition. The eggs were laid on walls of the glass troughs. In order to avoid the cannibalistic behaviour, the adult females were removed immediately after oviposition and the eggs were maintained in well aerated condition till eclosion.

**Structure of the Egg:** A mating pair of *T. fluviorum* was kept in a glass container and the eggs deposited on the walls of glass container were isolated, fixed in 70% alcohol (5 to 10 h), processed through the alcohol series (80, 90 and 100%) for 5 to 10 h each and later coated with gold to scan different sides of the egg chorion under scanning electron microscope (SEM). Different region of the egg chorion were chosen and magnified by 3 to 200 μ for the morphological observations.

**Morphometry of the Egg:** Ten eggs with were collected prior to eclosion, fixed in 70% alcohol and processed by dehydration, clearing and mounting. The mount was used to measure the length and width of the eggs by using stage and ocular micrometers.

**Percentage Success of Eclosion:** A mating pair was kept in a glass container and the eggs deposited by the female were maintained till eclosion. First nymphal stage that emerged out of eggs was maintained in the aquarium to record their life span. The percentage success of emergence of a nymph from its previous stage as well as the percentage success of attainment of adulthood from each egg was also derived. A total of ten trials were carried out for each experiment.

**Eclosion:** A few eggs were maintained under favourable room temperature (28±1°C) and salinity (1%) of water till eclosion. The sequences of eclosion were observed using hand lens.

## RESULTS

**Chorionic Structure:** The elongate and oval shaped egg of *T. fluviorum* has one flat and slightly depressed side, which is glued to objects such as stone surface or on leaves of floating vegetation and it is convex at the free side (Plate 1A). Electron micrograph of the adhesive surface is pad like having a number of pits. This pad like region is broad posteriorly and narrows anteriorly (Plate 1B). The magnified view of the central and marginal regions of the adhesive surface shows the presence of the pits, being predominant in the marginal region of the pad with the central region exhibiting the presence of shapeless glue like substance (Plate 1C). The blunt anterior pole bears a slight depression (Plate 1D). The centre of the depression possesses a single micropyle which runs slightly in a transverse direction and opens inward through the shell by short rectangular bend (Plate 1E). Chorion is smooth without a sculpture or hexagonal pattern. The magnified view of the posterior pole of the gerrid egg has sub-terminal serosal aeropyle (Plate 1F). Higher magnification of aeropylar region exhibits a number of long filaments intermittent on smooth surface of the aeropyle but arising from each individual shell of the aeropylar region (Plate 1G). The chorion in general is uniformly porous without any reticulation.

**Egg Morphometry:** The development of an egg in terms of length (mm) began with 1.190±0.022 (SE) on the first day followed by a gradual increase up to 1.243±0.017, 1.270±0.009, 1.302 ±0.016, 1.339 ±0.618 and 1.347 ±0.013 on the second, third, fourth, fifth and sixth day respectively. Similarly, on the first day, the width (widest point) of an egg was noted to be 0.355±0.014 followed by 0.399±0.013, 0.435±0.009, 0.491±0.033, 0.526±0.026 and 0.532±0.018 on the successive days.

**Eclosion:** The egg was small and the colour of the egg chorion was white at the time of oviposition, subsequently changing to yellow and then to brown. On the fourth day after oviposition, a pair of red spot appeared indicating the development of compound eyes (anterior region) and the entire egg turned black prior to the eclosion. During the final stage of eclosion, the egg burster present in all the eggs helps in the hatching process. It was either `T' or `Y' shaped.

The egg burster initiated hatching. The developing embryo showed contraction and relaxation as a result of which pressure developed inside the egg and first nymphal stage emerged out of the egg by making longitudinal slit till about two third the length of the egg from the egg burster. The opening site was thin and weak against the force given by the tiny nymphs. If the water in the rearing container (trough) was not frequently changed, the mortality rate was high in the first nymphal stage (to avoid accumulation of waste). Soon after its emergence from the eggshell, the first nymphal stages remained immobile and submerged in water. After 2 to 5 h they moved to the surface by spreading their thoracic legs; slowly flexed the legs and started to stride on the water surface.

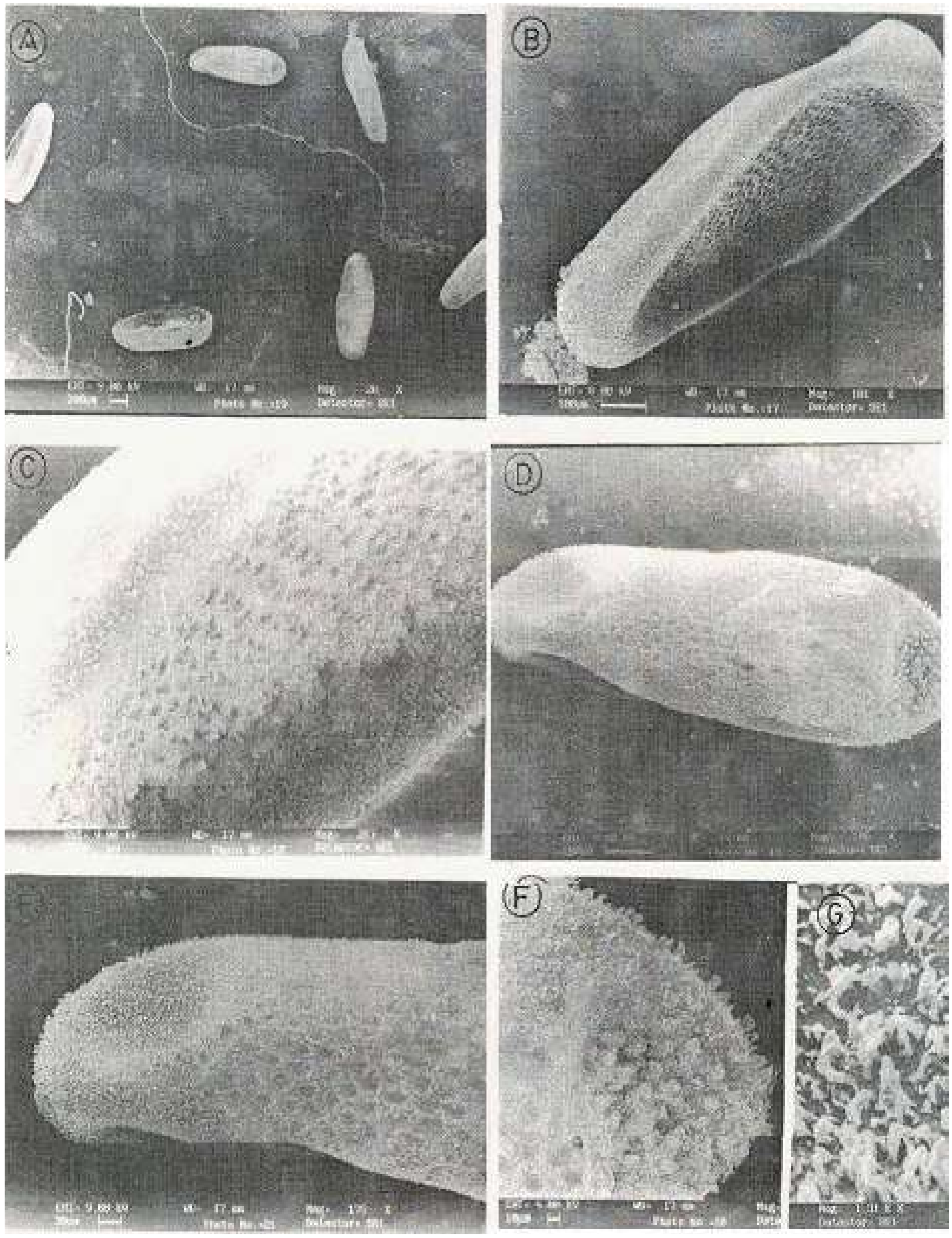


Plate 1: Scanning electron photographs of the eggs of *Tenagogonus fluviorum*

A. Egg entire view (X 20)
B. Egg ventral view (X 101)
C. Magnified view of adhesive side (X 267)
D. Egg dorsal view (X 106)
E. Micropylar region (X 175)
F. Aeropylar region (X 263)
G. Magnified view of tubular projection (X 465)

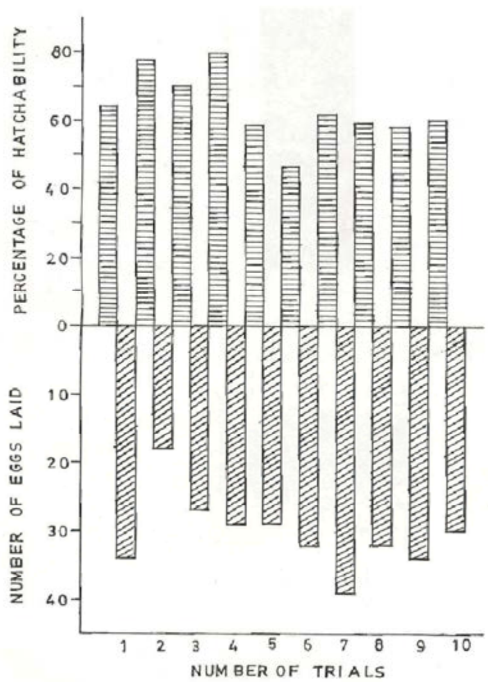


Fig. 1: Hatching success of *Tenagogonus fluviorum*

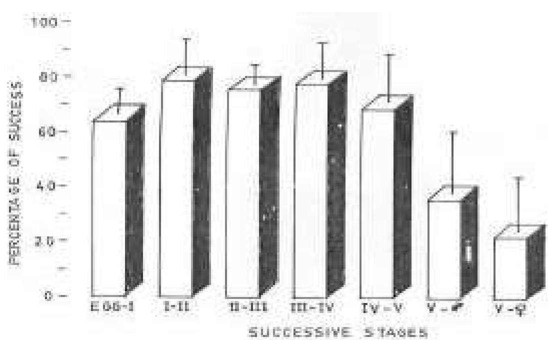


Fig. 2: Percentage success of successive stages of *Tenagogonus fluviorum*

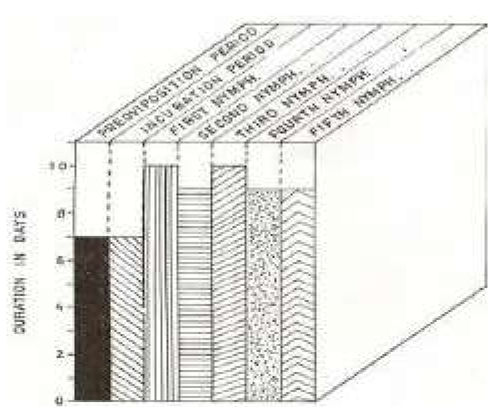


Fig. 3: Duration of pre-oviposition, incubation and nymphal instars of *Tenagogonus fluviorum*

**Hatching Success:** Number of eggs deposited by single female at a time ranged from 18 to 39. On average, eggs deposited by *T. fluviorum* was 30.4±5.222 per female and percentage hatchability was 63.74±9.758%.

**Percentage Success from Egg to Adult:** The female *T. fluviorum* laid eggs on the inner surface of the wall of glass container and were maintained till eclosion. Percentage success of emergence of adults from the egg and during the other ynmphal instars (egg to I, egg to II, egg to III, egg to IV and egg to V) were individually recorded (Figure 1).

**Percentage Success in Successive Nymphal Instars:** Degree of emergence of adults from eggs laid by female *T. fluviorum* through the post-embryonic development was relatively low. Data collected were reoriented to identify the percentage success of successive moults such as first to second nymphal instar, second to third, third to fourth, fourth to fifth and fifth to adult (either male or female) (Fig. 2).

During moulting the cuticle begins to split near the head region followed by `Y' shaped splits, which extended to the middle region of thorax and abdomen. Nymphal stage had a preparatory period for moulting, they appeared to be sluggish and were found attached to either the aquatic vegetation provided or the margin of the container. The transformation from first to second nymphal instar was relatively higher than any other instars. Among the sexes, number of males that were obtained from the fifth nymphal instar under laboratory condition was relatively higher than the females.

**Fecundity:** Pre-oviposition period of *T. fluviorum* was 7 to 9 days. A female laid 39 eggs after copulation. On an average, a female lays 150 to 200 eggs during its life under laboratory conditions. The time interval between successive brood was 8 to 10 days. The adult life span was observed to be maximum of 90 days under laboratory conditions.

**Duration of the Development of Egg and Nymphal Instar:** The duration of each stage after the eclosion period was recorded. Incubation period (eggs) of *T. fluviorum* was noted as 6 to 8 days. Among the post-embryonic stages, the time duration for first and third nymphal stages was 9 to 11 and for second, third, fourth and fifth nymphal stage it was 8 to 10 days. On an average, the total nymphal duration for transformation into an adult was 55 to 60 days under laboratory condition (Fig. 3).

## DISCUSSION

The electron micrography study on the eggs of *Tenagogonus fluviorum* reveals the presence of depressed micropylar region anteriorwards in the egg chorion, tapering down towards the adhesive side. It is pertinent to mention there is no micrograph taken through the micropylar region in gerrid [2]. The present work fills up the lacunae in viewing the micropyle at the higher magnification. Lack of chorionic architecture, as is noted in the micrograph reflects upon the primitiveness of gerrids in Amphibicorisae. Egg chorion was found to be smooth without hexagonal pattern. This was found to be in contrast with studies of Kannappan [14] in which hexagonal pattern on the egg chorion of water measurers (Hydrometridae) were reported. Though the egg is ovaid, limited regions of the ventral surface of the egg chorion assist in getting glued over the substratum. The firmness of adhesion may be due to the extent of the hollow region on the adhesive surface. Micrograph on the adhesive surface of egg chorion further indicates the extent of distribution of the egg glue. The spongy nature of the aeropyle at the posterior end of the egg chorion possessing number of filaments may enhance the gaseous exchange between the embryos developing within and the environment outside. Intermittent distribution of branched filamentous structures may promote increased surface of respiratory region in order to keep the eggs viable. However, the detailed work on micrograph of the egg chorion sections has further revealed the structural complexity of the inner layer of the egg chorion inner layer with which embryo has more intimate and immediate interactions.

The first and foremost is an increase in egg measurements from the time of oviposition till its eclosion, whose, average remains nearly as 6 days. The increase in width and length of such developing eggs were compared on different days of the development. The increase in egg length was phenomenal on the first day showing a declining trend on all other days but with a negligible rise on the last day of egg development. The rise in the egg width was distinct even on the third day of the development, though its increase was marginal in the later period indicating thereby not only the growth rate of the first nymph but also in its orientation in order to have the successful eclosion. Such an orientation may be of importance during eclosion. The nymph of *Trephobates knighty* managed to withdraw their bodies from exuviae but could not do so due to appendages [15]. Similar ecdysial failures were noticed in some individuals of *Gerris rufoscutellatus* [16]. At the time of emergence, the eggshell showed a simple longitudinal slit running from anterior pole to downwards along the median line of the egg. This primitive type of shell eclosion is also met with in Hydrometridae [14].

The data on the present study revealed the hatching success to the extent of 63.74% (Fig. 1). Among the egg laid by *T. fluviorum*, the rate of success for the emergence of the first nymph was high thereafter showing a declining trend with the mean value of 6.27% of males and 4.84% of females appearing from the egg to post-embryonic development (Fig. 2). Such an increased rate of ecdysial failure in the older nymphal instars than the early nymphal instars may be attributed to the difficulty in some individuals to wriggle out of the exuviae in the process of making their appendages free [17] or possibility of the eggs getting affected by the parasitoids. The former may be attributed to the fall in moulting success during various post-embryonic developments. The latter may be due to eclosion failure and parasitation of the gerrid's eggs by the parasitoid *Tiphodytes gerriphagus* was noted to the extent of 84% [18]. So in this context it would be of great interest if one could study the possible parasitation of the eggs of *T. fluviorum* to draw the conclusion as done by earlier researchers [18, 19]. Egg parasitism is a significant evolutionary force, shaping the life history patterns as well as behaviour of water striders. The low rate of eclosion failure may reflect upon the structure of local gerrid assemblage [19, 20].

Further the present study also showed nearly a uniform rate of ecdysial success of the first, second, third and fourth nymphal instars. The sudden fall in the eclosion success of fifth stage may be due to fact that the last nymphal instars develops adult characteristics, which may cause a greater stress on ecdysial mechanism and the possibility of incomplete appearance of such characters. A detailed study on the ecdysial mechanism of the fifth nymphal stage to adult may bring to light the cause for low rate of ecdysial success. The alternative as the possible development of fifth nymphal instar either to a male or female is also of great biological importance in the life history pattern of water bugs. Among the sexes that appear from fifth nymphal instar, the percent success with reference to male is relatively higher (36.26%) than the females (22.94%) Increased abdominal width in females would have caused low per cent of ecdysial success. Possibly ecological factors such as, temperature can eliminate a species by disrupting the emergence pattern in *Pteronarcys dorsata* [21].

Summary of the egg system and duration of the post-embryonic development throws light upon prolonged developmental period of the first and third nymphal stages than the others. The increased percentage of ecdysial success from first to second and third to fourth than the other instars is also well reflected in this study. In future studies, it will be pertinent to investigate the allometric growth pattern of *T. fluviorum* in order to verify whether prolonged period of development of first and third nymphal instars may cause any significant measurement values.

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