

Life cycle study of ragged sea hare, *Bursatella leachii* (Blainville, 1817) inhabiting the inter-tidal waters of Pulicat Lake, Tamil Nadu, India

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(Received 2 August, 2020; Accepted 18 September, 2020)

ABSTRACT

The species *Bursatella leachii* was collected from the intertidal waters of Pulicat Lake. The study was carried out at controlled room temp. in glass aquaria's; the mating occurred in the form of unilateral copulating with chain formation. Only the first animal acted as receptor, the second and succeeding animals acted as donors. It releases the fertilized eggs like spaghetti, packaged in capsules to form a cylindrical string called an egg mass. The number of capsule per cm of the egg masses varies from 25 to 55 capsules and each capsule contained 5 to 19 eggs. After spawning, the egg masses were light yellowish greenish, it changed to golden brown. The embryo developed into trochophore stage and began to rotate within the egg capsule in first 4-5 days; 6-8 days onwards it develops the precursor of the velum called prevelum. At 7- 8 days after spawning, the prevelum is transformed into the velum and attend veliger stage. Between 8-12 days the veligers hatched as free-swimming larvae. Metamorphic stage began: on 16 to 19 days shell get detached and villae start appearing whole body like spines, 19th to 20th days larvae become benthic and 30th to 40th days it transformed to adult stag.

Key words: *Aplysiidae*, *Bursatella leachii*, Embryo development, Veliger larvae

Introduction

Molluscs are the second largest animal phylum on earth and include an enormous diversity of species (Beesley *et al.*, 1998). The sea hares has around +/- 50 species worldwide and graze mainly in the tidal

and subtidal zones (Klussmann-Kolb, 2004). *Bursatella leachii* body is compact and rounded covers with numerous long branching fleshy papillae, gills covers with a pair of fleshy parapodia, it has two long retractile olfactory tentacles normally called rhinophores on the head and each side of the

mouth has two fleshy oral tentacles, head and neck are distinctly separated. Greyish green to white tan with dark brown blotches and spots throughout the body, displaying a fascinating range of colours, found in warm temperate to tropical marine environment (Rudman, 1998). It has been reported in inter-tidal zone depth 20 cm to 10 meter depth (Sethi *et al.*, 2015 and Kokane *et al.*, 2016) at Pulicat lake and shrimp farms in Thailand (Arkronrat, 2016). Otero *et al.* (2013) reported that *B.leachii* a hermaphroditic species has very fast life cycle and continuous reproduction.

Many species of opisthobranchia and pulmonatea are generally exhibits instantaneous gonochorism characters. The reproductive systems are comprised form of a single gonad, i.e., the ovotestis and a common genital gonoduct. During mating/copulation, one individual acts as a male and crawls over another to fertilize it (Beeman, 1970; Berry *et al.*, 1992; Vue, 2014). The types of copulation in opisthobranchs differ into reciprocal or unilateral according to facing direction (Rivest, 1984). *B.leachii* is a cross fertilizing simultaneous hermaphrodite (Kaplan, 1988). Fertilization is internal with one individual transferring sperm via an eversible penis located on the right side of the head to the dorsally located gonopore (genital opening) of second individual (Van Horn, 2005). The capsulated egg mass found in neritoposina and some caenogastropdas were as gelatinous egg masses are found in the heterobranchia and the structure and composition of egg masses differ among species (Przeslawski, 2004). Sea hares has multiple layered protection throughout ontogeny, since *Aplysia* have a relatively simple nervous system with many large neurons, they have been of a major interest to neurobiologists and physiologists (Kandel, 1979). Studies of sea hare (Opisthobranchia) species in various parts of the world has provided information on reproduction, metamorphosis, growth, fecundity, and seasonal abundance (Paigee, 1988, Yusa, 1996; Lee *et al.*, 2014; Vue *et al.*, 2014 and Arkronrat *et al.*, 2016). The present study was to investigate the reproductive biology of *B. leachii*, inhabiting the intertidal waters of Pulicat Lake, Tamil Nadu with particular reference to spawning behavior, structure of egg masses and egg development.

Methodology

The adult *B. leachii* species were collected from the

intertidal waters of the Pulicat Lake T.N, India (Lat 13.436221 ° N Long 80.324163 °E ± 0.25 °N/E (or) 13°26.1' N 80°19.4' E ± 1.5' N/E) (Accession number is M-1697 obtained from Zoological Survey of India, MBRC, Chennai, India dated 23.07.2015). The *B. leachii* species were maintained in 40 lits of glass aquaria and animals were fed daily with fresh seagrass, seaweed and dry prawn powder. 2-3 days later courtship behaviour has been observed and animal started lying of eggs mass strings on the glass aquaria. The newly spawned egg clusters were collected from the aquaria, rinsed, and inserted into 1-L flasks containing filtered seawater under continuous aeration at room temperature. The filtered seawater was changed daily and maintained at room temperature (25±0.5 °C) and salinity (30±0.6 PSU). The structure of egg mass and egg development of *B. leachii* were seen under ProcammTrue chrom HD RSMr / AxiocamMRC/Olympus SZX10 microscope at NCCR and ZSI.

Results and Discussion

Sea hare *B. leachii* is a simultaneous hermaphrodite in nature; eggs are produced by internal fertilization via unilateral copulation (Paige, 1988). When a pair of *B. leachii* copulated, the first animal attaching to the substrate acted as a female, and the second animal that is in close contact with the dorsal surfaces acted as a male. Male protruded penis inserted it into the common genital aperture of a female and sperm get released coupling chain is formed and deposited egg string on the walls of the rearing glass aquaria. The fertilized eggs were covered in capsules that are embedded in jelly to form a cylindrical string.

Pre-hatching embryo development and Post-hatching larval development

Matured sea hare (Fig. 1A) lays the fertilized eggs like spaghetti noodles and packaged in capsules that are embedded in jelly to form a cylindrical string called an egg masses which are generally adhesive in nature (Fig. 1B). The number of capsule per cm of the egg masses varies from 25 to 55 capsules and each capsule contained 5 to 19 eggs in general (Fig.1C). After 4 to 5 days of spawning, the embryo developed into trochophore stage and began to rotate within the egg capsule and start developing of foots, eye spot etc. (Fig. 1. D I, II & III). And next 6-8 days onwards it develops the precursor of

the velum, called the prototroch or prevelum (Fig.1 E). At 7- 8 days after spawning, the prevelum is transformed into the velum, and it developed into veliger stage (Fig.1F). Between 8-12 days after

spawning, the veligers broke out of the egg capsule, and hatched as free-swimming veliger larvae (Fig.1G). Metamorphic stage began after hatching of larvae from egg capsule; on 16 to 19 days shell get



Fig. 1. A. Adult Stage of *B. leachii*; B. Fertilized egg mass; C. Embryos inside egg capsule; D.Trochophore stage i,ii & iii; E. Prototroch or Prevelum Stage; F.Velum Stage (Ready to hatch); G. Hatched larvae - Velliger stage; H. shell get detached from the larvae; I. Villae appears like spines and J. Larvae become bethic and appear like adult

detached from the larvae (Fig.1H) and villae start appearing whole body like spines, two eye appears (Fig. 1I) and after 19th to 20th day larvae become benthic and appear like young *B. leachii* (Fig. 1J) and 30th to 40th day it start transformed to adult stage

Discussion

The larval stages of *B. leachii* are well documented and staging scheme are more or less similar to *Aplysia californica* and *Aplysia kurodai* (Paige, 1988 and Lee *et al.*, 2014). In this present study, the mating and courtship behavior of *B. leachii* occurred in the form of unilateral copulating with chain formation. In chain copulation, only the first animal acted as a female; the second and succeeding animals acted as males (sperm donors) to the animals in front and as females to the animals behind (Lee, 2014 and Vue, 2014). Laying of eggs and embryonic developments varies with the sizes of animal and climatic conditions. The fertilized eggs of the *B. leachii* are packaged in capsules that are embedded in layers of mucopolysaccharide jelly to form a cylindrical string like spaghetti noodles. In natural environment and artificial enclosed conditions, the number of capsules per unit length of egg masses and the number of eggs per capsule vary among *B. leachii* species and climatic conditions. It has been observed an inverse relationship between the size of the eggs and the number of eggs per capsule (Bridges, 1975).

It has been observed that, the number of eggs per capsule very from natural habitat to aquarium condition, wild collection it was 10-25 eggs per capsule were as in aquarium tank 5-19 eggs per capsule. Most molluscs undergo spiral holoblastic cleavage, and embryonic development varies by temperature, salinity and the egg size. In opisthobranchs, the egg diameter correlates positively with the size of the hatched veliger larvae, and hatching size also increases with increasing embryonic duration (Hadfield and Switzer-Dunlap, 1984). In the development of *B. leachii* species, the embryonic development periods are shorter or longer depends upon the size of eggs (Capo, 2002; Lee, 2014; Vue, 2014). In the case of *B. leachii* species, the fertilized eggs, like those of other molluscs, underwent spiral cleavage. The eggs hatched at 8-12 days after spawning, within the range of other aplysiids. The larvae of *B. leachii* was fed with microalgae and mixed diatoms, above 30-40 days larva could not survived. *B. leachii*

has small aquarium trade and ink gland has potential for pharmacological use (Otero *et al.*, 2013). Due to presence of anti cancer anti-tumour, anti-viral and anti-HIV protein compounds (Haefner, 2003; Rajaganapathi *et al.*, 2002). So, the species *Bursatella leachii* has potential as a candidate species for aquaculture.

Acknowledgement

The authors are grateful to the Officer In-charge, MBRC ZSI, Chennai and Director, NCCR, Chennai for providing the necessary support and lab facilities to carry out the experiments.

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