The Bioscan 15(1): 035-038, 2020
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

A REPORT ON COMPARATIVE MORPHOLOGY OF EGG MASSES AND EGGS IN PULMONATE GASTROPODS *INDOPLANORBIS EXUSTUS* (DESHAYES 1834) AND *LYMNAEA ACUMINATA* LAMARCK 1822

G. S. PANDE[1], S. P. CHAUHAN[1] AND R. D. TAK[2]*
[1]Department of Zoology, Ahmednagar College, Ahmednagar - 414 001, M.S., INDIA
[2]Department of Biochemistry, Ahmednagar College, Ahmednagar - 414 001, M.S., INDIA
e-mail: rajeshtak@gmail.com

KEYWORDS
Egg mass
Egg
Morphology
Indoplanorbis
Lymnaea.

Received on :
13.03.2020

Accepted on :
06.03.2020

***Corresponding author**

ABSTRACT
The present investigation deals with the comparative study of egg mass and egg morphology in freshwater snails *I. exustus* and *L. acuminata*. These snails lay eggs embedded in gelatinous strip called egg mass. The comparative morphometric measurements of egg masses and eggs showed significant variations with respect to morphology between two snails. The egg masses of *I. exustus* measured about 16.83 mm × 3.13 mm and were significantly smaller (P >0.05) than those of *L. acuminata* which measured about 18.28mm × 3.92 mm. The eggs of *L. acuminata* measured 1025.05μm × 723.44μm and were significantly smaller (P >0.05) than those of *I. exustus* which measured about 1149.17μm × 888.35μm. The number of eggs/egg mass was also found to vary significantly (P >0.05) between two snails studied. The snail *L. acuminata* had more eggs/egg mass with a mean number of eggs/egg mass 41.45 ± 22.10 as opposed to 22.05-± 4.88 in *I. exustus*. From the analysis of the results obtained, it can be concluded that there are significant differences between morphological parameters of eggs and egg masses of freshwater snails *L. acuminata* and *I. exustus*.

INTRODUCTION

The freshwater pulmonate snails *L. acuminata and I. exustus* are widely distributed in Indian sub-continent and are common inhabitants of freshwater bodies such as ponds, lakes, dams, streams, and rivers (Ramakrishna and Dey, 2007). Like many freshwater gastropods, the snails *L. acuminata* and *I. exustus* are simultaneous hermaphroditic animals and lay eggs in the form of gelatinous strip known as egg mass or egg capsule (Pechenik, 1983; Dillon, 2000; Mantale, 2009; Pande *et al.,* 2009, 2012). The studies in many gastropods have shown that the egg production occurs during specific periods of the year and the age of snail usually affects the quality and quantity of egg production (Norton and Bronson, 2006; Janssen and Baur, 2015; Norton and Newman, 2016). Within gastropods, egg capsule morphology varies tremendously (D'asaro, 1970, 1988; Pande *et al.,* 2012; Kyle *et al.,* 2013). It has been reported that the number of eggs per egg mass, egg, and egg mass morphology is related to a large number of factors in gastropods (Rawlings, 1990; Andrews, 2005). The hypothesis that underlies the present study is to know whether the eggs and egg masses of *L. acuminata* and *I. exustus* vary with respect to morphological parameters and the number of eggs in an egg mass. The study of the egg masses, eggs, and embryos in snails are considered important for studies on reproductive biology, dispersal, biogeography, and taxonomy of gastropods (D'asaro, 1988; Johannesson, 1988; Pastorino and Penchaszadeh, 2009; Gallardo *et al.,* 2012; Fukumori *et al.,* 2013; Srivastava and Singh, 2018). The freshwater snails *L. acuminata* and *I. exustus* serve as an intermediate vector for helminth parasites responsible for diseases of cattle and humans such as fascioliasis and schistosomiasis and control of vector snails is one of the strategies for reducing infection (Jordan *et al.,* 1993; Agarwal and Singh, 1988 and Mas-Coma et al., 2005; Singh *et al.,* 2013).

There have been a number of studies on egg and egg capsule in gastropods (Pechenik, 1983; Lam and Calow, 1988; Rawlings, 1989; 1990; Norton and Bronson, 2006; Mantale, 2009; Pastorino and Penchaszadeh, 2009). Our earlier work uncovered some details about egg and egg mass morphology, the effect of temperature and photoperiod on egg mass production and embryonic development of *L. acuminata* (Pande *et al.,* 2009, 2010a, 2010b, 2012). However, there is no published report on the comparative account on egg and egg mass morphology in *L. acuminata* and *I. exustus*. The variation in the egg mass and egg morphology are useful parameters in studies on taxonomy, evolution, dispersal and reproductive biology of freshwater snails. The present investigation was undertaken to investigate comparative aspects of the egg mass and egg morphology between snail *L. acuminata* and *I. exustus*.

MATERIALS AND METHODS

The methodology for conducting experiments was adopted from earlier works on eggs and egg masses of snails (Castilla and Cancino, 1976; Norton and Bronson, 2006; Neves *et al.,*

2010; Mahmoud *et al.,* 2013). The brief description of the methodology employed is as follows:

a) Experimental animal: The freshwater snail *I. exustus* (Planorbidae: Pulmonata: Gastropoda) and *L. acuminata* (Lymnaeidae: Pulmonata: Gastropoda) are widely distributed snails in India and are abundantly available in freshwater bodies throughout the year (Ramakrishna and Dey, 2007). They both are available in local freshwater bodies in and around Ahmednagar city, MS, India. Both of the snails have been reported to serve as an intermediate host for helminth parasites of veterinary and medical importance (Ramakrishna and Dey, 2007). There is no published record on comparative studies on egg and egg mass of these snails. Hence, these two snail species were selected as experimental animals for conducting the present research endeavor.

b) Identification of animals: Snail specimens were identified in the laboratory with the help of keys by Ramakrishna and Dey (2007).

c) Collection and maintenance: Live *I. exustus* specimen were collected from cement tanks near Ahmednagar fort and *L. acuminata* specimen collected from cement tanks in the botanical garden of Ahmednagar College campus. Specimen were collected with the help of aquatic net. The specimens were maintained in plastic tubs aerated with an aerator to maintain enough oxygen level. Mulberry leaves were chopped off into fine pieces and were added into tubs as a source of food. The amount of food provided was such that each snail specimen had access to the food. Water in the tubs was replaced daily with dechlorinated freshwater and the debris, if any, was removed from the tub. Egg masses laid onto mulberry leaves and tub surface was collected daily in the morning with the help of scalpel and observed under microscope to perform egg count and morphometric measurements. Afterward, egg masses were transferred in 70% alcohol for preservation.

d) Morphology of egg masses and eggs: Egg mass was placed onto laminated graph paper to measure its size. The dimension of eggs was measured using a stage and ocular micrometer (Neves *et al.,* 2010; Mahmoud *et al.,* 2013).

e) The number of eggs/egg mass: The number of eggs/egg mass was counted using a hand lens or a simple microscope (Norton and Bronson, 2006).

f) Statistical analysis: Statistical analysis was performed using Microsoft Excel 2007. Mean, range, and the standard deviation was calculated for each morphological parameter and number of eggs per egg mass.

RESULTS

I. Egg masses and eggs of *I. exustus:* *I. exustus* is hermaphroditic snail laying eggs throughout the year in laboratory conditions. It lays many eggs packed in the ribbon-like transparent gelatinous matrix called an egg mass or egg capsule.

a) The egg mass: The egg masses of *I. exustus* were faint yellowish, transparent, and in the form of long gelatinous strip. The deposited egg mass is usually curved around and looks semicircular to nearly circular when viewed from above. It is plano-convex in outline and attached to substrata like leaf surfaces, petioles, algae, dead shells, and small rocks. The length of egg mass varied between 7 to 25 mm while the width of egg mass varied between 3 to 5 mm. The size of an egg mass was found to be 16.83 ± 4.34. × 3.13 ± 0.35 mm (n = 184).

b) Eggs: Eggs are usually oval in shape. Eggs are isolecithal as reported earlier (Mantale, 2009). Eggs are transparent so that embryos within can be easily observed. The length of eggs varied between 840 µm to 1672 µm while egg width varied between 572 µm to 1686 µm. The size of eggs was found to be 1149.17 ± 10 µm × 888.35 ± 10 µm (n = 1486).

c) The number of eggs/egg mass: The number of eggs/egg mass was found to be 22.05 ± 4.88 (n = 183) and vary between 6 to 39. Eggs were arranged usually in two rows side by side within the egg mass of *I. exustus* (Plate I).

II. Egg masses and eggs of *L. acuminata*: The pond snail *L. acuminata* is a hermaphroditic snail laying eggs throughout the year in laboratory conditions. Its eggs are packed in an elongated gelatinous structure called egg mass.

a) The egg mass: The egg mass of *L. acuminata* is in the form of an elongated strip that is transparent and usually whitish or colorless. The egg mass has many eggs and is usually laid onto the aquatic vegetation, leaf surface, petioles, or surface of small rocks within the water. The size of the egg mass was found to be 18.28 ± 6.67 mm × 3.92 ± 0.89 mm (n = 53). The length of the egg mass varied between 5 to 40 mm while its width varied between 2 to 7mm.

b) Eggs: Eggs are usually oval in shape, however, few eggs may be rounded. Eggs are highly transparent allowing clear observation of embryos within the egg. Eggs are isolecithal as reported earlier (Pande et al., 2010b). The size of eggs was 1025.05 ± 14 µm × 723.44 ± 76 µm (n = 409). The length of eggs varied between 743 µm to 1329 µm, whereas the width of eggs varied between 129 µm to 1272 µm.

c) The number of eggs/egg mass: The number of eggs/egg mass was 41.45 ± 22.10 (n = 53). The number of eggs/egg mass varied between 7 to 90. Eggs were arranged usually in three rows side by side within the egg mass of *L. acuminata,* except for small egg masses having only a few eggs (Plate I).

III. Comparison of morphological parameters and no of eggs/ egg mass between snails: Except for egg mass length, all other parameters, viz., egg mass width, egg length, egg width, the number of eggs/egg mass differ significantly ($P > 0.05$) between I. exustus and L. acuminata (Table 1).

DISCUSSION

The results obtained show that there are significant variations between *I. exustus* and *L. acuminata* with respect to egg mass and egg morphology. Both the snails produce gelatinous egg masses as in many other freshwater gastropods (Dillon, 2000). Encapsulation of developing embryos is common among the more advanced gastropods (Pechenik, 1986). Egg capsules may protect embryos from such environmental stresses as predation, bacterial attack, osmotic changes, desiccation, temperature shock, and wave action (Pechenik, 1979, 1982, 1983). The eggs in both snails were observed to

Table 1: Data on egg mass and egg morphology in snails

Parameter	*I. exustus*	*L. acuminata*	'T-test' (P_{value} at 0.05 level)
Egg mass length (mm)	16.83 ±4.34	18.28±6.67	> 0. 05
Egg mass width (mm)	3.13±0.35	3.92±0.89	≤ 0.05
Egg length (μm)	1149.17 ± 101.90	1025.05±142.18	≤ 0.05)
Egg width (μm)	888.35± 109.71	723.44±76.52	≤ 0.05)
The number of eggs/egg mass	22.06±4.87	41.45±22.10	≤ 0.05)

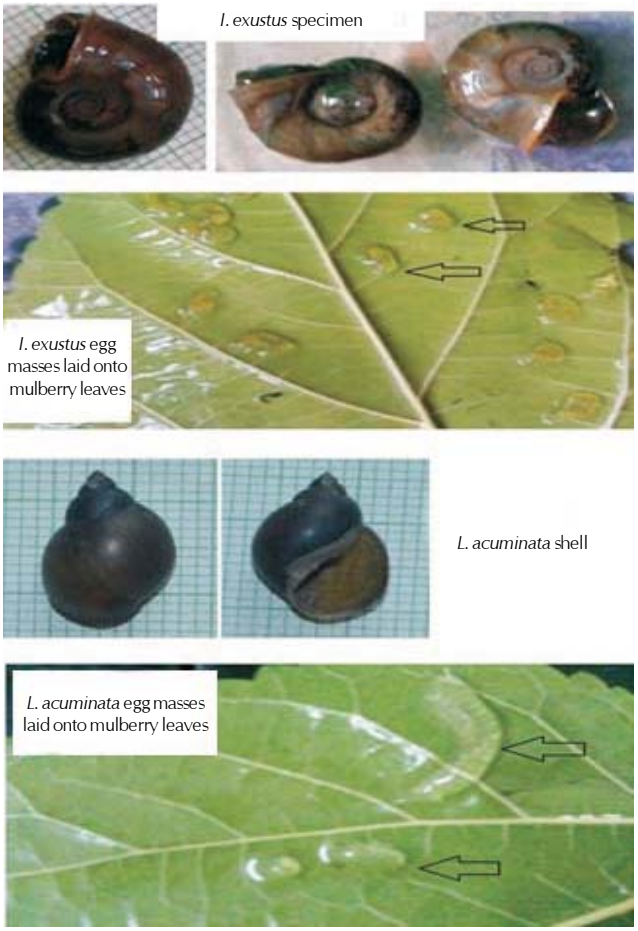

Plate 1: Image of egg masses of snail *I. exustus* and *L. acuminata*

be isolecithal type as reported earlier (Mantale, 2009 and Pande et al., 2010b). Except for egg mass length, all other parameters: the egg mass width, egg length, egg width, number of eggs/egg mass differ significantly (P >0.05) between *I. exustus* and *L. acuminata*. Similar observations were recorded in other gastropods. For example, in marine hydrobiid snail *Heleobia australis,* the number of eggs per egg mass varied between 10 and 15 whereas the egg diameter was about 80 μm and the capsule about 120 μm (Neves *et al.,* 2010). Castilla and Cancino (1976) reported that the number of eggs/egg capsule varied between 668 to 14250 in gastropod *Concholepas concholepas*. In a study on muricid gastropod *Chicoreus ramosus,* the average number of eggs per capsule ranged between 177.8 ± 3.58 and 214.3 ± 1.61 eggs/capsule whereas egg sizes ranged from 287 × 280.5 to 355.5 × 322 μm (Mahmoud *et al.,* 2013).

The variations in the egg mass morphology and the number of eggs in a capsule are of taxonomic importance as evident from the case of reassignment of *Trophon acanthodes* (Gastropoda:Muricidae) to new genus *Coronium* (Pastorino and Penchaszadeh, 2009). The study of eggs and egg capsules morphology in snails is significant in reproductive biology, dispersal, biogeography, and taxonomy (Pastorino *et al.,* 2007; Pastorino and Penchaszadeh, 2009). The results also suggest that there is a variation in the number of eggs/egg mass in the same species population and there are various factors responsible for variation in egg mass morphology (Kenji,1997; Norton and Bronson, 2006). The results obtained in the present study will be useful in the embryological, evolutionary, and taxonomic studies in freshwater snails.

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