

## Ultra structure of subitaneous and diapausing eggs of planktonic copepod *Sinodiaptomus (Rhinediaptomus) indicus*

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*Sinodiaptomus (Rhinediaptomus) indicus* produces subitaneous eggs in normal condition and diapausing eggs during unfavourable environmental condition. Diapausing eggs occurred both in the sediment samples from wild and laboratory culture system. The structure of subitaneous and diapausing eggs of the freshwater diaptomid *S. (R.) indicus* is described. Scanning electron microscopic (SEM) observations of these eggs showed marked variation in the surface ornamentation. Subitaneous egg has a single type of process, while diapausing egg has two different types of processes on its surface. Transmission electron microscopic (TEM) studies revealed that the plasma membrane of subitaneous egg is surrounded by a thin three-layered outer chorion, compared to highly complex, thick and four-layered outer chorion of the diapausing egg. Variation is also observed with regard to the nature of electron density of its layers.

PRODUCTION of diapausing eggs is a common trait of many species of marine copepods<sup>1</sup> and small temporary lake copepods<sup>2,3</sup>. Freshwater planktonic copepods of the family Diaptomidae are also known for the production of diapausing eggs<sup>3,4</sup>. The temporary lake copepods produce diapausing eggs in spring, apparently to avoid summer dry period<sup>5</sup>, whereas in permanent water bodies diapausing eggs are produced to avoid summer predation by fish<sup>6,7</sup>. Distinction of diapausing egg has been an ever-persisting problem. In many instances it has been difficult to distinguish diapausing egg from subitaneous egg under light microscope, as in the case of *Agalodiaptomus leptopus*<sup>8</sup>, *Onychodiaptomus sanguineus*<sup>10</sup> and *Loptodiaptomus minutus*<sup>10</sup>. However, a certain degree of identification is possible by observing the morphological features such as egg colour of *Agalodiaptomus clavipies*<sup>11</sup> and histological structure of egg chorion as in *Hemidiaptomus ingens*<sup>12</sup> and *Anomalocera patersoni*<sup>13</sup>. It is only by the electron microscope, the nature of the chorion of subitaneous and diapausing eggs can be distinguished reliably from each other<sup>14</sup>.

Morphological studies on calanoid eggs have been carried out on marine species and there is very little information on the egg morphology of freshwater copepod except for the studies on diaptomids<sup>14,15</sup> and centropa-

gids<sup>16</sup>. In spite of rich biodiversity of the diaptomids in freshwater bodies of the Indian subcontinent, diapausing eggs have not been reported for any of the species of this group. In this paper, morphology of subitaneous and diapausing eggs of *S. (R.) indicus*, with special reference to their surface ornamentation and nature of the egg membrane is reported based on SEM and TEM observations.

Multiple generations of *S. (R.) indicus* were reared in the laboratory by feeding with Baker's yeast solution (*Saccharomyces* sp.) and *Chlorella* sp.<sup>17</sup>. In this culture a fraction of the population switched on to the production of diapausing eggs, which were freely released into the medium and sunken to the bottom. The bottom sediment (debris) of the culture tank was siphoned out on alternate days. The eggs from the sediment were separated following the method as described by Chen and Marcus<sup>18</sup>. The sediment samples were suspended in 20 µm filtered water, sonicated for 30 min and filtered through 120 and 50 µm screen (Bolting silk cloth). The eggs and debris retained in 50 µm screen were resuspended in concentrated solution of sucrose (1 : 1, sucrose : distilled water) and centrifuged for 5 min. The material remaining in suspension was again filtered through 50 µm screen and the content was washed thoroughly and transferred to filtered water. Some of the eggs from this were transferred to a glass slide using fine pipette and observed under a compound microscope to study the morphological attributes and egg size. Occurrence of diapausing eggs in fish pond, Chetput hydrobiological station, was also investigated by obtaining sediment core (2 cm diameter, 10 cm length). Eggs from the core were also separated using the procedure mentioned above. Subitaneous eggs were collected from ovigerous female two hours after spawning. The ovisac was removed and eggs were isolated after cut opening the ovisac with the help of fine pair of needles.

The eggs that were separated from the sediment were distinguished from the subitaneous eggs based on diameter and colour. Further, eggs were maintained in the same medium (from which the eggs were collected), eggs that had neither hatched nor had begun to decay within two weeks were designated as diapausing egg. This method is more than 95% efficient in distinguishing egg types<sup>19</sup>. Generally freshwater diaptomids carry the eggs in ovisac (which is attached to the genital segment) until they hatched into free-swimming nauplii. However, eggs are also released directly in the medium (free spawned lose eggs in the sediment), which are found to be diapausing eggs. To test their viability and hatching percentage, these eggs (both collected from Chetput pond and laboratory) were washed and incubated in 100 ml rainwater with continuous aeration and hatching success was observed. Simultaneously, identical batches of eggs were maintained in the same medium (from where the eggs were collected) without aeration, to test their survival.

For light microscopic (LM) studies, eggs were either directly studied or counter stained with haematoxylin and

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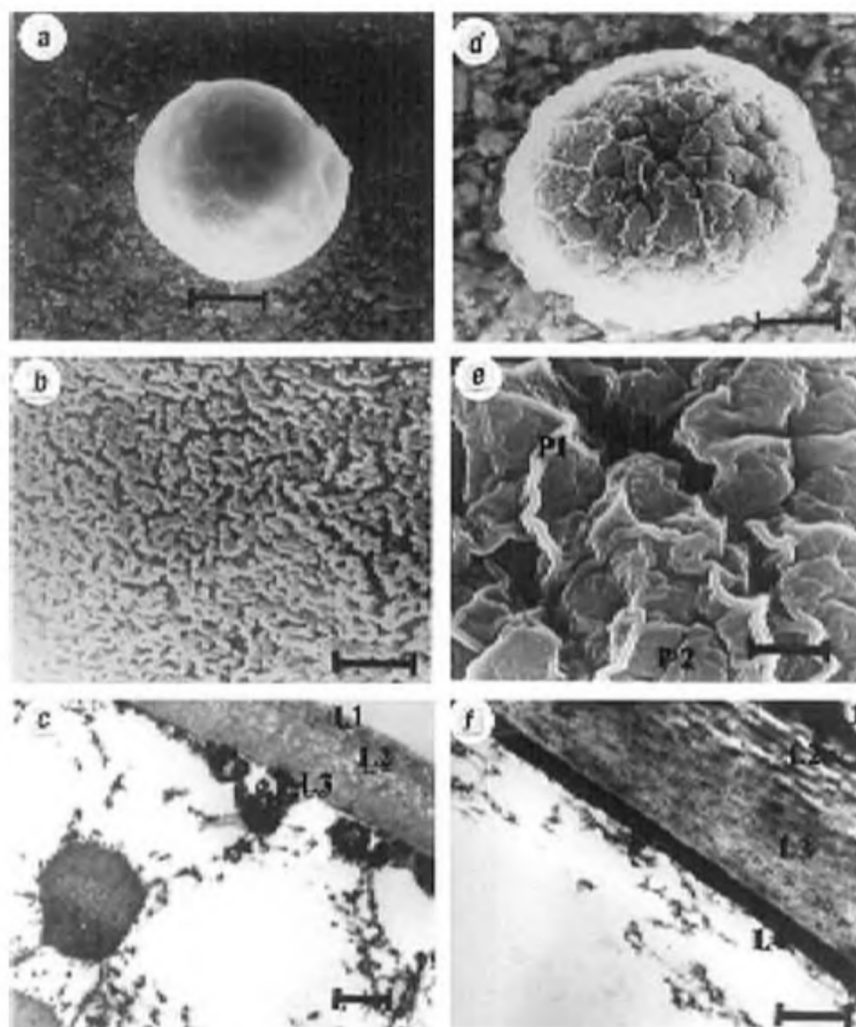
eosin. For SEM studies freshly collected eggs were washed in distilled water and fixed in 2.5% glutaraldehyde solution at 4°C for 48 h and then in 1 M phosphate buffer solution, osmicated in 1% osmium tetroxide for 2 h and dehydrated in ascending series of ethyl alcohol. The speci-

mens were further treated with propylene oxide, air-dried below the room temperature and coated with palladium gold to a thickness of 200 Å in ion sputtering device (JEOL JFC). The specimens were scanned under a scanning electron microscope (Philips 501B Model PSEM) at 15 kV. For TEM studies, propylene oxide-treated materials were embedded in epoxy resin and ultra thin sections of 60 to 90 nm were taken using ultra microtome (ULTRA CUT JEOL). Sections were double stained in lead citrate and uranyl acetate, and studied under a transmission electron microscope (PHILIPS CM 10).

The important differences in the characteristics of subitaneous and diapausing eggs of *S. (R.) indicus* are presented in Table 1. The outer surface of subitaneous egg has small process (minute folds) with distinct space between

**Table 1.** Differences in the structure of subitaneous and diapausing eggs of *S. (R.) indicus*

Characters	Subitaneous egg	Diapausing egg
Appearance	Transparent	Whitish
Diameter	108 µM	90 µM
Surface ornamentation	Single type of processes	Two type of processes
Fertilization membrane	Thin	Very thick
Chorion	3 layers	4 layers



**Figure 1 a-f.** *a*, SEM of *Sinodiaptomus (Rhinediaptomus) indicus* subitaneous egg (bar = 20 µm); *b*, Surface ornamentation of subitaneous egg (bar = 5 µm); *c*, TEM of the subitaneous egg chorion showing three layers L1-L3 (bar = 5 µm); *d*, SEM of diapausing egg (bar = 10 µm); *e*, Surface ornamentation of diapausing egg showing two types of processes P1 & P2 (bar = 5 µm); *f*, TEM of the diapausing egg chorion showing four layers L1-L4 (bar = 5 µm).

them (Figure 1 *a, b*). The outer and inner layers of chorion of these eggs are dense, while the middle layer is less electron dense in nature (Figure 1 *c*).

The first type of process on the diapausing egg are broad and plate-like (Figure 1 *d*), while the second type are thin and blister-like processes which occur over the first type process (Figure 1 *e*). Of the four layers (L1–L4) of diapausing eggs (Figure 1 *f*), the outer and innermost layers are electron dense in nature. The outer layer has a blister-like process which is a fringed structure and forms a projected periphery of the egg. The second and third are less electron-dense layers and the former layer is striated in nature.

It is observed that the early developmental stage of the diapausing egg contains cytoplasm filled with yolk material (Figure 2 *a*). During the course of development, cleavage takes place and as a consequence of this, blastomeres are concentrated towards one pole of the egg (Figure 2 *b*). At the other pole a vacuole is formed, which progressively develops along the periphery of the egg causing the gastrula to get condensed into a small spherical structure (Figure 2 *c*). In this stage, development is arrested and the egg remains dormant. The diapausing eggs collected from Chetpet pond and laboratory showed 54% and 81% hatching success respectively. In many cyclopoid copepods, it is reported that the copepodid instar or even adult secretes an organic, cyst-like covering and remains inactive within under unfavourable conditions like drought and extreme cold<sup>21</sup>.

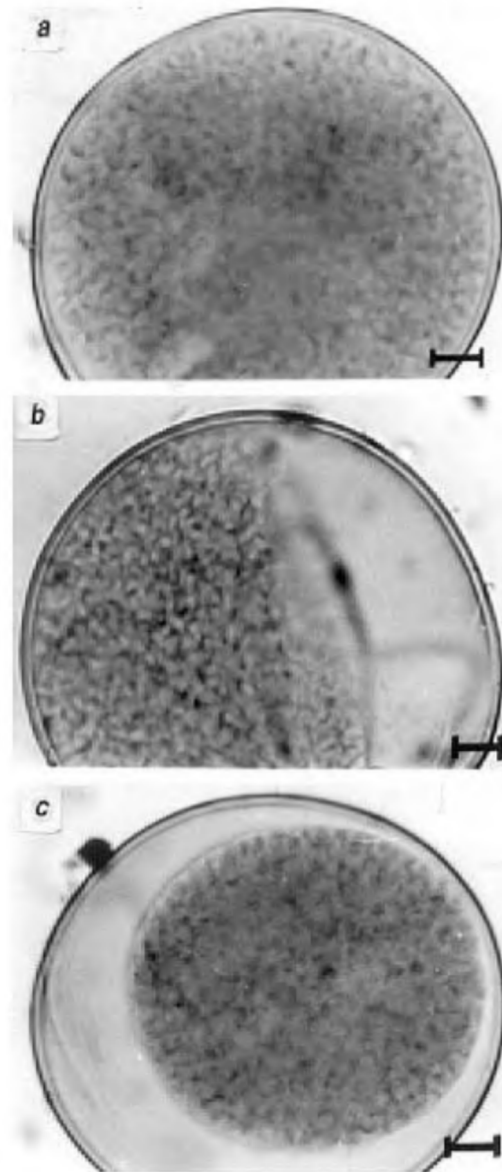
Resting egg production is supposed to be a common life history strategy adopted by the calanoids for survival in unfavourable environments<sup>21</sup>. The production of diapausing eggs in large and permanent inland lakes and ponds may be due to deterioration of environment seasonally without drying up or alternatively, diapausing might be a vestigial trait indicating that the historical origin of population lies in more rigorous seasonal environment<sup>22</sup>. Induction of diapausing egg production may be governed by a number of factors like photoperiod, temperature, food scarcity, over-population, pH, conductivity and gradual reduction of water quality or it may be genetically programmed into the phenotype of these organisms for their sustainability in smaller water bodies.

The size of diapausing eggs of *S. (R.) indicus* was comparatively similar to that of different species of *Acartia* than those of *Epilabidocera amphitrites* and *Tortanus discaudatus*<sup>21</sup>, *Calanus sinicus*<sup>23</sup> and *Pontella mediterranea*<sup>24</sup>. The surface attributes of subitaneous and diapausing eggs of *S. (R.) indicus* do not reveal marked variation under LM examination. SEM examination shows considerable topographical variation. Such variations in the surface attributes have also been reported in the freshwater calanoids *Diaptomus sanguineus*<sup>14</sup>, *Eurytemora affinis*<sup>25</sup>, in marine calanoids *Anomalacera pattersoni*<sup>13</sup> and *Centropages velificatus*<sup>26</sup>.

The outer layer of subitaneous eggs although reveals the presence of small processes, its surface is much smoother compared to diapausing eggs. Many calanoids, in

which eggs are laid in the ovisac are known to possess smooth surface<sup>15</sup>. However the presence of smooth and spiny surfaces among diapausing eggs has been reported in many copepods<sup>21</sup>. It appears that development of egg envelopes and surface ornamentation in *S. (R.) indicus* follows closely the pattern described for diapausing eggs of *Hemidiaptomus ingens*<sup>15</sup>.

The processes similar to the subitaneous and diapausing eggs of *S. (R.) indicus* were reported in A and B type of eggs of *Calanus sinicus*<sup>27</sup>. Compared to this type of ornamentation, the diapausing egg of *Pontella mediterranea* has spiny ornamentation<sup>24</sup>. Apart from the surface



**Figure 2 a–c.** LM of diapausing egg in (a) cleavage stage (bar = 10 µm); (b) early gastrula stage (bar = 10 µm); (c) gastrula stage (bar = 10 µm).

ornamentation, considerable variations were also observed with regard to the thickness of different layers of subitaneous and diapausing eggs of *S. (R.) indicus*. The structure of fertilization membrane in *S. (R.) indicus* resembles that of *Calanus sinicus*<sup>27</sup>. However, this structure differs from that of many other calanoids<sup>14,28,29</sup>. The role of surface ornamentation of subitaneous egg is not clear. The surface ornamentation of the diapausing eggs may be an adaptive strategy<sup>21,26</sup>, to overcome adverse environmental conditions and may prolong the viability of the egg and help in the rejuvenating of the species, once suitable conditions are restored. Further, the diapausing eggs possess a thick chitinous chorion and blisters. The chitinous chorion may permit the egg survive from both desiccation and passage through the gut of predators<sup>14</sup>. The blisters may be concerned with anti-predation. The diapausing egg of *S. (R.) indicus* might be kept dormant by an endogenous factor, which may be inherited from the female<sup>19</sup>. However, hatching of dormant eggs in fresh and aerated water suggests that the exogenous factors may also serve as vital cues for the induction of hatching of the dormant eggs. Further studies on the factor governing diapausing egg production and their hatching induction would be useful for understanding the life history strategies and mass culture of this species for their use as live feed in aquaculture.

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