

Gametogenic cycle in *Villorita cyprinoides* and the influence of salinity

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Abstract. *Villorita cyprinoides* is gonochoristic and showed no signs of sex reversal and hermaphroditism. During the inactive stages of reproductive cycle the gonad is hardly visible through the visceral mass, but at advanced level it observed as a thick transparent tissue through the visceral epithelium. In *Villorita cyprinoides* the reproductive stages is classified into undifferentiated, early gametogenesis, late gametogenesis, mature, partially spent and spent stage. In undifferentiated phase the sexes are not able to distinguish even in smear preparation and histological sections. At both stations the number of females are higher and *Villorita cyprinoides* breeds twice a year, minor spawning during December-February and major during May to July. There is a period of rest between two reproductive cycles; change in salinity is found to be the major factor which triggers reproduction and temperature was not at all a factor governs reproduction. Induced breeding of the species is possible either by sudden drop in salinity or increase in pH. Construction and periodical opening of Thannermukkom bund drastically affects the reproductive pattern of *Villorita cyprinoides*.

Key words: reproductive cycle, gametogenesis, induced breeding, sex-ratio, black clam.

വില്ലോറിറ്റ സിപ്രിനോയിഡ്സ് പ്രത്യുല്പാദന അവയവങ്ങൾ ഉള്ളതും, ലൈംഗിക മാറ്റങ്ങൾ കാണിക്കാത്തതും, പ്രത്യേക ആൺപെൺ വിഭാഗങ്ങൾ ഉള്ളവയുമാണ്. പ്രത്യുല്പാദനനന്തര വിശ്രമാവസ്ഥയിൽ പ്രത്യുല്പാദന അവയവങ്ങൾ തിരിച്ചറിയാൻ കഴിയുകയില്ല. എന്നാൽ പ്രത്യുല്പാദന വേളയിൽ അവയവങ്ങൾ എളുപ്പം തിരിച്ചറിയാം. വില്ലോറിറ്റയുടെ പ്രത്യുല്പാദന സമയത്തെ പല ഘട്ടങ്ങളായി വേർതിരിക്കാം. അവ ഇവയാണ്, പ്രത്യുല്പാദന അവയവങ്ങൾ തിരിച്ചറിയാൻ കഴിയാത്ത അവസ്ഥ, പ്രത്യുല്പാദന അവയവങ്ങൾ ഉണ്ടായി തുടങ്ങുന്ന അവസ്ഥ, അവ നല്ലരീതിയിൽ ഉണ്ടായിക്കൊണ്ടിരിക്കുന്ന അവസ്ഥ, പ്രത്യുല്പാദന അവയവങ്ങൾ പൂർണ്ണ വളർച്ചയെത്തിയ അവസ്ഥ, പ്രത്യുല്പാദനം നടത്തിക്കൊണ്ടിരിക്കുന്ന അവസ്ഥ, പ്രത്യുല്പാദനം കഴിഞ്ഞവ. പ്രത്യുല്പാദനാനന്തര വിശ്രമാവസ്ഥയിൽ ആൺജീവിയെയും പെൺജീവിയെയും തിരിച്ചറിയാൻ സാധിക്കുകയില്ല. പാന്നം നടത്തിയ രണ്ടു സ്ഥലങ്ങളിലും പെൺജീവികളുടെ എണ്ണം ആൺ ജീവികളെക്കാൾ കുടുതലും അവ വർഷത്തിൽ രണ്ടു തവണ പ്രത്യുല്പാദനം നടത്തുകയും ചെയ്യുന്നു. അതിൽ ചെറിയ രീതിയിലുള്ള പ്രത്യുല്പാദനം ഡിസംബർ-ഫെബ്രുവരി മാസത്തിലും, വലുത് മെയ്-ജൂലൈ മാസത്തിലുമാണ്. വെള്ളത്തിലെ ഓരിന്റെ അളവ് കക്കകളുടെ പ്രത്യുല്പാദനത്തിൽ നേരിട്ട് ബന്ധപ്പെട്ടിരിക്കുകയും, താപത്തിന് അതിൽ ഒരു ബന്ധവും ഇല്ലാതിരിക്കുകയും ചെയ്യുന്നു. വെള്ളത്തിലെ ഓരിന്റെ അംശം പെട്ടെന്ന് കുറയുന്നതും അമ്ളാംശം പെട്ടെന്ന് കൂടുന്നതും കക്കയെ ഉത്തേജിപ്പിച്ച് പ്രത്യുല്പാദനം നടത്തുവാൻ സഹായിക്കുന്നു. തണ്ണീർ മുക്കം ബണ്ടിന്റെ നിലനിൽപ്പും വല്ലപ്പോഴുമുള്ള തുറക്കലും ഈ കക്കയുടെ പ്രത്യുല്പാദനത്തെ ബാധിക്കുന്നു.

താക്കോൽ വാക്കുകൾ: പ്രത്യുല്പാദനചക്രം, പ്രത്യുല്പാദന അവയവങ്ങളുടെ ഉണ്ടാകൽ, ഉത്തേജിപ്പിച്ചുള്ള പ്രത്യുല്പാദനം, ആൺ-പെൺ അനുപാതം, കറുത്ത കക്ക

Rezumat. *Villorita cyprinoides* este o scoică gonochorică și nu a arătat semne de inversiune sexuală ori hermafroditism. În timpul stadiilor inactive ale perioadei de reproducere, gonadele sunt abia vizibile în masa viscerală, dar, în stadiile mai avansate, ele pot fi observate ca un țesut subțire, transparent prin epiteliul visceral. La *Villorita cyprinoides*, stadiile reproductive pot fi clasificate în nediferențiat, de gametogeneză timpurie, de gametogeneză târzie, maturat, parțial îmbătrânit și îmbătrânit. În faza nediferențiată sexele nu pot fi distinse nici măcar în preparatele microscopice de tip frotiu sau secțiuni microscopice. La ambele puncte de lucru la care noi am studiat specia, numărul de femele este mai mare, iar *Villorita cyprinoides* se reproduce de două ori pe an, reproduceri puțin intensive, în perioadele Decembrie-Februarie și Mai - Iulie. Există o perioadă de pauză între cele două cicluri reproductive; s-a constatat că modificările de salinitate sunt principalul factor care declanșează reproducerea, iar temperatura pare să nu fie, deloc, un factor important în reproducerea speciei. Reproducerea poate fi indusă fie prin scăderea bruscă a salinității, fie prin creșterea pH-ului. Construirea și deschiderea periodică a barajului Thannermukkom afectează drastic tiparul reproductiv al scoicii *Villorita cyprinoides*.

Cuvinte cheie: ciclu reproductiv, gametogeneză, reproducere indusă, sex-ratio, scoica neagră.

Introduction. *Villorita cyprinoides*, popularly known as black clam, occurs abundantly in all the major estuaries of Kerala. In the Cochin backwater it is found in areas, where the annual variation in salinity is between 0% and 23%. The shell of clam provides the raw material for the manufacture of cement and lime and the flesh forms a source of proteinacious food for a large section of people inhabiting the coastal areas. Clam meat is used for human consumption and also as a protein supplement for prawn and poultry feed. Thousands of families are depending on this species for their livelihood. Annual harvest of clam from the natural resources from India was estimated as 45,412 t (Narasimham 1991). The black clam, *Villorita cyprinoides* tops the clam production forming 67 percent (29,077 t) of the total estimated annual landing. Major production centers in Kerala are the Vembanad and Ashtamudi lakes. Achari (1988) has described the characteristics of clam resources of Vembanad lake and estimated a total production of 2,500 tones of clam. Arun (2002) concluded that the total landings of *Villorita* from Cochin estuary is nearly 1,300 tonnes.

Cochin Estuary extends from about 9° 30' to 10° 20' Lat N and 76° 13' to 76° 5' Long E. This estuary is about 96 km long and 3–4 km wide on an average; it is a part of Vembanadu lake. ie., the largest estuary in Kerala. Thanneermukkom bund (see Plate 1, see also Arun 2009) was constructed (in 1974) to prevent salt-water incursion and to promote two crops of paddy in about 50,000 Ha of low lying fields of Kuttanadu area. The bund has been functional since 1976 and remains closed from January to May every year. This has resulted in a drastic ecological changes in the lake, particularly south of bund (Zone A), affecting the ecology, distribution, survival and abundance of the living resources in the estuary, and causing depletion of many estuarine organisms especially *Villorita cyprinoides* (Plate 2) in several localities. The complex estuarine ecosystem has one perennial opening to the sea at Cochin in the south. Recently the Cochin estuary has undergone vast anthropogenic environmental alterations, leading to an estimated reduction of its extend by about 35% as a result of construction of bunds and reclamation for agriculture, harbour, and urban development.

Knowledge of reproductive behaviour and factors determining breeding are central to the understanding of life history, ecology and suitability of organism for culture. It also helps in predicting animal recruitment and their growth, which leads to successful aquaculture or management of commercially important bivalves. The sexes are separate in Pelecypoda (Frether & Graham 1976) and eggs and spermatozoa are released into water where fertilization takes place (Webber & Giese 1969). Works on estuarine bivalve molluscs in Indian waters have shown that different species fall into different categories ranging from animals having prolonged or continuous breeding period to those having restricted spawning season. Rand (1973), Giese & Pearse (1974) have postulated year round spawning activity for tropical marine organisms, though various exogenous factors which initiate and synchronize breeding activity, also restrict it to certain periods of the year. "Reproductive difficulty" has been considered a major factor limiting species distribution (Hutchins 1974; Fritchman 1962).

The major objectives of this study are (1) to trace the annual gametogenic pattern in *Villorita spp.* (2) to elucidate the probable influence of salinity, temperature and other important factors on reproduction.

Materials and Methods

Hydrographic Parameters. Regular fortnightly sampling was carried out from zones A and B (situated on either side of bund) for a period of two and half years (November 2005 to October 2008). Only bottom samples were taken for the study. All the analysis was carried out by standard procedure, temperature (ordinary thermometer), salinity by Mohr's titration method (Strickland & Parsons 1968) and sediment texture by Carver (1971).

Histology. During 2005–2008 *Villorita cyprinoids* samples of different sizes were collected weekly from the clam bed. The clams were maintained in the laboratory for 24 hours in ambient water. Detailed microscopic observations of individual gonads through histological techniques were made in both sexes for the description and classification of the developmental stages of the gonad. Hundred animals arbitrarily selected with respect to age and visible stages of gonad development and fixed in Bouins fixative for 24 hours. The tissues then washed for 5 minutes in running water, dehydrated in graded ethanol, embedded in paraffin wax, cut into serial sections of 5 μ and spread on slides smeared with Mayer's albumen. Slides were stained using Mayer's hematoxylin, counterstained with eosine and mounted in D.P.X mounting media. Slides were examined under light microscope and classified into different developmental stages. Examination of the sections was made from sample preparations at regular intervals furnished detailed information on the reproductive cycle including the actual period of spawning in *Villorita cyprinoides*.

Smear Preparation. Mature clams after maintaining in the laboratory for 48 hours were employed for sex determinations. Clams were cut open and simple smears were prepared by taking samples from gonad area. These smears were examined under microscope and the sex was determined.

Induced Breeding. Induced breeding experiments were conducted with either sudden increase in pH or decrease in the salinity. After maintaining the clams in higher ambient salinity (10–12ppt) in the laboratory for one month induced breeding experiment was carried out. Clams were fed with *Chlorella* in sufficient quantity (1000 ml/50 organisms, twice in a day, con.10⁷ cells/ml) regularly. To drop the salinity rapidly, ordinary tap water was used; and for change in pH 1 N NaOH was used.

Results. Temperature varied between 33.8⁰C and 27.1⁰C at zone A and 33.4⁰C and 26.1⁰C at zone B (Figure 1), Zone A has comparatively low average temperature than zone B especially during pre-monsoon (closed period of Thaneermukkom bund). Zone B showed high salinity fluctuation when compared to zone A (P>0.001) especially during the closed period of bund. Salinity varied from 0 ppt to 8.54 ppt at zone A and 0 ppt to 10.18 ppt at zone B (Figure 2). At zone A, from January to April there was an increase in the percentage of silt and clay and corresponding decrease in sand (Figure 3–5; Plate 3). At zone B the sediment was mainly sandy.

Description of Gonadal Development. Morphology of Gonad. Visual observation of gonad area showed that during the inactive stages of reproductive cycle the gonad was hardly visible through the visceral mass, but at a slightly more advanced stage it could be observed as a thick transparent tissue through the visceral epithelium. During advanced stages of gametogenesis the gonad diffused into the visceral mass and occupied a major part of it as well as small portion of the foot. The epithelial wall of the viscera at this stage was opaque and ivory in colour. The genital tissue did not invade the mantle lobes. At spawning and post-spawning periods the visceral mass became translucent and no sexual differences were observed externally, both male and female gonads had the same colouration and morphology.

Indifferent Phase or Undifferentiated Phase (Plates 4 and 5). During early development, gonads of male and female clams were indistinguishable even in histological examinations. The term indistinguishable, hence forth referred to as indifferent, applied to gonads of those specimens that could not be sexually differentiated i.e., with low levels of spermiogenic or ovogenic activity and correspondingly low levels of recognizable sex cells. Either spermatogonia or oocytes are required to determine sex

with certainty. Most of the gonad consists of interfollicular connective tissue or Leydig cells, which are specialized for lipid and glycogen storage.

Each follicle was bound by an outer follicular wall and the it was usually expanded and wall was dominant, where the gametogenesis occurred. In the early stages of development, all regions of the gonad look similar, as the gross anatomy of gonadal follicle of both the sexes were similar. No gametogenesis was discernible and the sex was indistinguishable. However the individuals failed to reveal their true sex even in freshly prepared smears. This indicated that these indeterminates may be either males or females in dormant stage or in the stage of minimum gametogenic activity.

Male – Early Spermatogenesis (Plate 4). Gemetogenesis in male clams began only after the follicles were cleared off residual matter of the previous spawning. The gonad rests here before meiosis and it is composed of connective tissue cells. Gametogenesis started after the proliferation of follicles and gametes developed from the stem cells present in the follicle cells. Most of the spermatids were found to form lightly staining centripetal bands inside the follicle. Fully developed spermatozoa were not seen at this stage. Proliferation of follicle became more conspicuous and interfollicular tissues were present but reduced and definite spermatogonia and spermatocytes were separated from follicle cells. Increase in gonad size making digestive gland to have a restricted space; gonad somewhat flabby; containing water; definite spermatogonia and spermatocytes appear from follicle walls. Primary and some time secondary spermatogonia were in close contact with the germinal epithelium. No spermatozoa was present.

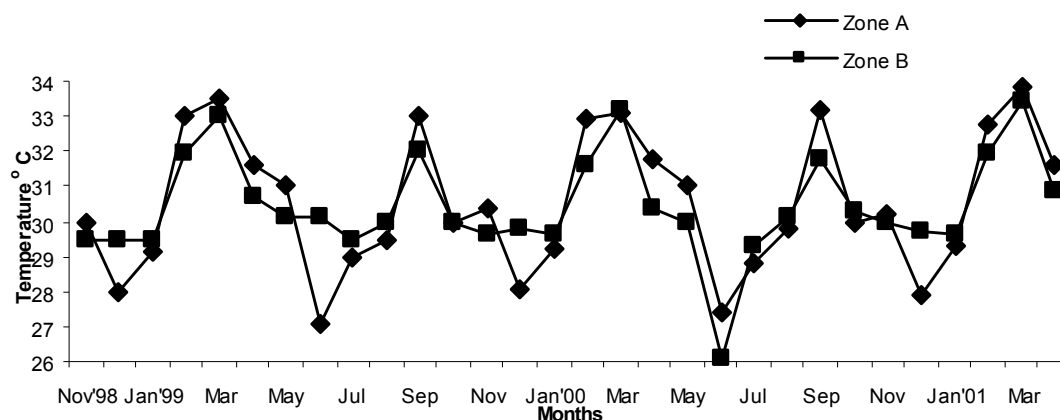


Figure 1. Monthly variation in temperature at Zone A and B.

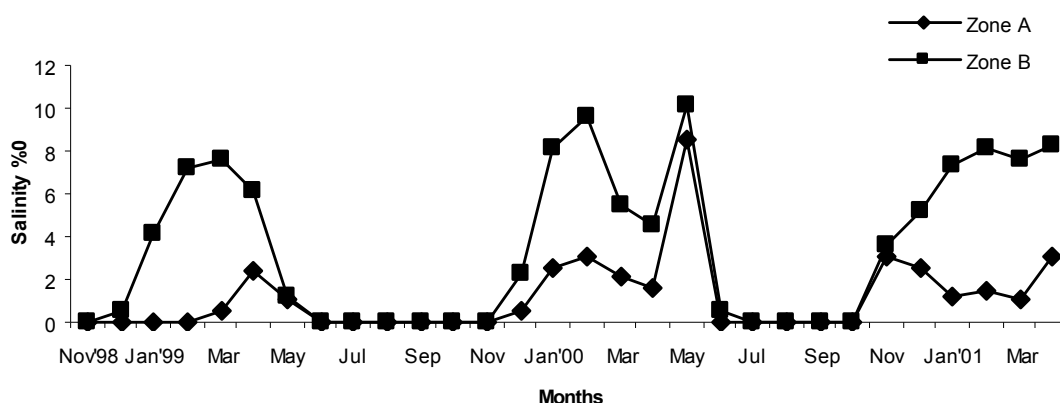


Figure 2. Monthly variation in salinity at Zone A and B.

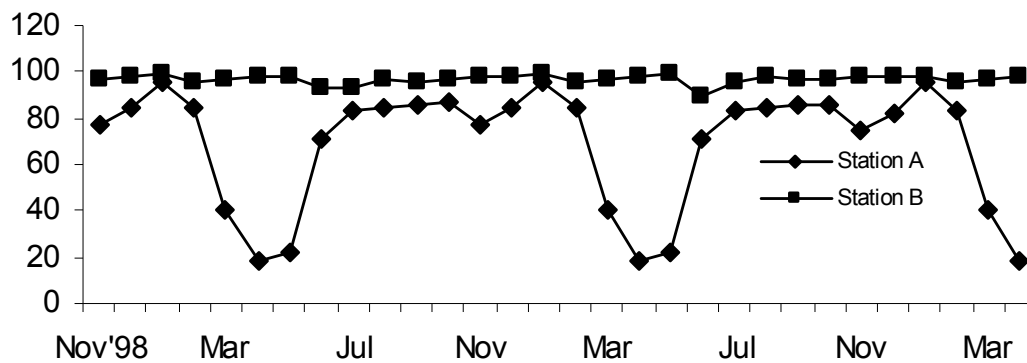


Figure 3. Monthly variation in % of sand at Zone A and B.

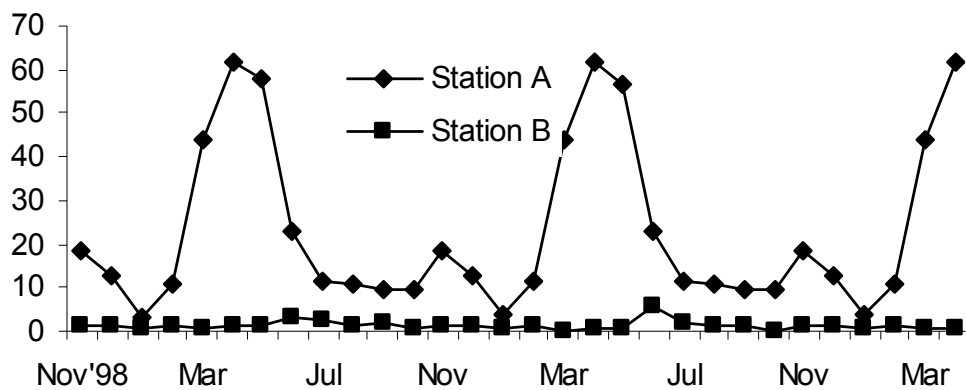


Figure 4. Monthly variation in % of silt at Zone A and B.

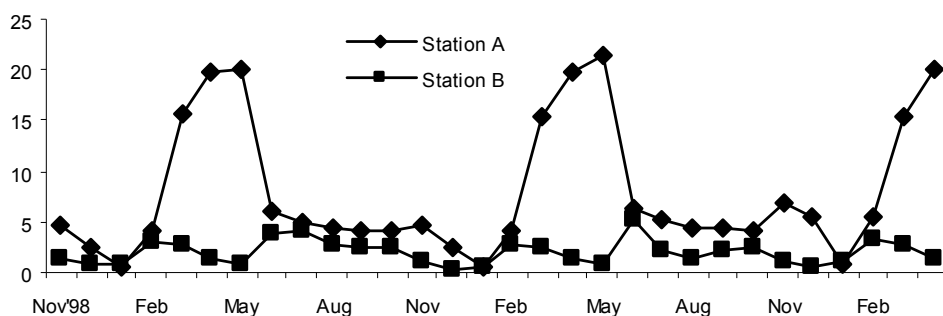


Figure 5. Monthly variation in % of clay at Zone A and B.

Male – Late Gametogenesis (Plate 4). In late gametogenic stage, most of the spermatocytes were observed to have developed into spermatids, which in turn were in the process of development into spermatozoa. Spermatozoa which developed were assembled at the centre of the follicles. There was a rapid turnover of cells, the stages between primary spermatocyte and spermatozoa being of very short duration. The

follicles deeply penetrated the visceral mass and the follicular walls contained predominantly spermatogonia with spermatocytes and a few spermatids radiating in to the lumen of follicle. In male follicles, lumen contained free spermatozoa, which were becoming closely packed, and spermatogonia arranged in centripetal bands; free spermatogonia and spermatocytes remain near walls of follicles, showed the characteristic swirling pattern of spermatozoa.

Male – Mature (Plate 4). The male gonad was termed mature when the follicle contained mainly spermatozoa. In keeping with the rapid increase of follicular content, the follicle wall expands and the connective tissue is occluded so that walls of adjacent follicles are opposed to each other. Spermatozoa aggregate in bands projecting into the lumen with their basophilic heads directed towards the periphery and sperm tail directed away from the wall towards the center. No spermatocytes were seen during this stage, when gonads were filled with spermatids. Clams were in a ready-to-spawn condition at this stage. Lumen of large follicles wall contained spermatozoa, which sometimes lose continuity with rest of cell content and sometimes form “plug”. Gonad became creamy in colour, full and plumpy.

Male – Partially Spent or Early Regression (Plate 4). In some follicles, the lumen was often seen empty due to the discharge of sperms while in other follicles, gametogenesis continued and the central part of the follicle was still filled with spermatozoa. Connective tissue started developing between the follicle. Spermatozoa tend to concentrate towards the centre of the gonad, forming thick bands. Gonads thin, collapsed, flabby, loose in consistency and grayish in colour.

Spent stage or Late regression (Plate 4). Spawning was almost complete and follicles were empty except for a few residual gametes and were filled with a watery fluid. Reabsorption of residual gametes was in progress. Gonads of this phase were characterized by contracted follicles and residual spermatozoa in the process of being cytolysed. Interfollicular tissue appeared to occupy the space between the follicles.

Female – Early Oogenesis (Plate 5). The earliest identifiable female follicles contain oogonia, primary oocytes and follicle cells. The follicles are still shrunken and leydig tissue predominates in the gonad. Sex differentiation starts with the differentiation of the germ cells in the connective tissue. Oogonia are the initial female germ cells which proliferated from the large resting cells, ‘the stem cells’ found around the follicular wall. Gonad was somewhat flabby; enlarged in size, interfollicular tissue still present but getting reduced.

Female – Vitellogenesis (Plate 5). Oocytes get attached to the follicle walls by means of stalks and they attained pear shape. Thin vitelline membrane was seen around some oocytes. Oocytes were seen to increase in size and yolk accumulated. Follicular wall was lined with very few oogonia and young oocytes, interfollicular tissue was seen. A small nucleus began to appear in the center, very few follicle walls still having primary germ cells. As yolk accumulates, the cytoplasmic volume increases and it loses its basophilic nature though the nucleus is still clear. Vitellogenesis commenced soon after the nucleus enters the vegetative phase.

Female – Mature (Plate 5). Follicles fully developed, filling the whole lumen and were completely filled with mature oocytes of maximum size. The gonad was considered mature when the number of detached oocytes was greater than the number of attached oocytes. Growth of the lightly packed follicles obliterates the interfollicular connective tissue. Gonad became creamy, plumpy and follicles were closely packed without interspace.

Female - Partially Spent Stage (Plate 5). Active discharge of ripe ova takes place and as it proceeds, the central portion of the follicle remains vacant. This stage was characterized by the reduction in density of ova. As the release of oocytes continues, free space appeared towards the distal part of the tightly packed follicles with most of the follicles remaining empty and few collapsed. Gonad was flabby, loose in consistency and colour turned to gray.

Female - Spent Stage (Plate 5). Almost all oocytes were found to be released and the follicles contained a watery fluid and few residual oocytes which were found to be in the

process of reabsorption. Females were not complete spawners and residual oocytes were always found in the spawned gonad. Gonad remarkably shrunken and distorted.

Sex-ratio. It is difficult to determine sex ratio during the spent and early gametogenic phase, because females often retain oocytes and thus the observed sex ration would be biased in favour of females with many males being classified as indifferent. Besides this, during non-breeding season (6 months) it is not easy to detect sex by any method. To overcome these the sex ratio was determined by computing only those samples during the mature and spawning periods when majority of the clams could be sexed from smear examination. It has been observed that females were more numerous (54.6%) than males (45.4%) at zone B and at zone A it was 50.2% (females) and 49.8% (males) (see Table 1).

Table 1

The sex-ratio of the studied species

<i>Locations</i>	<i>Number of individuals</i>	<i>Males</i>	<i>Females</i>
Station I	500	249	251
Station II	500	227	273

Annual Reproductive Cycle. In annual reproductive cycle a minor spawning occurred during late December to February and a major spawning during late May to July – ie., two spawning were demarked by a resting period between them. The annual reproductive cycle of this species studied over two and half years showed some sort of similarity in the development of gonads in two sexes.

During April, the clams were in late gametogenesis phase, most of the clams were in the developing or mature condition. In early May mature individuals constituted the majority, but in late May reproduction started and population contained both mature and partially spent individuals. Almost equal percentage of mature as well as spent clams was observed during June. During July, majority of the population was in a fully spent condition. Spawning during this period (late May to July) coincided with the onset of south-west monsoon. Salinity during late May to July is supposed to be dropped due to monsoon showers.

After a break of few months, during September-October most of the clams observed were in indifferent stage, only few organisms were in developing stage for next breeding. During November clams with mature gonads increased in number and during late November and early December mature clams in late gametogenesis phase were noticed, whereas during late December mature and partially spent individuals contributed to the major share. The peak in second reproduction was observed to be during January, which coincided with the arrival of saline water in to the clam bed. It has been observed that both reproductive periods coincide with either sudden decrease in salinity (late May-February) during Monsoon showers or increase in salinity (late December-February) due to the intrusion of saline water in to clam bed.

Induced breeding. Early success in the induced breeding of *Villorita spp.* was achieved by decreasing the salinity suddenly (10 - 12 ppt to 0 ppt) or increasing the pH from 7 to 8.5.

Discussion. According to Coe (1943), about 965 of the species included in the class Bivalvia have separate sexes eg. *Donax cuneratus* (Nagabhushanam & Talikhedkar 1977a), *Marcia opima* (Maqbool 1993) and *Donax incarnatus* (George 1998). Black clam *Villorita spp.* found to be gonochroistic, showed no sings of sex reversal and hermaphroditism. The classification of different stages in the reproductive cycle in *Villorita spp.* was very similar to the allied genus of the bivalve. It showed different maturity stages, which is prominent in their reproductive cycle namely early gametogenesis, late gametogenesis, mature, partially spent and spent stage. This is in agreement with the classification of Nagabhushanam & Mane (1975a) and Katticaran (1988).

The pattern of reproduction of *Villorita cyprinoides* with two spawning peaks per annum is exhibited by other tropical bivalves like *Donax faba* (Alagarswami 1966), *Katelysia opima* (Nagabhushanam & Mane 1975ab), *Mercenaria spp.* (Hesselman et al 1989), *Villorita cyprinoides* (Modassir 1991) and *Marcia opima* (Maqbool 1993). It has been observed that there was a period of resting (1-2 months) between two reproductive cycles in *Villorita cyprinoides*. Gonad of bivalves usually is seen in a resting stage after spawning (Loosanoff 1962). Nagabhushanam & Mane (1975a) in *Katelysia opima*, Salih (1977) in *Meretrix casta*, Maqbool (1993) in *Marcia opima*, and Victor & Subramaniam (1988) in *Donax cuneatus* noted a period of rest after each reproduction and before the next gametogenesis.

Sastry (1968) noted that reproductive cycle of a species as a genetically controlled response to components of the environment especially temperature, salinity, light, food and endogenous factors. In higher latitudes, variation in temperature has been recognized as the main stimulus for maturation and spawning (Galtsoff 1930, 1932; Loosanoff 1937; Chipperfield 1953; Giese 1959; Bayne 1975). However in a tropical environment, temperature is relatively stable and variations between maximum and minimum is too insignificant to elicit major physiological responses. It may be suggested that temperature may not play a direct role in the maturation and spawning of bivalves of Indian waters (George 1998). But in tropical estuaries and coastal waters salinity variations have direct influence on spawning (Nagabhushanam & Talikhedkar 1977ab; Victor & Subramoniam 1988; Narasimham 1988; Maqbool 1993). Gonadal development especially in *Villorita cyprinoides* from the Cochin estuary coincides with ambient salinity changes. It possesses two peaks of spawning (1) late December to February (2) late May to July. The first peak coincided with sudden increase in salinity due to the intrusion of saline water from sea and the second with the sharp decrease in salinity during early monsoon showers. Second spawning takes place during May – July, the rapid gametogenesis for this phase of reproduction take place during April – May coincides with higher ambient salinity. This result confirms without doubt that salinity is a major factor, which controls maturation and spawning in *Villorita cyprinoides*. Higher salinity has been found to stimulate spawning in *Donax cuneatus* (Rao 1967; Nagabhushanam & Dhamne 1977; Victor & Subramoniam 1988) in *Paphia laterisulca* (Nagabhushanam & Talikhedkar 1977b) in *Marcia opima* (Maqbool 1993). Salinity variations acting as a “natural spawning stimulus” (Panikkar & Aiyar 1939). Qasim (1956) formulated “Crisp’s Rule” to signify the relationship between spawning season and availability of food for larvae. Here one reproductive peak is during May - June, once reproduction happens during this period the young ones will get sufficient quantity of food during the phytoplankton bloom produced by South-west monsoon during late May to September.

Environmental factors are found to influence the sex ratio of the clam. Increased organic load can be noted as a common feature for male dominance in the clam population (Maqbool 1993; Ajithkumar 1984). Percentage of male was higher at Zone A compared to Zone B, where accumulation of organic matter occurred during the closure of Thanneermukkom bund. It is concluded that regular opening and closing of thanneermukkom bund leads to variation in salinity, which significantly affects the reproduction of *Villorita cyprinoides* in the Cochin Estuary. This study gives a basic knowledge, which helps the fishermen community to practice clam ranching experiment of *Villorita cyprinoides* in Cochin Estuary.

Conclusion. *Villorita cyprinoides* did not show any signs of sex reversal and hermaphroditism. In *Villorita cyprinoides* reproductive stages is classified into undifferentiated, early gametogenesis, late gametogenesis, mature, partially spent and spent stage. It is very difficult to identify sexes during undifferentiated phase, even in smear preparation and histological sections. *Villorita cyprinoides* breeds twice in a year, minor spawning during December-February and major during May to July. Change in salinity is found to be the major factor which triggers reproduction. Induced breeding of the species is possible either by sudden drop in salinity or increase in pH. Construction and periodical opening of Thanneermukkom bund drastically affects the reproductive pattern and survival of the larval stages of *Villorita cyprinoides*.

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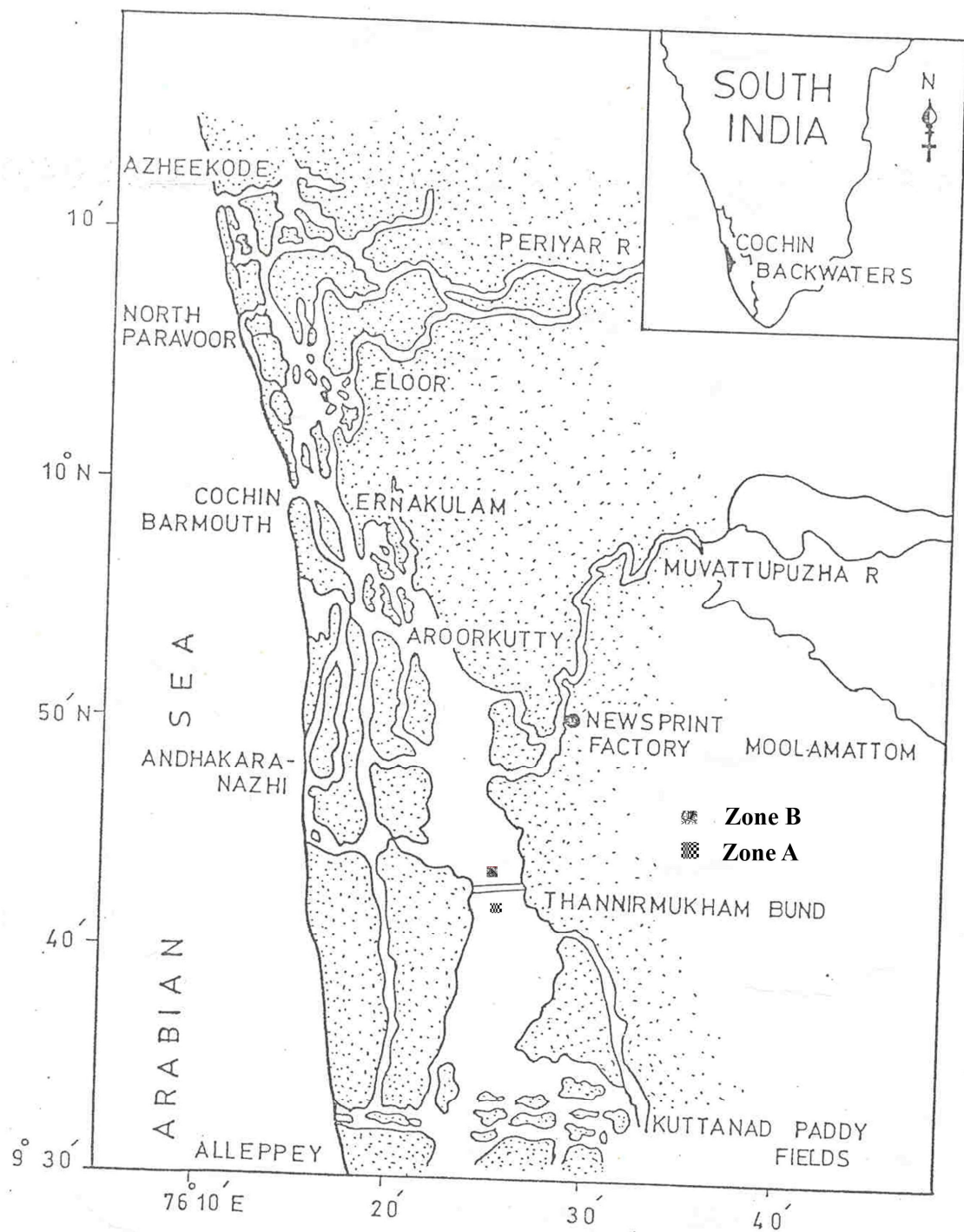


Plate 1. Thanneermukkom bund



Villorita cyprinoides



Plate 2. The black clam, *Villorita cyprinoides*

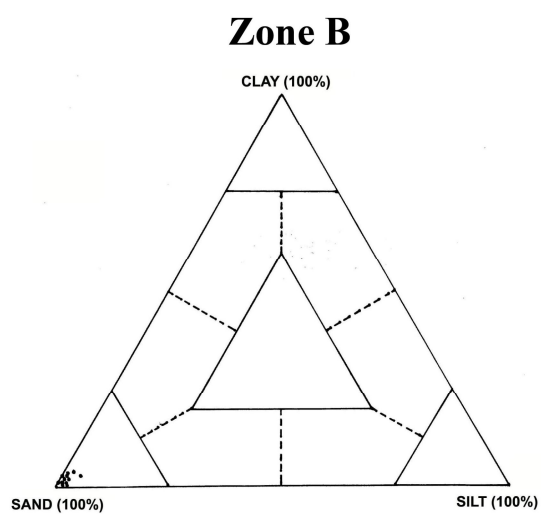
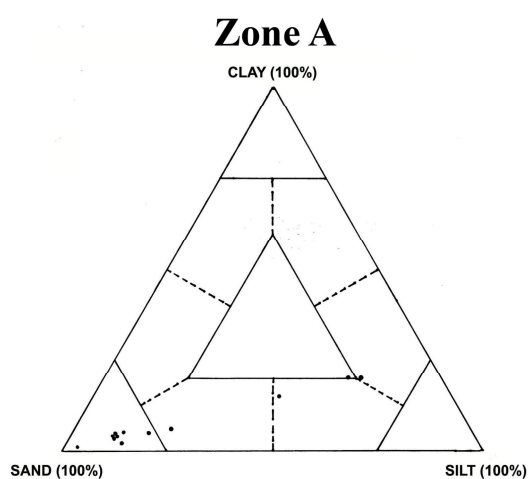
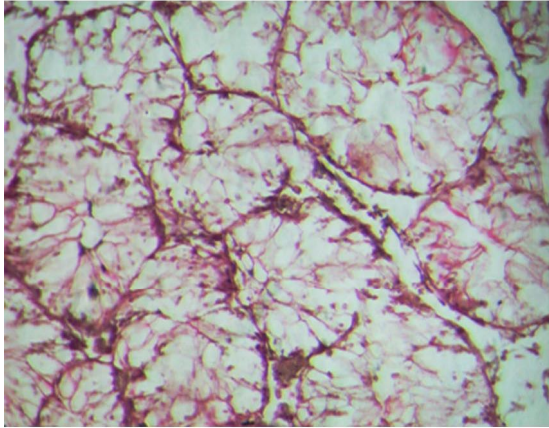
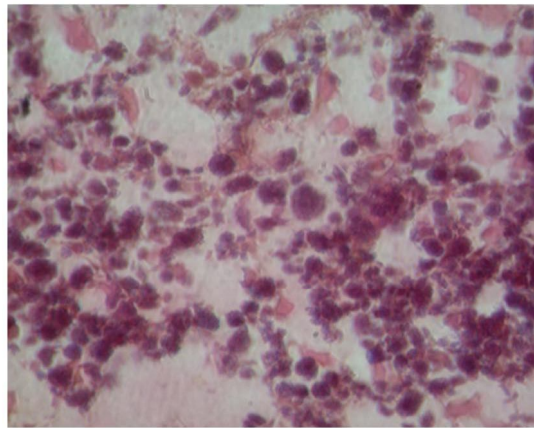


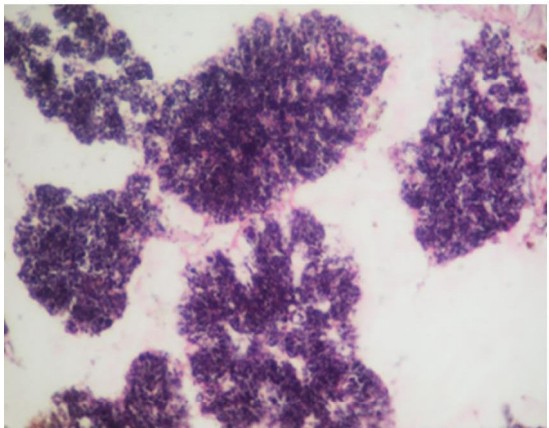
Plate 3. Sediment Texture at Zone A and B



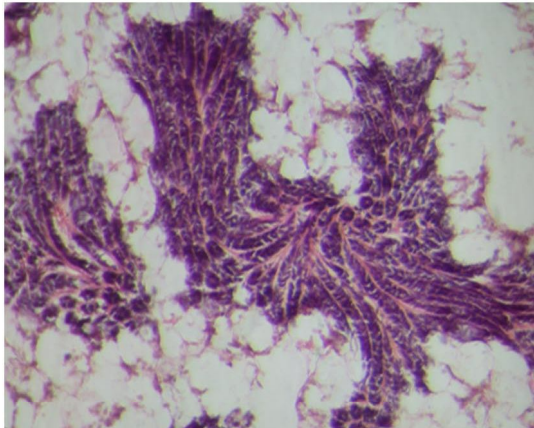
UNDIFFERENTIATED PHASE



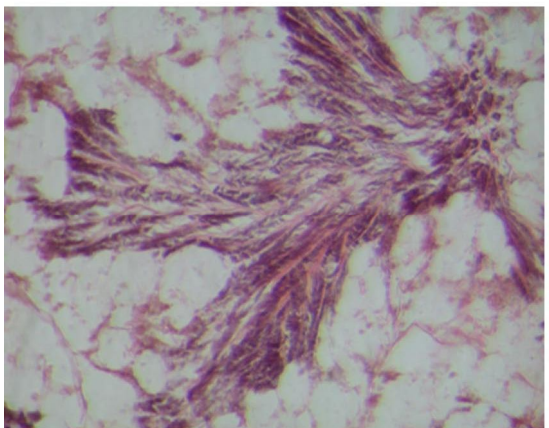
EARLY SPERMATOGENESIS



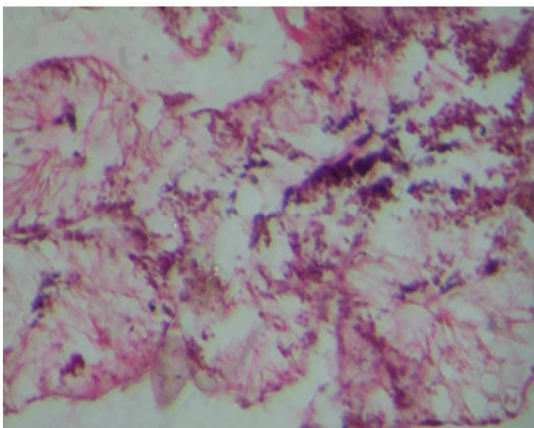
LATE SPERMATOGENESIS



MATURE GONAD

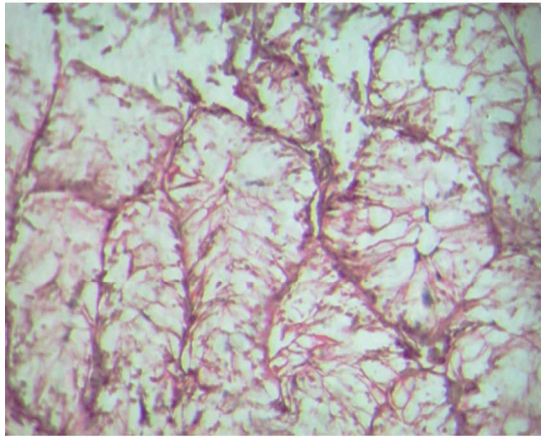


PARTIALLY SPENT GONAD

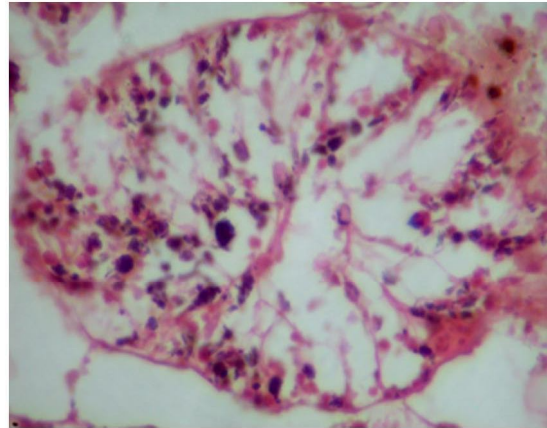


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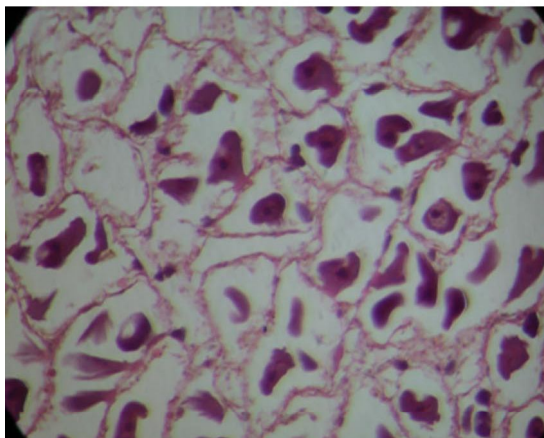
Plate 4. Male reproductive cycle



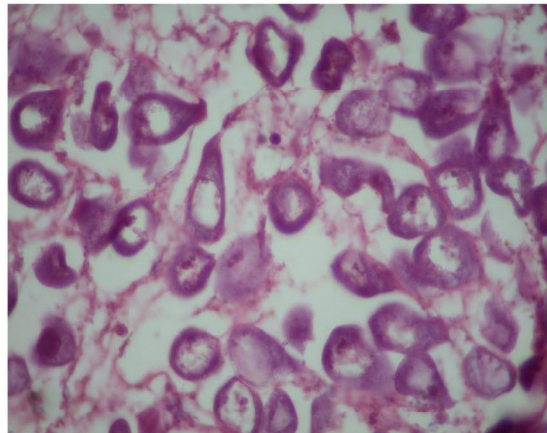
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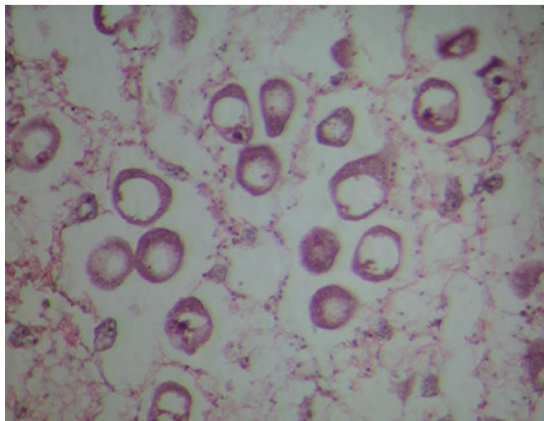
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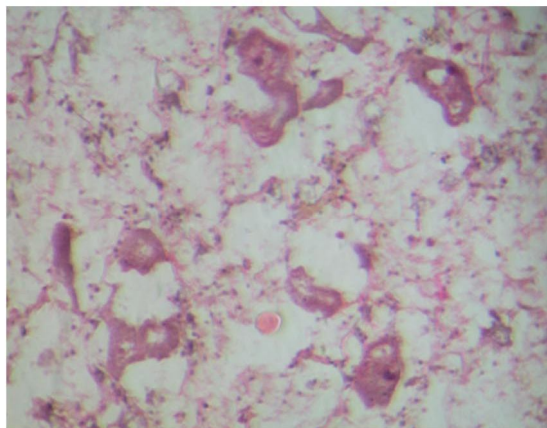
VITELLOGENESIS



MATURE OVARY



PARTIALLY SPNT OVARY



SPENT OVARY

Plate 5. Female reproductive cycle

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