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RESEARCH ARTICLE

GONADAL DEVELOPMENT IN *MAYDELLIATHELPHUSA. MASONIANA* (HENDERSON) USING MACRO AND MICROSCOPIC TECHNIQUES FROM GHO MANHASAN STREAM OI J&K STATE, INDIA

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ABSTRACT

The gonadal development in fresh water Crab *Maydelliathelphusa masoniana* (Henderson) is being described based on macrosophic and microscopic analysis for a period of two years viz (Jan 2012- Dec 2013). *Maydelliathelphusa masoniana* (Henderson) is a biannual breeder exhibiting two breeding peak seasons (June-July & Dec-Jan). The gonadal stages observed macroscopically by volume and colour were validated through histological analysis and proved to be useful method for the rapid identification of sexual maturity in the species.

Size at sexual maturity was 3.5 cm and 4.5 cm of carapace width for females and males respectively. The histological description was based on 48 specimens (24 each sex). Four gonadal development stages were found for females: immature, ripening, mature and spawned. Three development stages were found for males immature, maturing and mature.

The present study offers first report on histological aspects of *M.masoniana* under study since no work on this aspect of the species is on record from Jammu region.

INTRODUCTION

Reproductive cycles of a crustacean include a series of morphological & physiological events. This cycle is basic to all sexually reproducing crustaceans but the time relationship between certain events and duration of these events varies for different species.

The gonads of fresh water crabs are located in the cephalothoraxes above the hepatopancreas. The reproductive system in males consists of a pair of testis, a pair of vas deferens, 2 pairs of gonopods (modification of the first and second pair of pleopods) and a pair of penises (Cumberlidge 1999). The female reproductive system consists of a pair of ovaries, a pair of spermathecae, which terminate into two genital pores. After copulation the ovules develop, resulting in an increase of the ovary size and a change in colour depending on the development stage & species (Chen *et al* 1994, Rostant *et.al* 2008).

Taking into account, the degree of gonad development, the macroscopic characterization of the gonads among decapods have been commonly investigated to determine the onset of the sexual physiological maturity or histological maturity. (Costa & Negreiros – Fransozo, 1998; Castiglioni & Santos, 2001; Castiglioni & Negreiros-Fransozo, 2006).

Morphological gonadal variations are the index of underlying histological changes, therefore present investigation was done to study macroscopic & microscopic analysis of gonad development of *M. masoniana*. Review of literature on reproductive status of these lesser known crabs reveled that not much is available on detailed histology variations.

M. masoniana fresh water crab from Jammu region of J&K (North India) has remained unexplored, barring a few reports on its occurrence & abundance (Gupta, 2012) nutritional status (Manhas, 2012) & parasitic infection (Anjum, 2012). A lacuna is clearly evident particularly with regard to the various biological aspects related to the maturity and breeding of the species in general and reproductive biology in particular. The present studies therefore were under taken with the main objective of histological description of the gonads of *M. masoniana*.

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MATERIAL AND METHODS

The specimens of *M.masoniana* were sampled from Gho-manhasan stream, a tributary of river Chenab, J&K State India, for period of two years on monthly basis viz Jan 2012- Dec 2013. For sampling crabs were collected by following procedure of catch per unit effort (C.P.U.E) and live specimens were brought to the laboratory. In the laboratory crabs were anaesthetized by deep freezing them for 30 minutes. They were then washed and segregated sex wise. The size of male and female crab i.e. carapace width (CW) was measured with vernier calliper scale. The study is based on a total of 48 specimens per year (24 each sex). After making macroscopic observations they were dissected out for microscopic studies in order to observe seasonal variations in gonads on monthly basis. The gonadal developmental stages for both sexes of *M. masoniana* were investigated by following methods.

(a) Macroscopic examination of the consistence, volume and coloration of the ovaries and testis in relation to thoracic cavity (Mantelato & Fransozo, 1999)
(b) Microscopic examination of cell types (Castilho *et al.* 2008)

For macroscopic & microscopic analysis 24 gonads of each sex were randomly selected from specimens ranging in CW from 2.0 to 5.5 cm, encompassing maturity to spawned specimens. For macroscopic analysis, the shape, size and coloration of gonads were analyzed & photographed & classified as immature, maturing, mature & spawned following the pattern of Haefner (1976) and Fransozo *et al.,* (2002) For histological description, gonads were fixed in Bouins fixative for 24 hrs, at 20^0C. After post fixation treatment gonads were kept in 70% alcohol and subsequently dehydrated through alcohol series (70% to 100%), diaphanized in xylol, infiltrated and embedded in paraffin. Finally, the sections were prepared using a microtome (3 to 4 µm) and stained using haematoxylin-eosin. The thin sections of ovaries and testis were analyzed with an optical microscope and photo micro graphed using Olympus CH20iBTMF attached with Sony SSC-DC 378P Camera.

RESULTS

Seasonal variations on morphology of gonads of male and female crabs of *M. masoniana* with respect to size and colour have been found to be given in Table 1:-

Table 1 Display the size data for each sex (N=24) in different developmental stages on monthly basis (Jan 2012-Dec 2013)

Months	Developmental Stages	Carapace Width (Cm)	
		Females (Min - Max)	Males (Min - Max)
Jan- Oct	Immature	2.1 - 2.9	3.0 - 3.5
Feb- May, Nov	Ripening/Maturing	3.0 - 3.4	3.5 - 4.2
June-July, Dec-Jan	Mature	3.5 - 5.0	4.5 - 5.5
Aug- Sept, Feb - March	Spawned/Spent	5.2 - 6.0	-

Table 2 Macroscopic & Microscopic variation in size, volume, colouration and cell type in different gonadal stages of females crabs (N=24)

Development Stages	(Size) Carapace Width	Volume occupied	Colour	CellType (Histology)
StageI (Immature) (Jan-Oct)	2.1-2.9	1/6thofthe Body cavity	Transparent	Oocytes absent Rounded oogonioa and follicular cells present
StageII (Maturing) (Feb-May, Nov)	3.0-3.4	1/4thof the body cavity	White to yellowish	Oocytes surrounded by follicular cells with flat aspect visible
StageIII (Mature) (June-July, Dec-Jan)	3.5-5.0	Full body cavity	Orang red colouration	Mature oocytes with few follicular cells present.
StageIV (Spawned) Aug-Sept, Feb-March	5.2-6.0	¼ of the body cavity	Pale yellow colouration	Atresic oocytes visible.

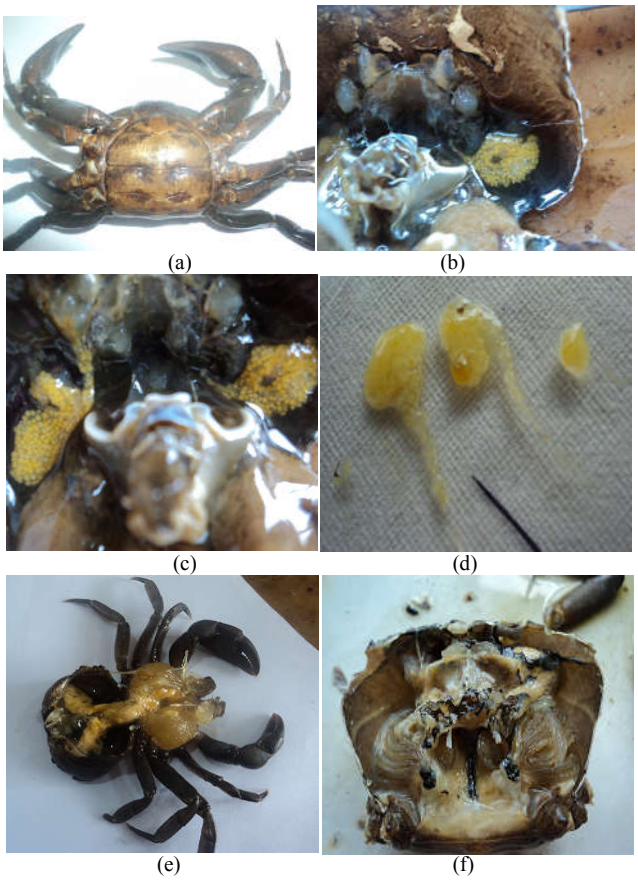

Fig 1 (a) Dorsal view of Female *M.masoniana* (b & c) Thoracic showing ovaries at different stage of reproductive cycle. (d) Yellow coloured ovaries (e) Dorsal view of Male *M.masoniana* (f) Thoracic cavity showing male reproductive part with whitish colouration and Gelatinous aspect.

From table 1, it is evident that carapace width of the specimens ranged from 2.1-6.0 Cm Cw. The size at sexual maturity was found to be 3.5 cm CW for females and 4.5 cm CW for males. Mature individuals within the range of mean size 3.5 cm (females) and 4.5 cm CW (males) were evident only during the monthly collection of June-July and December-January. Macroscopically, the extent of occupancy of body cavity by gonads as given in table 2 and 3 clearly reveled that difference

in gonadal stage varied in accordance with size colouration & volume occupied in the body cavity.

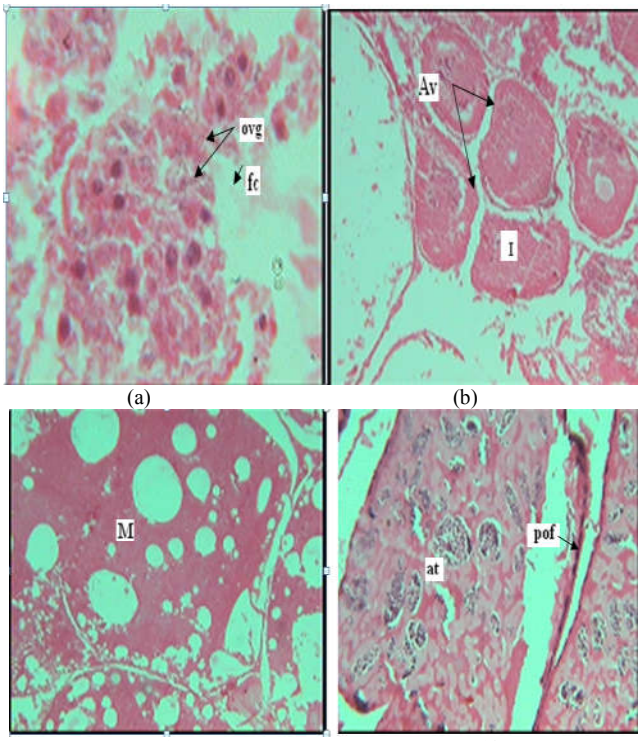

Fig: 2 Ovary of the Crab *M.masoniana*; a immature stage: presence of ovagonia (ovg), follicular cells (fe), 400x, b ripening stage: presence of occcytes in initial vitellogenesis (1) and advanced vitellogenesis (Av), 100x; **c** mature stage presence of mature oocytes (M), 100x; **d** spawned stage: presence of post-ovulatory follicles (pof), mature oocytes (M) and atrestic oocytes (at), 100x.

The macroscopic and microscopic examination of the ovaries allowed the identification of four gonadal stages.

Stage- I (Immature)

Ovaries during this stage have been observed to occupy 1/6th of the body cavity and were transparent in appearance with no oocyte visible to naked eyes Table: 2. Histologically ovaries shows presence of rounded oogonia and follicular cells. Oogonia are small oval cells enclosed in a thin linning of germinal epithelium undergoing mitosis to form more oogonia or meiosis to form oocytes. (Fig 2a).

Stage-II (ripening)-

Ovaries occupied 1/4th of the body cavity with white to yellowish in colour (Table: 2) Histologically some oocytes could be seen surrounded by follicular cells. Primary oocytes are larger than oogonia with no longer mitotic division. Ooplasm is characterized by absence of yolk. (Fig 2b).

Stage – III (mature)

Ovaries occupied full body cavity and were orange red in colouration (Table: 2) Vitellogenesis begins with originating of yolk globules from perinuclear yolk complex. Mature oocytes become enlarged further and were fully laden with yolk vesicles. Follicular cells with nuclei surrounded the oocytes (Fig 2c)

Stage-IV (spawned)

Ovaries occupy ¼ of the body cavity with pale yellow colouration & flaccid appearance (Table: 2). Atresic oocytes showing atresia were observed. Atresic stage is characterized by oocytes disintegration or fusion of nucleus with sinking of ooplasm and collapsing oocyte membrane (Fig 2d)

Males: The testis exhibited varied colouration and consistency & were made up of semniferous tubules (Fig 3) Table 3)

Table 3 Macroscopic & Microscope variation in size (cm) volume, colouration & cell types in gonadal stage of male crab (N=24)

Development Stage	Size (Cw) in Cm	Volume	Colouration	Cell Type
Stage I (Immature)	3.0-3.5	1/6th of body cavity	Transparent gelatinous	Spermatogonia spermatocytes visible
Stage II (Maturing)	3.5-4.2	1/4th of body cavity	Creamy white gelatinous	Spermatocytes & Spermatids visible
Stage III (Mature)	4.5-5.5	Entire body cavity	Milky white in colour	Spermatozoids visible.

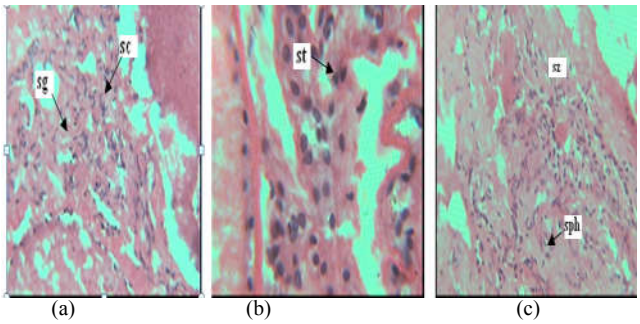

Fig: 3 Testicle of the crab *M.masoniana*: **a-** immature stage: presence of spermatogonia (sg) and spermatocytes (sc) 400x; **b-** maturing stage: presence of spermatocytes and spermatids (st) in cell division 1000x; **c-** mature stage: presence of spermatozoids & spermatophores, 400x.

The macroscopic & microscopic examinations allowed the classification of the testis in 3 stages:

Stage I (immature)

The testes & vasa differentia occupy 1/6th of body cavity & had transparent coloration with gelatinous aspect (Table: 3) Testicular follicles lined up by a single layer of germinal epithelium giving rise to spermetogonial cells are observed. Each primary spermatogonia under go mitotic division to form secondary spermatogonia (Fig 3a).

Stage II (maturing)

The testes occupy 1/4th of the body cavity & had creamy white colouration but with gelatinous aspect. (Table: 3) Secondary spermatogonia differentiate into spermatosites by undergoing mitotic division but few resting spermatogonia are observed forming new crop of germ cells for next breeding seasons. (Fig 3b)

Stage III (mature)

The testis occupy entire body cavity and had milky white colouration. Spermatozoids were found in all seminiferous

tubules (Table: 3) Histological analysis of these stage indicated various cell types viz spermatogonia, spermatocytes and large number of spermatids and spermatozoids in the lumen of tubules. No germinal area were observed in semniferous tubules (Fig 3c)

DISCUSSION

The reproductive cycle in crustacean has been studied by many workers including investigations on reproductive biology in relation to the monthly occurrence of mature male and females and monthly distribution of individuals according to size, classes and sex. (Colby & Fonseca 1984, Conde and Diaz 1989, Lopez-Greo et al 2000, Moura and Coelho 2000, Negreiros-Fransozo and G Bertini, 2002)

In the present investigations, based on availability of data on carapace width Table -1 it has been observed that *M.masoniana* is a biannual breeder with the two breeding peak seasons viz June-July & Dec-Jan. Maximum numbers of ovigerous females observed, found correlation with maximum development of ovaries in the present studies on crab. Maximum numbers of males with mature testis and maximum carapace width were observed in the same seasons. The size at sexual maturity for female was 3.5 cm of CW and 4.5 cm for males. These findings are in agreement with the work done by Sukamaran and Neelakantan. (1996) on relative growth and sexual maturity in the marine crabs *Protonus pelagicus*.

In comparison to male crabs of the present study with size 4.5 cm , female crabs are smaller viz 3.5 cm of CW, which may be attributed to the reason that female crabs spend large amount of energy in their gonadal cycle than male counterparts. Similar to present observations Mantelato (2003) also recorded differential changes in CW of male and females indicating gonadal maturity in *Mithraculus forceps* of his studies. Our findings are also in accordance with pattern proposed by Shine (1988) for brachyurans. According to this pattern, the requirement for reproduction in two sexes. When females allocate their energy for reproductive purpose, such as spawning and egg incubation, they tend to mature at smaller size than males, who invest their resources in somatic growth and reach maturity at greater size.

Observations on reproductive output per brood for brachyuran crab as advocated by Hartnoll, (1985). To simply uphold present view point of strong correlation of body size and weight within species. Our observations get further strengthened by work of Mantelato & Fransozo, (1997) on fecundity in crab *Callinectes ornatus*, who held that the wide variability in carapace shape as well as abdomen width affects the volume reserved for gonadal development. Observations on presently studied crab reveled a series of morphological and physiological events. (Table 1, 2 & 3) and (Fig 1, 2 & 3)

When external morphological characteristics of the gonads were compared to histological descriptions, modifications that characterize the process in different developmental stages of gonadal cells throughout the gonadal cycle could be clearly demarcated. The macroscopic analysis of the present studies revealed that gonads witness changes in volume as well as colour during the course of maturation (Table 2 & 3)

It was observed that during maturation phases from stage I & IV the volume occupied by maturing gonads in both male & female crabs ranged from $1/6^{th}$ of body cavity to entire occupancy of the body cavity. This is in accordance to the findings of Adiyodi & Subramonian (1983). Arculeo *et.al* (1995) who also held that during the course of gonadal maturation the ovaries undergo a sequence of macroscopic changes in its morphology viz relative size, which are easily delectable by naked eye.

The present study revealed that in females the colouration of the ovaries ranged from transparent when immature (Stage I) to white in (Stage II) and yellow (Stage III) and orange tones in (Stage IV). Present observations are in tune with the finding on ovarian colour change during vitellogenesis as recorded for C*yrtograpsus angulatus* by Castiglioni santos (2001). Variations in colouration has also been used to elaborate macroscopic scales in the ovarian development (Arculeo 1995 & flores *et.al* 2002.)

In the present study too, the ovaries of *M. masoniana* showed a pronounced macroscopic differentiation in size and colouration during the maturation process of the gonad. During the vitellogenesis the amount of oocytes in secondary stage increases in the ovaries as a result of yolk deposition resulting thereby a change in colouration from yellow to orange red. On similar pattern Charniaux-cotton (1980) observed that ovarian colouration is a result of the storage of vitellogenine presenting carotenoid pigments. Goodwin (1951) also held that change in colour is the result of modification in carotenoid content occurring during oogeneis. The sequence in the change on colour of ovaries evidenced by the accumulation of yolk has also been described for the fresh water crabs *Eudaniela garmani* and *Sinapotamon yangtsekiense* by Rostant *et.al;* (2008) and Chen *et.al* (1994) respectively.

Histological analysis of female gonads of *M. masonian* in the present studies revealed a gradual process of oocyte development where cell types viz oogonia, oocytes, mature oocytes, atresic oocytes along with follicular cells were observed (Fig 2 a, b, c & d). These observations indicating the description of cellular stages agrees with the cell types observed in other female decapods crustaceans by Castiglioni *et.al* (2007); Rostant *et.al* (2008), Souza & Silva (2009).In this context, the presence of post ovulatory follicles in fresh water crabs as reported by Rjeibi *et.al* (2010) and presence of these follicles responsible for synchronic maturation ensuring the necessary amount of mature oocytes for spawning gets authenticated by observations of Shinozaki-Mendes *et.al* (2011). Histological analysis of the modification observed in the oocytes during the process of gonad maturation as observed presently are similar to the description in the literature for other females of decapods crustaceans (Adiyodi & Subramonian, (1983), Lopez *et al* (1997), Elorza & Dupre, (2000).

The general layout of reproductive system of *M.masoniana* was similar to those found in other decapods Cronin, (1947); Ryan

(1967) Joshi & Khana, (1982) Castilho *et.al* (2008). The system shows the bilateral symmetry and H shape, characteristic of many brachyuran crabs. This arrangement is found in many crabs and crayfishes (Krol *et.al* (1992); cumberlidge, (1999).

The morophological analysis of male gonads during course of maturation process form stage I to III also indicated colour change from transparent to milky white (Table 3). Whereas, the histological analysis of these gonadal stages exhibited cell types viz spermatogonia, spermatocytes, spermatozoids which are responsible for the modification of the coloration ranging from transparent in immature individuals to milky white in mature ones. These observations are in accordance with the findings of Mota Alves (1975) and Castilho *et.al* (2008) who held that testicular lobules are filled with large number of spermatocytes in different stages of development.

Seasonal changes in morphology & physiology in testies of *M. masoniana* is in tune with findings of Joshi & Khanna (1982) who also reported similar changes in the testis of fresh water crab *Potamon koolooense,* with different stages of development in semniferous tubules. The changes in the colouration and cell types of male gonads of *M. masoniana* under observation followed the pattern described for other decapods species viz *Ucides cordatus* and *Armases rubripes* by Castilho *et.al* (2008) and Santos *et.al* (2009).

Summary

In the present study the morphological analysis of the gonads of both the sexes of *M.masoniana* when compared to histological description exhibited modification during process in different development stages throughout gonadal cycle.

Gonadal stages of *M. masoniana* exhibited that size ranging from 3.5-5.5 cm CW in case of both males & females indicates the size at sexual maturity. Such sexually mature specimens (stage III) are found twice in a year viz (June, July and December-January) exhibiting *M. masoniana* as a biannual breeders. Morphological observation of gonads at stage III exhibit (a) orange red colouration of ovaries in female (b) milky white colouration of testies in males occupying full cephalothorax cavity. Histological evidence further authenticated the gonadal maturity at stage III with the presence of nature oocytes in ovaries & spermatozoids in testis.

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