Annual Reproductive Cycle of the Marine Pulmonate, *Onchidium verruculatum* (Cuvier) & Its Control by Environmental Factors

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Annual reproductive cycle of the protandric hermaphrodite marine pulmonate, *Onchidium verruculatum* has been studied with seasonal changes in gonad and accessory sex organ indices and histology of the gonad. *O. verruculatum* breeds during December to March. Though the development is protandric it becomes simultaneous hermaphrodite during the reproductive peak. Life span of *O. verruculatum* seems to be 1 y and hence the population exhibits asynchrony in the reproductive status during the postbreeding season. In July to September, slugs with the mature male phase reproductive system are abundant in the population. At the same time older slugs exhibit degeneration of all the gametes. Increase in the photoperiod stimulates male maturation whereas increase in temperature during summer terminates the breeding season.

Reproduction in marine pulmonates is cyclic with seasonal fluctuations in sexuality as these pulmonates are hermaphrodite^1. Marine pulmonates are known to reproduce during the restricted part of the year^2 - 4, and/or continuously throughout the year^5 - 9. Similarly, annual cycles of protandric sex changes have also been demonstrated^9 - 10.

The above reports are for the marine basomatophoran snails and no information appears to exist on the reproductive physiology of the marine systellommatophoran pulmonates to which onchidid slugs belong. These slugs form the link between the sea and the land pulmonates in the evolutionary continuum of phylum Mollusca^1. Hence, the work on the reproductive physiology of these slugs is important.

In the present paper, annual reproductive cycle of the marine pulmonate, *Onchidium verruculatum* has been delineated with the seasonal fluctuations in the gonad and the accessory sex organ indices and histology of gonad proper.

Specimens of *O. verruculatum* were collected every month (Oct. 1977 - Sept. 1978) during low tide from Veraval coast (lat. 20°35'N; long. 70°35'E). In the laboratory they were soaked with filter paper, weighed and dissected. Gonad and accessory sex organs (albumen gland, mucous gland and prostate gland) were excised and weighed. Gonad (GO) and accessory sex organ (ASO) indices were calculated on wet weight basis^12. For histological observations, gonad tissue was fixed in seawater - Bouin's fluid, dehydrated, embedded in paraffin wax (56°C-58°C) and sectioned (10 μm). Sections were stained with eosin-haematoxylin method.

Oocytes and ova, on the basis of their cytomorphological characters and size are classified into 5 stages as avitellogenic oocytes (I), maturing ova (II), vitellogenic ova (III), postvitellogenic ova (IV) and degenerating ova (V). Percent frequency distribution of each oocyte-ova size class per gonadal follicle was the mean of 5 standard follicles per animal and of 5 replicates per month (Oct. 1977 to Aug. 1978). Percent frequency distribution for each oocyte-ova size class is plotted against the time^13. Seasonal changes in spermatogenesis were recorded by histological observations of gonad sections.

One way analysis of variance and student t test were applied to pool the significance of the collected data^14. Monthly changes in GO and ASO indices cycled in the similar pattern (Fig. I) and all the fluctuations are significant ($P < 0.05$) against time. GO and ASO indices were maximum through December to March. Percent avitellogenic oocytes (I) were more in October-November and declined from January onwards (Fig. 2). Maturing oocytes (II) were abundant from November to January whereas vitellogenic ova (III) increased during February to March. In June

![Fig. 1](image-url) Changes in GO and ASO indices of *O. verruculatum* during different months
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postvitellogenic ova (IV) were dominating over other oocyte-ova size classes. By the end of July, 90 to 95% ova were in degenerating stage. No evidence for the proliferation of new oocytes was observed after degeneration of the gametes (July-August).

Spermatogenesis was at the peak during September to December (Fig. 3A). During egg laying season, many developmental stages of spermatogenesis were observed. The incidence of spermiation was rare and it increased during January to February as evident from the old Sertoli cells in the gonad follicles (Fig. 3B). Spermatogenesis decreased during April to June (Fig. 3C). The total degeneration of sperm stages was evident through July-August (Fig. 3D).

Fig. 2—Percent frequency distribution of oocyte-ova size classes during different months [I, avitellogenic ova; II, maturing ova; III, vitellogenic ova; IV, postvitellogenic ova; and V, degenerating ova].

Fig. 3—L.S. of gonad follicle at different periods [A, September to December; B, January to March; C, April to May; D, June to August, AO, avitellogenic oocytes; DO, degenerating ova; OS, old Sertoli cells; PO, postvitellogenic ova; S, spermatozoa; SS, developing sperm stages, and VO, vitellogenic ova].
Annual cycles of protandric sex change have been reported in a number of marine basommatophoran pulmonate snails\(^5,6\). Though, \textit{O. verruculatum} is a restricted breeder, there is no definite annual shift of sexuality. However, like \textit{Ovatella myosotis}\(^7,8\) there is only one shift in the sexuality due to protandric development. Newly hatched young ones of \textit{O. verruculatum} exhibit initiation of spermatogenesis within 2-3 months, at the time when the female reproductive parts are immature. This is followed by appearance of oocytes in the gonad and rapid growth of albumen gland.

With the observations on the GO and ASO indices and histology of gonad, annual reproductive cycle of \textit{O. verruculatum} can be delineated as (1) sperm production (September to December), (2) initiation of oogenesis (September to November), (3) mating (November to February), (4) first egg laying cycle (December), (5) total maturation and accumulation of ripe gametes (January to March), (6) final egg laying (March-April), and (7) degeneration of relict gametes (May-July). Though the development of \textit{O. verruculatum} is protandric it becomes simultaneous hermaphrodite during the peak reproductive period. As the development is protandric, male maturation is induced during the periods of long day day-lengths (14h) following the summer solstice in March. This suggests that different environmental factors control the different aspects of reproductive cycle of \textit{O. verruculatum}. Similar observations have been made in marine snails\(^8-10\). However, all these snails, unlike \textit{O. verruculatum} reproduce throughout the year.

Since egg laying in \textit{O. verruculatum} commences by early December and juveniles appear as early as in the last week of December, June-July population exhibited heterogeneity in the reproductive status. The young slugs exhibited mature male characters whereas older slugs often showed either simultaneous hermaphroditism and/or gametes were in degenerating state. The monthly analysis of size frequency also showed decline through April to July as the young ones are recruited in the population\(^16\). Such an asynchrony in the reproductive status of a population has been reported in marine basommatophoran snails like \textit{Cassidula aurisfe/is} and \textit{Lemmodonta punctigera} which reproduce throughout the year\(^17\).

The incidence of young slugs with small albumen gland but with enlarged male reproductive organs is more in the population during March to July\(^16\). However at the same time older slugs with the spent gonads never revealed reinitiation of gametogenesis. Hence, possibly, \textit{O. verruculatum} survives only one breeding season.

The egg laying is commenced in 2 cycles and the young slugs attain maturity approximately in 2 to 3 months. Naturally, juveniles appearing in the last week of December will be mature males during March egg laying cycle. This could be to provide the young males for copulation to obtain maximum genetic variations since these slugs copulate in pairs. Lastly, the pressure of natural selection in the form of only one breeding cycle per life span could be the reason for the protandric development followed by simultaneous hermaphroditism in \textit{O. verruculatum}.

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