Gametogenic activity in three internally fertilized prosobranchs from southern Africa, with notes on the egg capsules of *Burnupena lagenaria*

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Patterns of gametogenic activity in three prosobranchs with internal fertilization, *Burnupena lagenaria*, *Nerita albicilla*, and *N. plicata*, were determined. No seasonal trends were evident in the histological appearance of *N. albicilla* gonads, indicating that gametogenesis was a continuous process. Four distinct reproductive phases, inactivity, gametogenic build up, active vitellogenesis or spermiogenesis, and postspawning were recognizable in *B. lagenaria* and *N. plicata*. Although mature gametes could be found throughout the year in the gonads of *N. plicata* the prevalence of post-spawned individuals between March and September suggested that the breeding period may be less protracted. Gametogenic development in *B. lagenaria* was found to be poorly synchronized, although an intensification in reproductive activity was evident between April and November. Egg cases were also found in the field during this period. A brief description of these cases plus their contents is presented. Since gamete accumulation and storage prior to copulation could not be ruled out it is suggested that the time spans identified be regarded as 'potential' rather than 'effective' breeding periods.

Patrone van gametogeniese aktiwiteit in drie prosobranche, *Burnupena lagenaria*, *Nerita albicilla*, en *N. plicata*, wat interne bevrugters is, is bepaal. Geen seisoenale patrone is gevind in die histologiese voorkoms van die gonades van *N. albicilla* en dit blyk dat gametogenese 'n aaneenlopende proses is. Vier duidelike reproduktiewe fases, onaktiwiteit, gametogeniese opbou, aktiewe dooiervorming of spermiogenese, en 'n post-kuitskiet-fase was egter waarneembaar in *B. lagenaria* en *N. plicata*. Alhoewel volwasse gamete reg deur die jaar gevind is in die gonades van *N. plicata*, het post-kuitskiet-indiwidue oorheersend tussen Maart en September voorgekom, wat daarop dui dat die broeiperiode wel korter mag wees. Gametogeniese ontwikkeling is swak gesinkroniseer in *B. lagenaria* maar 'n verhoging in reproduktiewe aktiwiteit was wel waarneembaar tussen April en November. Eieromhulsels is gedurende hierdie periode in die natuurlike omgewing gevind. Die eieromhulsels en hul inhoud word ook beskryf. Aangesien opeenhoping en storing van gamete voor kopulasie kan plaasvind, word voorgestel dat die geïdentifiseerde broeiperiodes beskou word as 'potensiële' eerder as 'effektiewe' broeiperiodes.

Previous studies on the reproductive biology of rocky intertidal molluscs from southern Africa have been restricted to broadcast spawners (Newman 1967; Branch 1974; Griffiths 1977; Joska & Branch 1983; Lasiak 1986a & b, 1987a, b & c). With the exception of sandy beach whelks of the genus *Bullia* (Brown 1971; McGwynne & van der Horst 1985) little is known of reproduction in gastropods which copulate and deposit egg capsules. The present study, based on gonadal histology, examines the gametogenic cycles of three numerically abundant internal fertilizers: the archaeogastropods *Nerita albicilla* and *Nerita plicata* and the neogastropod *Burnupena lagenaria*. The egg capsules of the latter species are also described.

Methods

Thirty specimens of *N. albicilla*, *N. plicata* and *B. lagenaria* were collected, at approximately monthly intervals between August 1982 and September 1983, from intertidal sandstone platforms within the Hluleka Nature Reserve, Transkei (31°49'S / 29°19'E). Additional samples of *B. lagenaria* collected from Schoenmakerskop, near Port Elizabeth (34°00'S / 25°30'E) were also examined. All collections were made at spring low tide. The total length of each animal was recorded before removing the shell. Portions of the gonad and associated digestive gland were fixed in either Bouin's or 10% formol/saline prior to routine histological preparation. The wax-embedded material was sectioned at 7μm, stained with Delafield's haematoxylin, counterstained with eosin and later examined microscopically.

Results

Burnupena lagenaria

Gametogenesis was found to be asynchronous within individual whelks, the proportional representation of the different gametogenic stages varied along the length and breadth of the gonad. With the exception of the peripheral tubules all stages of spermatogenesis were evident within each tubule. Groups of spermatogonia were found just inside the basement membrane, aggregations of spermatocytes and spermatids were present throughout and clumps of spermatozoa were centrally located around the lumina. The ovarian tubules were lined by a thin layer of follicle cells with which oogonia and previtellogenic oocytes were intimately associated. Yolk vesicle formation was apparent in some of the larger (40–60 μm) pre-vitellogenic oocytes. Two distinct phases of vitellogenesis were discernible: small basophilic primary yolk globules were evident in oocytes of approximately 70–85 μm diameter, and secondary yolk deposition was observed in oocytes of > 100 μm diameter (range 100–190 μm). Most vitellogenic oocytes were freely situated in the tubules. Relatively few intact mature oocytes were found in sectioned material. Most of the tubules examined contained numerous free yolk globules resulting from the rupture of mature oocytes during processing.

Asynchronous gametogenic development within individuals and the break-up of mature oocytes precluded the use of quantitative methods for determining reproductive periodicity. However, on the basis of microscopic inspection, four reproductive phases: inactivity (S1) (Figures 1a & 2a), gametogenic build-up (S2) (Figures 1b & 2b), active vitellogenesis or spermiogeneis (S3) (Figures 1c & 2c), and post-spawning (S4) (Figures 1d & 2d), could be distinguished. A distinct resting period was evident in inactive male *B. lagenaria*. Ovarian development, however, appeared to be continuous. Even the so-called 'inactive' females had a few previtellogenic oocytes within their tubules. Gametogenic development was more synchronous in male than female whelks. Distinct periods of inactivity (December–January) and gametogenic proliferation (February–March) were evident in male *B. lagenaria*. Inactive females were recorded throughout the study period, and, although gametogenic build up (S2) peaked in February to March females at this stage of development were found through to August. The histological evidence suggested that *B. lagenaria* may have a protracted breeding period from April to November (Figure 3). Field observations confirmed the presence of egg capsules within this period. No differences were discernible in the reproductive activity of *B. lagenaria* from Algoa Bay and the Transkei coast.

The egg capsules of *B. lagenaria* are tough, compressed cases with a leaf-like flap at the apex and a stalkless base. They are found attached in clusters to mussel shells and to the rocky substratum of pools and crevices between low and high water neap tide levels. Females do not congregate to lay eggs, consequently each cluster represents part of the reproductive output of a single female. The clusters, which are almost spherical in shape, vary in diameter from 20 mm–40 mm, and

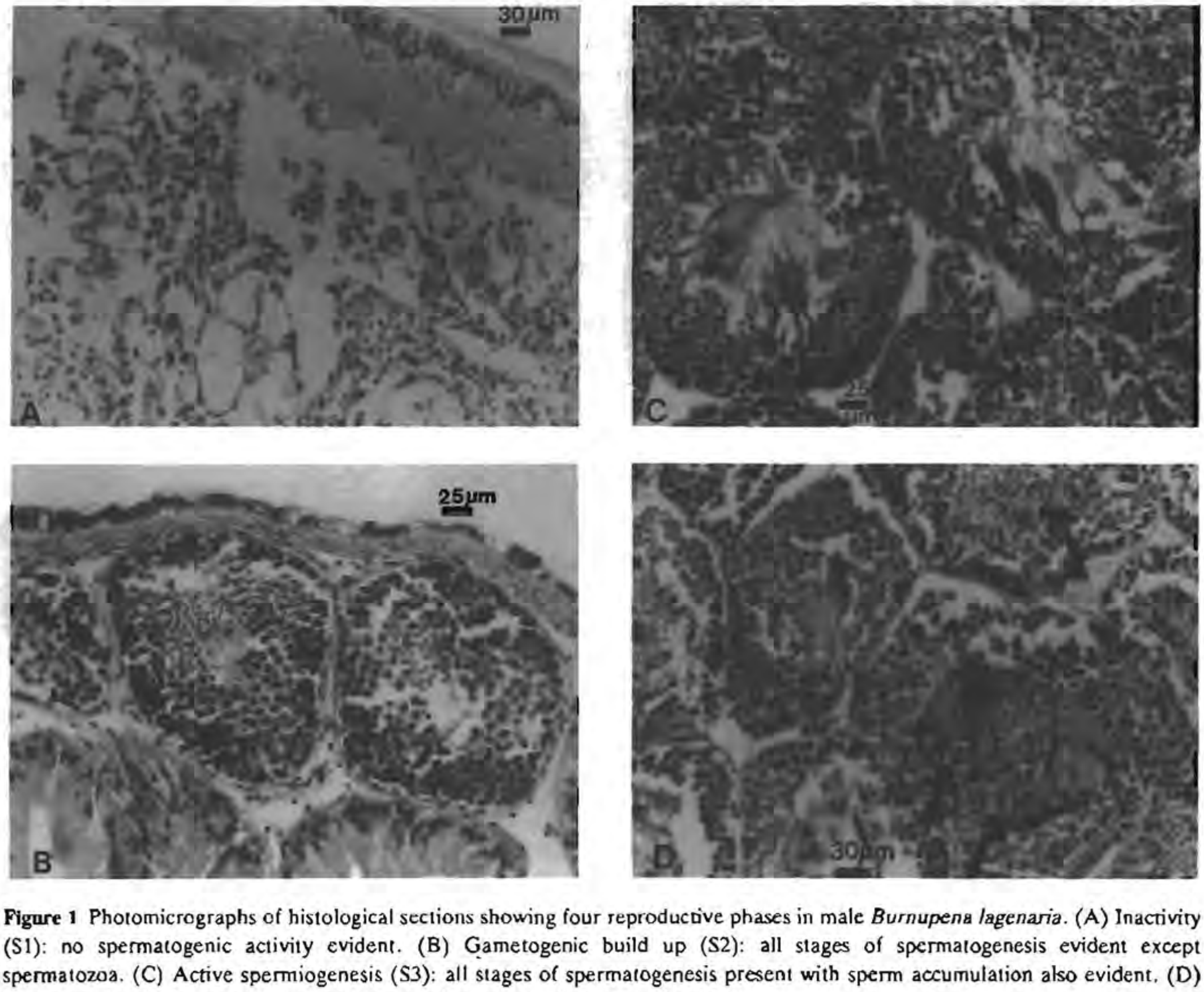

Figure 1 Photomicrographs of histological sections showing four reproductive phases in male *Burnupena lagenaria*. (A) Inactivity (S1): no spermatogenic activity evident. (B) Gametogenic build up (S2): all stages of spermatogenesis evident except spermatozoa. (C) Active spermiogenesis (S3): all stages of spermatogenesis present with sperm accumulation also evident. (D) Post-spawning (S4): sperm degeneration and phagocytosis (see arrows) evident.

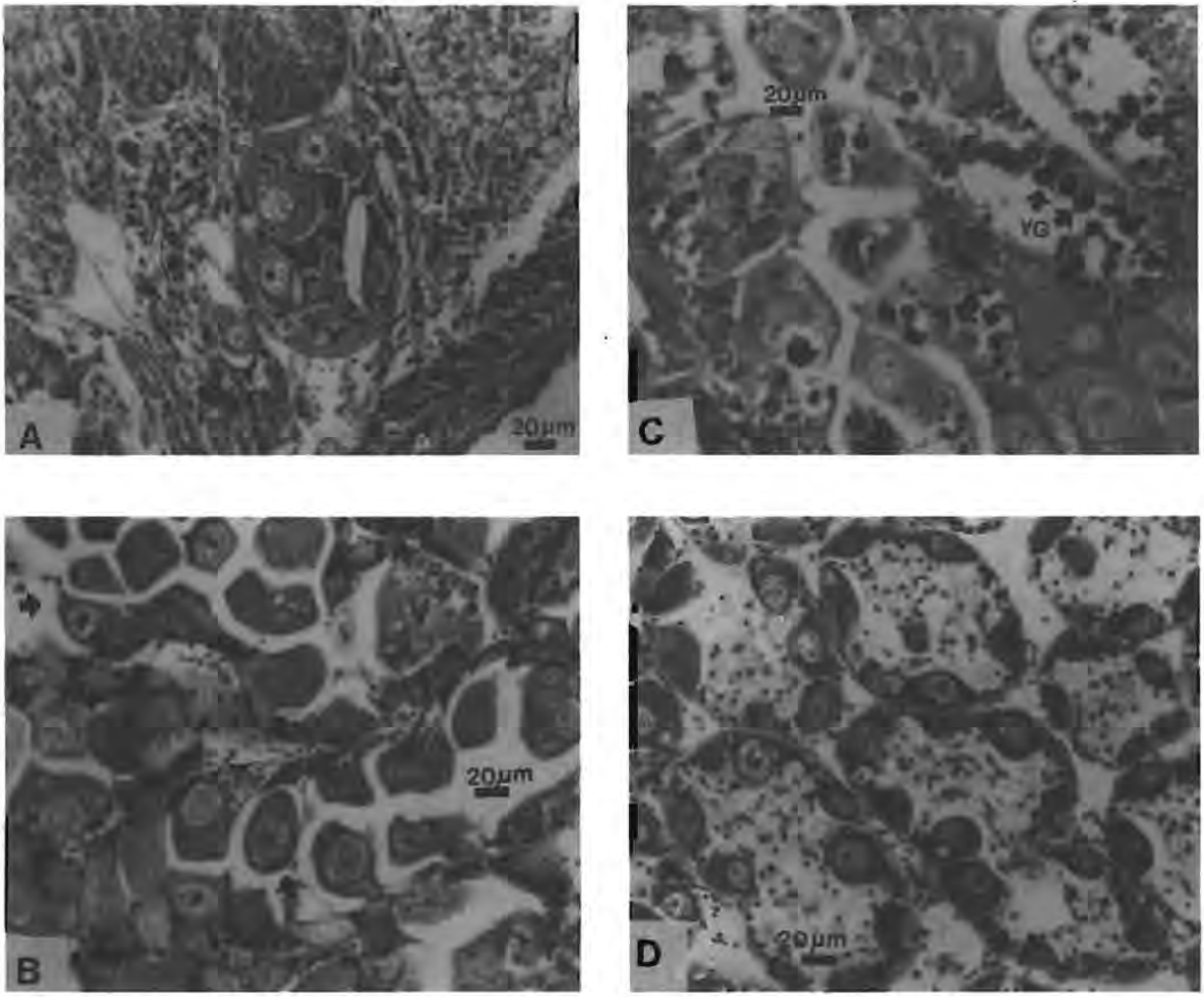

Figure 2 Photomicrographs of histological sections showing four reproductive phases in female *Burnupena lagenaria*. (A) Inactivity (S1): few pre-vitellogenic oocytes present in small groups. (B) Gametogenic build up (S2): pre-vitellogenic oocytes predominate (see arrows), V = vitellogenic oocyte. (C) Active vitellogenesis (S3): vitellogenic oocytes with large secondary yolk globules (YG) dominant. Great difficulty is experienced in sectioning these oocytes as can be observed. (D) Post-spawning (S4): large follicles which become infiltrated with connective tissue present as well as a peripheral layer of pre-vitellogenic oocytes.

consist of 23–52 transparent capsules ($n = 36$) (Figure 4). The outermost capsules of each egg mass were either empty or contained only a few eggs, whereas those from the interior contained on average 302 ± 61 eggs each ($n = 100$). Relatively few of these appear to develop into embryos, most becoming nurse eggs. The number of developing embryos observed per capsule varied from 1 to 10 ($\bar{x} = 3{,}4$; $S.D. = 1{,}9$; $n = 418$). Therefore, on average, 1,1% of the embryos within each capsule were viable. The production of nurse eggs as a nutritive source for developing embryos is a characteristic feature of several neogastropod families (Webber 1977). In the Buccinidae it has generally been observed that nurse eggs outnumber developing embryos to a considerable degree (Table 1). Juvenile snails with pigmented, calcified shells were evident in some of the clusters examined which suggests that hatching takes place at the crawling stage. The total number of embryos or hatchlings found within a cluster varied from 27–133 ($\bar{x} = 72$; $S.D. = 29$; $n = 19$).

In principle the reproductive phases observed in the neritids were similar to those of *B. lagenaria*, therefore no additional photomicrographs are presented.

Nerita plicata

Throughout the entire study period most males exhibited active spermiogenesis, indicating a potential for continuous breeding. However, the presence, between March and September, of several individuals at the gametogenic proliferation and post-spawning stages suggests that the effective breeding period may be less protracted (Figure 5). The scarcity of inactive males may indicate a resting stage of short duration. Gametogenic activity was evident in all the female *N. plicata* examined, with no evidence of a resting phase.

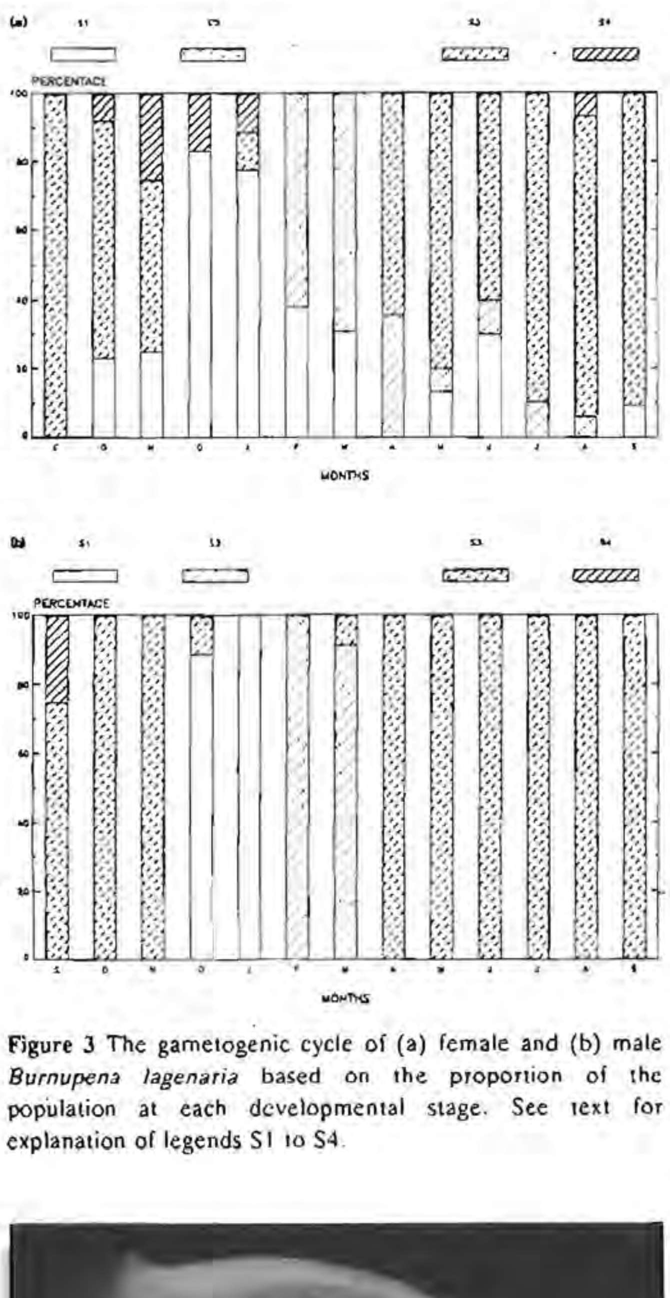

Figure 3 The gametogenic cycle of (a) female and (b) male *Burnupena lagenaria* based on the proportion of the population at each developmental stage. See text for explanation of legends S1 to S4.

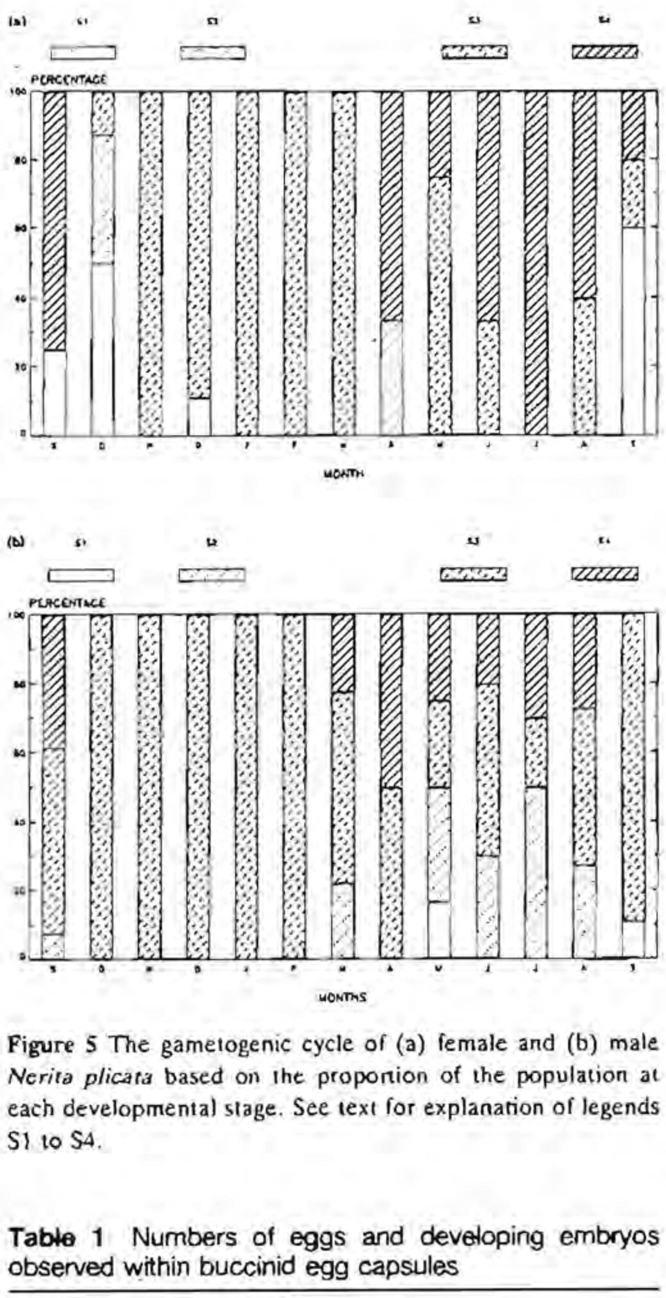

Figure 5 The gametogenic cycle of (a) female and (b) male *Nerita plicata* based on the proportion of the population at each developmental stage. See text for explanation of legends S1 to S4.

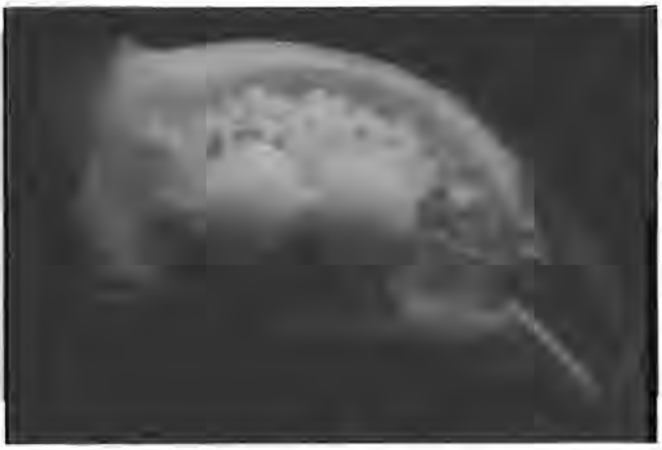

Figure 4 Single egg capsule of *Burnupena lagenaria* showing two embryos surrounded by nurse eggs.

Table 1 Numbers of eggs and developing embryos observed within buccinid egg capsules

Species	No. eggs	No. embryos	% Viability	Source
Burnupena lagenaria	302	1–10	1,1	This study
Colus stimpsoni	5000	1–8	<0,1	West 1981
Neptunea antiqua	5000	1–4	<0,1	Pearce & Thorson 1967
Neptunea lyrata	2400	1–4	–	Rivest 1983
Searlesia dira	132	7,2–3,4	4,7	Rivest 1983

Gametogenic proliferation in females took place between August and October. Active vitellogenesis was evident in all the ovaries sectioned between November and March, thereafter post-spawning individuals predominated (Figure 5). From November to June there was some cytolysis of mature oocytes.

Nerita albicilla

In contrast to *N. plicata* the histological appearance of the gonads showed no seasonal progression, which suggests that gametogenesis is continuous. Within the testis spermatogenic progression was evident across the width of each tubule. In most instances the tubules were densely packed with spermatogenic cells. With the exception of the peripherally located ovarian tubules, which consisted of numerous oogonia and small previtellogenic oocytes, post-vitellogenic oocytes were found in all the tubules sectioned. Although cytolysis of mature oocytes was apparent in some individuals no seasonal trends were observed.

Discussion

Histological investigations of gonadal development have often proved to be the most reliable methods for determining the reproductive periodicity of marine invertebrates (Underwood 1974; Ernest & Blake 1981; Sebens 1981; Grant & Tyler 1983). In external fertilizers, which tend to rely on the simultaneous release of ripe oocytes and spermatozoa for successful fertilization, the sudden loss of large quantities of gametes from mature gonads is considered to be indicative of breeding activity (Giese 1959). Histologically such gonads appear to consist of large empty spaces with a few patches of residual gametes in some tubules. As a result of copulation and sperm storage the large-scale emission of gametes is not essential in internal fertilizers, consequently breeding activity may not be accompanied by drastic changes in gonadal appearance. Furthermore, as breeding does not always immediately succeed active gametogenesis (Webber 1977) the presence of numerous gametes within gonadal follicles may be an indication of the 'potential' rather than the 'effective' breeding period (Garwood & Kendall 1985).

Cycles of gametogenic development were found to be similar in male and female *B. lagenaria*. Previous studies on the gonadal histology of buccinid gastropods have, however, shown ovarian and testicular development to be poorly coupled (Takamuru & Fuji 1981; Martel, Larrivee, Klein & Himmelman 1986a). The latter have attributed this lack of synchronous activity to the long-term storage of sperm in the seminal vesicle prior to copulation. The present study, based solely on observations of gonadal histology, suggested that *B. lagenaria* was capable of breeding from April through to November. The discovery of egg capsules in the field during this period supports this. Rather than indicating an extended breeding period the protracted occurrence of ripe gametes in this whelk may point to a lengthy period of gamete accumulation and storage prior to copulation, as reported in other buccinids (Takahashi, Takano & Murai 1972; West 1979; Takamuru & Fuji 1981; Martel *et al.* 1986a). To take this possibility into account studies of gonadal histology need to be combined with observations on the contents of the bursa copulatrix, receptaculum seminis, and seminal vesicle, as demonstrated recently by Martel, Larrivee & Himmelman (1986b). Field observations on the incidence of copulation and egg deposition would also help to distinguish between 'effective' and 'potential' breeding periods.

Ripe gametes were found to be present throughout the year in gonadal tissue of the two neritids. Although there was some evidence of dispersion of spermatozoa and cytolysis of residual mature oocytes, no definite trends in the gametogenic development of *N. albicilla* could be detected. Seasonal variations were, however, evident in the rate of gamete production by *N. plicata*. This was most pronounced in the females, which showed distinct periodicity in gametogenic proliferation, active vitellogenesis, and post-spawning. The prevalence of post-spawned individuals between March and September suggested that the 'potential' breeding period may be less protracted than the prolonged presence of ripe gametes appeared to indicate.

Berry, Lim & Sase Kumar (1973) and Underwood (1974) also noted the protracted occurrence of ripe gametes in neritid gastropods. The former found ripe gametes throughout the year in *N. birmanica*, which led them to conclude that this species bred continuously. Although they described the morphology of the associated reproductive organs no attempts were made to examine their contents seasonally. This could, for reasons outlined earlier, have enabled the differentiation of 'potential' and 'effective' breeding periods. From an assessment of changes in the relative proportion of pre- and postvitellogenic oocytes, Underwood (1974) was able to show that *N. atramentosa* accumulates and stores mature oocytes for a six-month period prior to spawning. A sharp decline in the per cent mature oocytes between December and February enabled him to pinpoint the spawning period, which was confirmed by the appearance of egg capsules in the field. Others (Adegoke, Dessauvagie & Yoloye 1969; Govindan & Natarajan 1972) have inferred breeding periods of neritids solely from observations on the occurrence of egg capsules. The tendency for these capsules to persist long after the embryos have completed development, however, necessitates the examination of capsules for fresh contents, a requirement which is frequently overlooked (Berry *et al.* 1973).

Finally it is interesting to note that an inverse relationship apparently exists between number of eggs and percentage viability of buccinid egg capsules (Table 1). This may have implications for breeding strategies within this family.

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