Reproductive periodicity in *Evechinus chloroticus* in the Hauraki Gulf

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Abstract Test diameter and gonad volume were recorded over 1 year for bimonthly samples of *Evechinus chloroticus* (Valenciennes) from each of 3 subtidal populations 10 km apart in the Hauraki Gulf. Histological analysis was used to relate cellular events during gametogenesis to the annual cycle of change in gonad size. Mean gonad sizes of the different sea urchin populations were compared by analysis of covariance. Gametogenesis began in the spring, and major spawning occurred in mid to late summer. The proportion of the gonads taken up by nutritive phagocyte cells increased from autumn to spring. During proliferation and growth of gametes the nutritive phagocytes declined in abundance and globulation, suggesting that reserves stored in these cells were transferred to developing gametes. Gonad size doubled during gametogenesis, declined after spawning, and remained low in autumn and winter. Differences in gonad size between populations were significant during most of the year, and were most pronounced in mid summer. Although synchronous gametogenesis occurred in the 3 populations studied, spawning occurred at different times. This suggested that spawning in this species was induced by factors acting either within sea urchin populations or over distances of a few km or less.

Keywords *Evechinus chloroticus*; gonads; histology; gametogenesis; spawning; population sampling; Hauraki Gulf; Echinometridae; phenology; Rangitoto Island; Noises Islands; Crusoe Island; seasonal variation; reproduction; kina; echinoderms.

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INTRODUCTION

In temperate regions marine invertebrates generally reproduce only when environmental conditions are favourable (Giese 1959). In some asteroids, seasonal fluctuations of photoperiod can control initiation of gametogenesis (Pearse & Eernisse 1980); and in the sea urchin *Strongylocentrotus droebachiensis* spawning is triggered by the spring phytoplankton bloom (Himmelmann 1975). Other possible causes of reproductive synchrony should be sought.

The New Zealand sea urchin *Evechinus chloroticus* (Valenciennes) has an annual reproductive cycle with spawning occurring in summer or early autumn (Dix 1970). Populations may differ in mean gonad size and in the degree and timing of spawning activity (Dix 1970). This study outlines reproductive periodicity at the cellular level in *E. chloroticus* in the Hauraki Gulf, and relates the cellular events of gametogenesis to changes in gonad size and timing of spawning.

STUDY AREA AND METHODS

Samples were collected from 3 subtidal reefs in the Hauraki Gulf: north-west Rangitoto Island (36°47′S, 174°52′E; The Noises islands (36°43′S, 175°00′E; and Crusoe Island (36°49′S, 174°59′E). At Rangitoto sea urchins were collected from a shallow-water platform and slope which supported dense mixed stands of laminarian kelp (*Ecklonia radiata*) and fucoids (*Carpophyllum* spp.). The sea urchin population comprised large individuals (mean test diameter (t.d.) 84 mm, range 55–107 mm), usually found singly or in small groups. At The Noises, low mixed stands of brown algae occurred on the shallow subtidal reef and *E. radiata* dominated the deeper region. This sea urchin population was the densest studied, and individuals found ranged from 10 to 100 mm t.d., though individuals sampled were in the range 59–97 mm (mean 75 mm). Crusoe Island samples were taken from a shallow-water platform dominated by dense stands of *E. radiata*. Sea urchins were very large (sample range 67–113 mm, mean 96 mm), and almost always solitary.

Between April 1975 and March 1976, 20 sea urchins were collected at 2-month intervals from each of the 3 sites. All individuals collected were

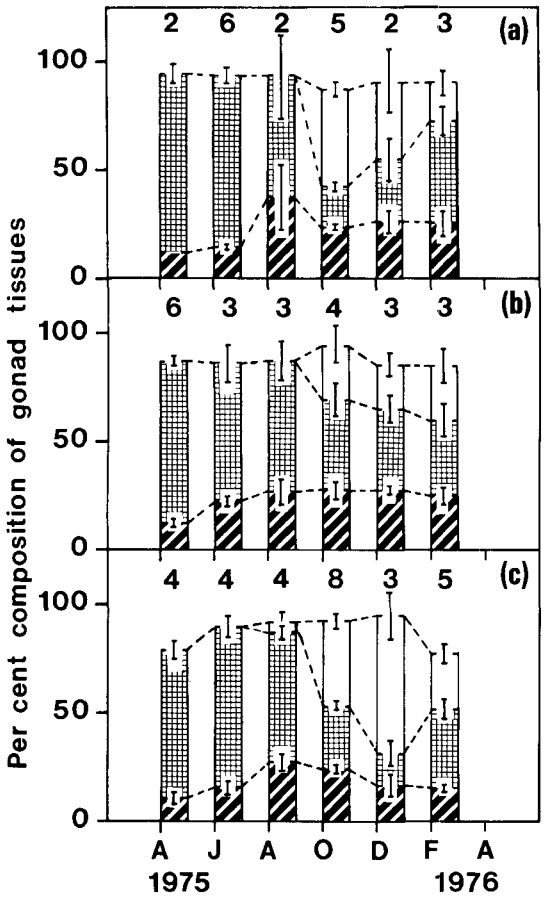

Fig. 1 Seasonal variation in proportional composition of testis tissue of *Evechinus chloroticus* from (a) Rangitoto I., (b) The Noises, (c) Crusoe I. Diagonal bands, spermatogonia and spermatocytes; cross-hatched, nutritive phagocytes; open, spermatozoa free in central lumen of gonad. Numbers over histograms are numbers of individuals examined. Vertical bars within histograms indicate standard errors of mean tissue proportions within sections.

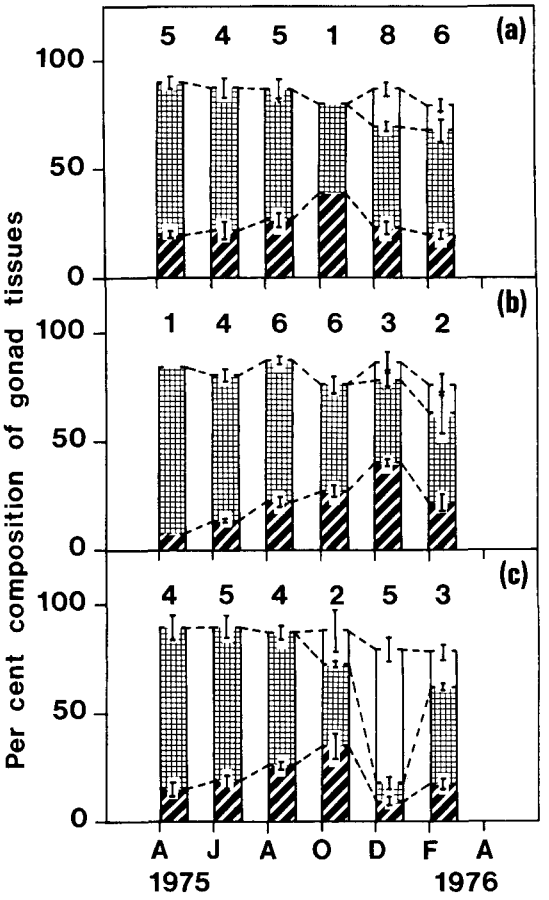

Fig. 2 Seasonal variation in proportional composition of ovarian tissue of *Evechinus chloroticus* from (a) Rangitoto I., (b) The Noises, (c) Crusoe I. Diagonal bands, oogonia and oocytes; cross-hatched, nutritive phagocytes; open, ova free in central lumen of gonad. Other conventions as for Fig. 1.

adults (>40 mm t.d.). Test diameters (in mm) were measured with vernier calipers shortly after sample collection, and gonad volumes were determined by displacement in a measuring cylinder. One of the major lobes of the gonad of each sea urchin was removed, fixed in Bouin's solution, and embedded in paraffin wax. Ten embedded gonads from each population sample were sectioned at 7 μm and stained with Mallory and Heidenhain's azan stain.

Histological analyses of gonads were carried out by estimating the proportion of the cross-sectional area occupied by different cell types. A representative section from each urchin was selected and analysed by recording the cell type falling under each internal cross of an eyepiece grid at ×100 magnification. Cell types recognised in the analyses were germinal cells and developing gametes (oogonia, oocytes, spermatogonia, spermatocytes, spermatids), nutritive phagocytes (non-germinal cells), and gametes (ova, spermatozoa) held in the central lumen of the gonad lobes.

Analysis of covariance was used to examine habitat-related effects on gonad size. The analysis calculated a regression of log. gonad volume on log. test diameter. Adjusted mean gonad sizes were computed by solving the regression for the mean test

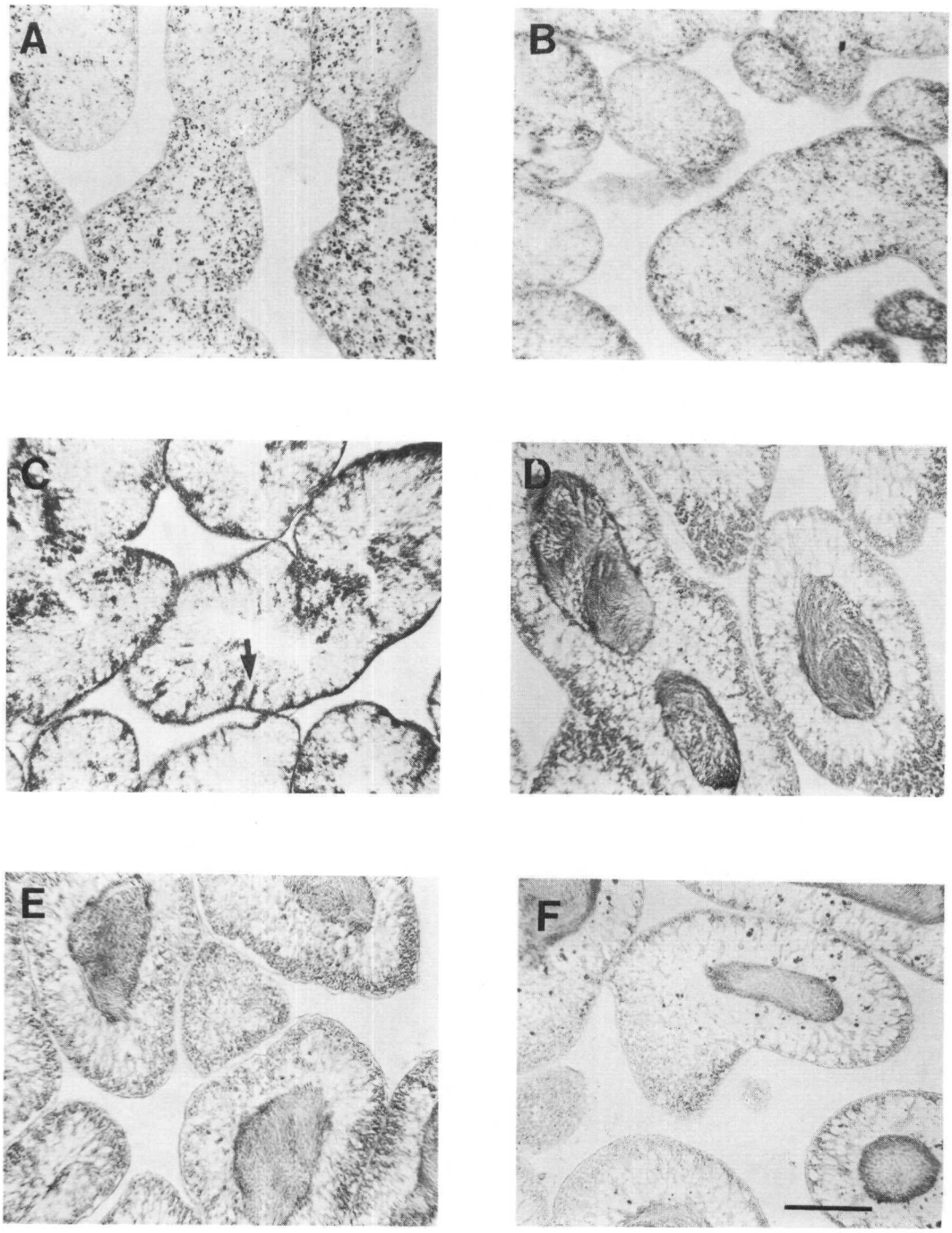

Fig. 3 Histological sections of testes of *Evechinus chloroticus* from the Noises: **A,** 30 April 1975, abundant nutritive phagocytes; **B,** 28 June 1975, small clusters of spermatogonia around peritoneal walls of testis lobes; **C,** 13 September 1975, abundant spermatocytes in clusters (*arrowed*); **D,** 11 November 1975, ripe, with spermatozoa in central lumen of lobes; **E,** 17 January 1976, ripe; **F,** 28 February 1976, ripe. Scale line 200 μm for all sections.

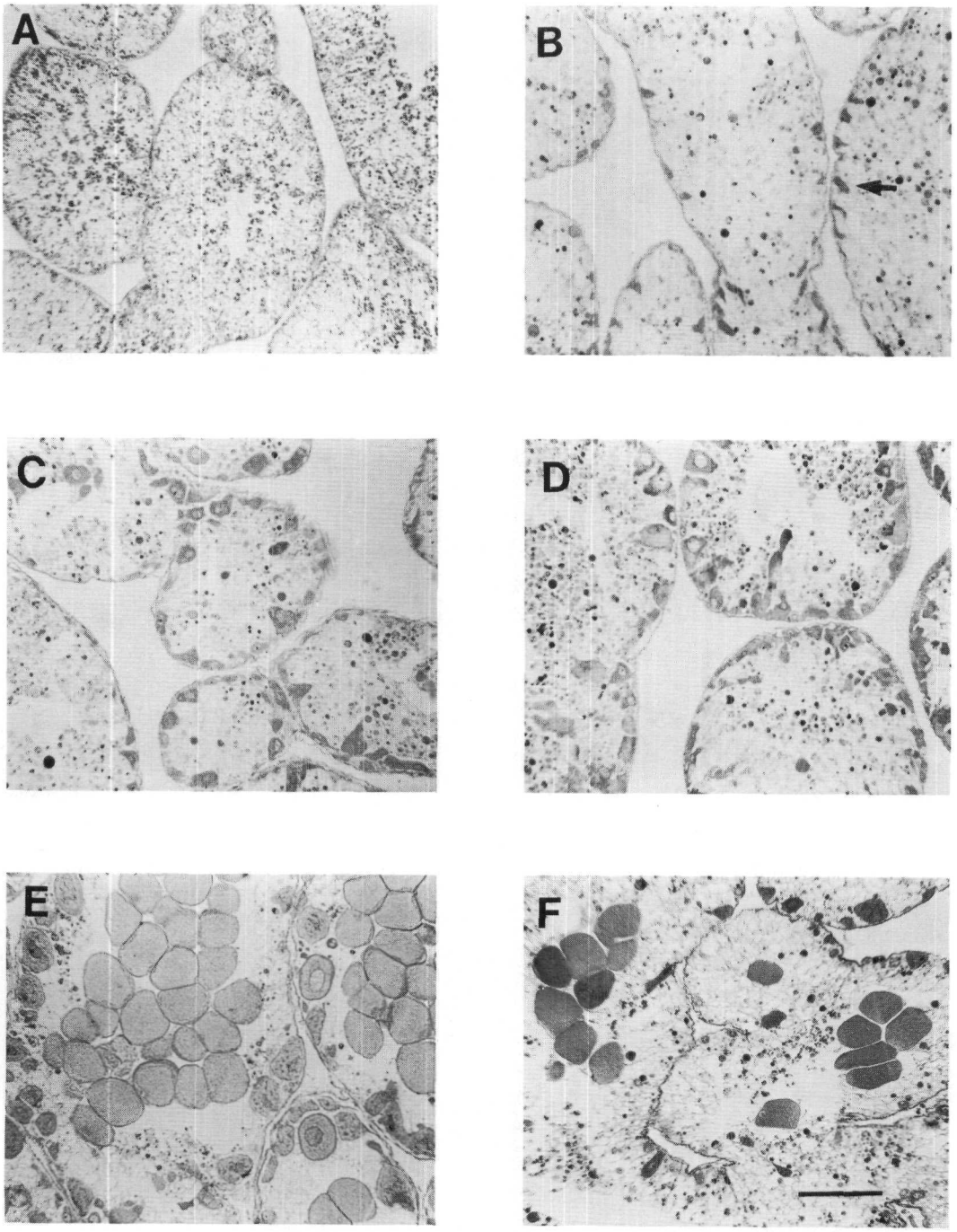

Fig. 4 Histological sections of ovaries of *Evechinus chloroticus* from The Noises: **A,** 30 April 1975, abundant nutritive phagocytes; **B,** 28 June 1975, small oocytes against peritoneal wall of ovary lobes (*arrowed*); **C,** 13 September 1975, abundant oocytes developing against ovarian wall; **D,** 11 November 1975, ripening, with oocytes surrounded by nutritive phagocytes; **E,** 17 January 1976, ripe, with ova free in central lumen of lobes; **F,** 28 February 1976, spawned, with relict ova in lumen. Scale line 200 μm for all sections.

diameter of all the sea urchins taken on a sample date. This approach took account of the effect of test size on gonad size and allowed for easy graphic presentation of habitat-related effects on gonad size. The analysis also avoided many of the statistical problems associated with the use of gonad indices to compare gonad sizes of sea urchins from different habitats (Sokal & Rohlf 1969).

RESULTS

Gametogenic cycle

Between April and September 1975 nutritive phagocytes occupied most of the sections of the gonads of both sexes in *E. chloroticus* (Fig. 1 and 2). During this time these cells contained many darkly staining globules as well as pale brown globules which did not take up stain (Fig. 3 and 4). During spring and early summer these cells became less prominent in both sexes, and the globules in the cells disappeared. By mid summer the nutritive phagocytes were reduced to a thin, lightly staining layer between the germinal tissues and the gamete-filled central lumen of the gonad (Fig. 3 and 4). After spawning, the nutritive phagocytes invaded and filled the empty central lumen of gonad lobes and again became highly vacuolated.

Spermatogonia occurred in clusters, and formed a thin layer at the peritoneal edge of the testicular lobes. They first appeared in sections in the June 1975 sample (Fig. 3). By September 1975 a darkly staining layer of spermatocytes had developed. Extensions of spermatogonia and spermatocyte clusters protruded through the nutritive phagocyte layer toward the central lumen. By September 1975 at Crusoe Island, and by November 1975 at the other sites, free spermatozoa were present within the central lumen of gonad lobes. Spermatozoa were found in gonad sections until the end of sampling in late February 1976, although the percentage of gonad cross-sectional area they occupied varied between sites and between samples.

Oocytes were found in the region of the peritoneal wall of ovarian lobes throughout the year (Fig. 4). During autumn and winter 1975 oocytes were found only near the peritoneal wall, except when they appeared to be disintegrating. By September 1975 a continuous layer of oocytes had formed around the outer walls of ovarian lobes. Some larger oocytes were surrounded by nutritive phagocytic cells and were evidently migrating toward the central lumen. By November 1975 at Crusoe Island, and by January 1976 at the other sites, all females sampled had mature ova within the central lumen of gonad lobes. Mature ova were also found in all females sampled in February 1976.

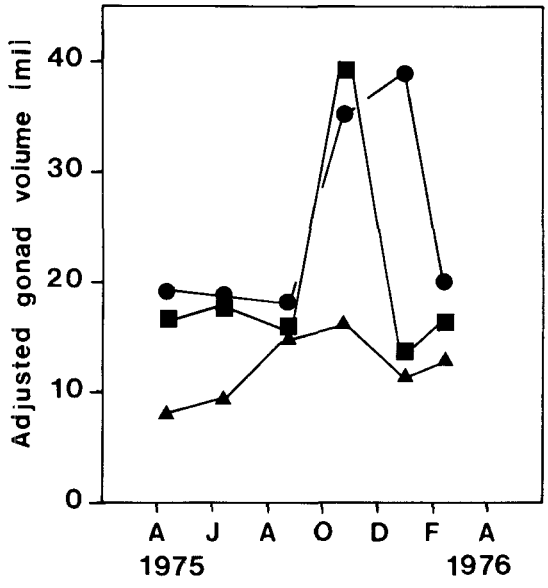

Fig. 5 Seasonal variation in gonad size, *Evechinus chloroticus*. Values shown are adjusted from regression of log. gonad volume on log. test diameter to take account of effect of test size on gonad size (see text). ●, Crusoe I.; ■, Rangitoto I. ▲, The Noises.

Thus, females in the study populations may have been capable of spawning for at least 2 and perhaps as many as 4 months from mid summer to autumn.

Spermatogenesis preceded oogenesis in all 3 study populations. Maximum percentages of cross-sectional area occupied by germinal tissues and appearance of free gametes in gonads occurred in September and November respectively in males (Fig. 1), but not until November and January respectively in females (Fig. 2). Variability in gametogenesis in both sexes within populations was low, suggesting close synchronisation.

A major spawning at Crusoe Island was indicated by histological analysis. Between the November 1975 and January 1976 samples the proportion of tissue sections occupied by free gametes nearly doubled in males and more than quadrupled in females (Fig. 1 and 2). By late February 1976 free gametes occupied only small proportions of sections. This suggested that a major spawning effort had occurred in this population between the January and February 1976 sampling dates. This did not occur in the other 2 populations, perhaps because of the timing of sampling relative to that of spawning.

Gonad size cycle

Major changes in gonad size during the year were associated with spawning. Adjusted mean gonad

volumes doubled during proliferation and growth of gametes in spring in the Rangitoto and Crusoe populations and between mid winter and spring at The Noises (Fig. 5). Gonad volume was halved between the January 1976 and February 1976 samples at Crusoe Island (Fig. 5). This evidence for spawning between these dates coincides with histological indications of spawning recorded above. Gonad volumes declined similarly between the November 1975 and January 1976 samples from both The Noises and Rangitoto. Spawning probably occurred in these populations between these dates, although this was not corroborated by histological analysis.

Adjusted mean gonad volumes differed between habitats (Fig. 5). These differences were significant for all except the September 1975 samples (analysis of covariance – Fs 2,56 >11.5, P <0.001 except February 1976 when Fs 2,56 = 3.14, P = 0.05). Adjusted mean gonad volumes for the 3 populations were almost identical in September 1975. Rapidly changing gonad sizes associated with initiation of gametogenesis in individual sea urchins may have been responsible for the disappearance of habitat-related differences in gonad size in September 1975.

DISCUSSION

Gametogenesis in *E. chloroticus* followed very closely the patterns recorded for sea urchins elsewhere (Boolootian 1966, Holland 1967, Pearse 1969). Cellular events during gametogenesis occurred uniformly throughout the gonads, and included proliferation, differentiation, and growth of spermatozoa in the spring and summer. This was accompanied by an apparent change in the role of the nutritive phagocytes from nutrient storage to transfer of nutrients to the developing gametes.

Nutritive phagocytes are probably not directly involved in the regulation of gametogenesis. There was no evidence of gametogenesis in the April 1975 sample (Fig. 3 and 4). Spermatocytes and oocytes were first observed around the walls of gonad lobes in the June 1975 sample. Between the winter (June 1975) and spring (September 1975) samples the proportion of gonad volume occupied by both germinal tissues and developing gametes increased in all the populations studied. Continuous layers of spermatogonia and oogonia which had formed around the testicular and ovarian walls in September 1975 were found until the end of sampling in February 1976. This suggested that the production rate of spermatocytes and oocytes changed at initiation and cessation of gametogenesis. The nutritive phagocytes therefore appeared to play relatively passive roles, storing nutrients in vacuoles, perhaps destroying relict gametes in the autumn and winter (Pearse 1965), and transferring nutrients to the gametes after initiation of gametogenesis (Pearse 1969).

Histological analyses (Fig. 1 and 2) and gonad size changes (Fig. 5) showed that spawning occurred between 17 January and 28 February 1976 at Crusoe Island, but that these sea urchins were capable of spawning in November 1975, and evidently retained mature gametes for at least 2–3 months. In contrast, histological study suggested that sea urchins from both Rangitoto and The Noises were not capable of spawning in November 1975 (Fig. 1 and 2), but changes in gonad size suggested that both groups had spawned by January 1976. While there is synchrony in initiation of gametogenesis in *E. chloroticus*, the period between appearance of mature gametes in the gonads and spawning can vary between populations.

E. chloroticus, like other echinoids, can retain mature gametes for extended periods before spawning (Holland 1967). The adaptive significance of this ability is that it may allow synchronous spawning independent of annually varying factors. Himmelmann (1975, 1978) demonstrated the importance of the spring phytoplankton increase for synchronising spawning in populations of *Strongylocentrotus droebachiensis* separated by considerable distances. However, there were 1–2-month differences in timing of spawning between *E. chloroticus* populations in the Hauraki Gulf. This suggests that a factor or factors other than a single rapid phytoplankton bloom occurring over a large area initiates spawning in this species. Spawning may be triggered either by cues operating within sea urchin populations or else by factors which can operate over distances of no more than a few km. Experimental studies are necessary to identify these cues.

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