Marine Biology 63, 51–66 (1981)

MARINE BIOLOGY

Reproduction and Genetic Variation in the Deposit-Feeding Sea Star *Ctenodiscus crispatus*

J. M. Shick, W. F. Taylor and A. N. Lamb

Department of Zoology, University of Maine; Orono, Maine 04469, USA

Abstract

The gonad index in the deposit-feeding asteroid *Ctenodiscus crispatus* (Retzius) in the Gulf of Maine (USA) is seasonally less variable than in any other sea star, ranging from 2.99 to 4.98% of dry body weight in females and from 2.28 to 3.42% in males, and varies in concert with, rather than reciprocal to, the pyloric caecum index. Biochemical composition and, hence, caloric content, of the gonads also show little seasonal change, suggesting that reproduction is aseasonal and continuous in this population. Oocyte development is asynchronous, all females having a full size range of oocytes (from less than 30 μm to greater than 400 μm diameter) throughout the year. Seasonally determined size-frequency distributions of juveniles, oocyte cytology and size-frequency distributions, responsiveness of adult females to 1-methyladenine, and oxygen uptake rates indicate that variations in reproductive intensity are superimposed on continuous reproduction, and seem related to changes in phytoplankton production rather than to temperature. The rich neutral lipid content (ca. 50% of total lipid) and large egg size (> 400 μm) in the ovaries suggest that development is direct. The population is extremely variable genetically, polymorphism among 13 enzyme-coding genes being 77% and average heterozygosity being 0.174. The reproductive pattern and genetic variation in the eurybathic *C. crispatus* are similar to those in deep-sea echinoderms. This may be related to the constancy of the population's detrital food source, to small-scale heterogeneity of its physical environment, and to low individual vagility.

Introduction

The sea star *Ctenodiscus crispatus* (Retzius) is an infaunal deposit feeder on clayey silt in the Gulf of Maine (Edwards, 1980; Shick *et al.*, in press). Such sediments represent an organically rich, complex, and stable food source, which should be manifested in the reproduction (Boolootian, 1966; Giese and Pearse, 1974) and perhaps genetics (Valentine, 1976; Ayala and Valentine, 1978; Nelson and Hedgecock, 1980) of this population. *C. crispatus* is similar in morphology and feeding habits to the Porcellanasteridae, a quantitatively important group of truly deep-sea asteroids (Madsen, 1961a, b). Hence, study of the biology of this stenothermal, boreo-arctic species may aid in understanding that of less-accessible deep-sea forms as well.

Much attention has centered on the size and biochemical composition of the gonads and pyloric caeca, the latter being important nutrient storage organs in many asteroids. The frequently observed inverse relationship between the size and energy content of the gonads and pyloric caeca (reviewed by Lawrence, in press) has been interpreted as reflecting the transfer of materials and energy from the caeca to support gonad development during periods of low food availability, an interpretation experimentally verified in *Patiriella regularis* by Crump (1971). The lack of reciprocal changes in pyloric caecum and gonad size in *Patiria miniata* (Farmanfarmaian *et al.*, 1958; Nimitz, 1971) and *Odontaster validus* (Pearse, 1965) has been attributed to their omnivorous, scavenging habits and to lack of seasonality of food availability or feeding activity. Body component indices apparently have not been determined for deep-sea deposit-feeding sea stars, which similarly might be expected to show little seasonal variation in these parameters owing to their occupation of extremely stable environments.

Changes in gonad size and composition give a general picture of the course of gametogenesis and spawning, but more precise information may be obtained using histological techniques (Lawrence, 1973; Giese and Pearse, 1974). Even then, the presence of "ripe" gametes may not necessarily define the spawning season (e.g.

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Farmanfarmaian *et al.*, 1958; Cognetti and Delavault, 1960), which can rarely be observed directly. In the absence of observations of spawning in the study population of *Ctenodiscus crispatus* at a depth of 70 m, we have used several lines of evidence to assess its seasonal pattern of reproduction. These include gonad and pyloric caecum indices and biochemical composition, gonad histology, sensitivity to 1-methyladenine (an inducer of final oocyte maturation and spawning), seasonal rates of aerobic metabolism, and population size-frequency distribution.

Finally, data on the population genetics of *Ctenodiscus crispatus* are presented. These are discussed in conjunction with those available for 8 species of deep-sea asteroids and in relation to several factors purportedly affecting genetic variability in deep-sea invertebrates, as part of a larger study of the physiological ecology of this species (Shick *et al.*, in press).

Materials and Methods

Specimen Collection

Specimens of *Ctenodiscus crispatus* (Retzius) were collected off Damariscove Island and The Cuckolds, Maine, USA (43°44'N; 69°39'W), at water depths of 60 to 76 m, from June 1976 through March 1979. Seasonal data were obtained from collections made from February to November 1977. Mud stars were taken by trawling, using an otter trawl with 0.75 in. (1.9 cm) mesh through 1977, and a Blake trawl containing a 0.1 in. (0.3 cm) bag liner thereafter. Juveniles were obtained by sieving the sediment retained in the Blake trawl through a 1 mm mesh screen. Bottom (seabed) temperatures were taken in coincident sediment samples obtained with a Shipek grab (Edwards, 1980; Shick *et al.*, in press). The mud stars were maintained at the field temperature and salinity in recirculating seawater in the laboratory for a maximum of 60 h before being processed.

Body Component Indices

The distances from the center of the mouth to the tip of the longest arm (R) and from the mouth to the madreporite interradius (r) were measured, and only specimens having $R \geqslant 2.6$ cm were used in the component index determinations. The gonads and pyloric caeca were removed, the sex of each individual recorded, and the organs and eviscerated body dried at 60° to 65 °C for 4 to 7 d. Gonad and pyloric caecum indices are expressed as the percentage of total body dry weight accounted for by each organ (Giese, 1966).

Biochemical Analyses

Subsequent to component index determination, tissues were stored at −20 °C until being ground to a homogeneous powder (or paste, in the case of ovaries). Pooled samples were prepared by combining equal weight aliquots of tissues from each individual in a collection (Pearse, 1965).

Tissue extraction and analysis of levels of protein, total and neutral lipid, free reducing sugars, and total carbohydrate were performed after Holland and Gabbott (1971) and Holland and Hannant (1973), with the following modifications: Owing to the high lipid content of the tissues, extraction was done by homogenizing 10 mg of tissue in 500 µl of methanol:chloroform (2:1). To 200 µl of the homogenate were added 550 µl of methanol-chloroform and 200 µl of water, after which the procedure of Holland and co-workers was again followed. Protein was determined by the microbiuret method (Itzhaki and Gill, 1964).

Caloric composition of the tissues was estimated by using the values 5.7, 9.5 and 4.1 cal mg^{-1} dry weight for protein, total lipid, and total carbohydrate, respectively (Brody, 1945).

Histological Procedures

Separate groups of individuals were used for histological analysis of the gonads in each collection because of the small size of the organs. Gonads were fixed in Bouin's solution, and subsequently washed and stored in 70% ethanol. They were embedded in paraffin at 61 °C after dehydration with ethanol and clearing with xylene, and sectioned at 10 µm (females) or 3 µm (males). Sections were stained in phosphotungstic acid hematoxylin (PTAH) (Luna, 1968) or in aqueous periodic acid Schiff (PAS) with alcian blue and fast green counterstains (Humason, 1972).

Oocyte size-frequency distributions were compiled for 5 individuals from PAS-stained slides from each collection. Over 5 to 10 serial sections, the first 50 oocyte sections encountered displaying nucleoli were measured. Each oocyte cross-section was classified as an ellipse, rectangle, or triangle, and two appropriate diameters were measured to calculate the oocyte cross-section area. The diameter of a hypothetical sphere with the same cross-sectional area was then calculated, and is referred to as the oocyte diameter. All measured oocytes were scored either PAS-negative or PAS-positive. Size-frequency distributions, resticted to mature oocytes (as determined by staining characteristics: see "Results"), were also obtained. The first 18 to 25 (generally 20) mature oocyte sections with visible nucleoli were measured and scored for the absence or presence of atritic regions. Oocyte size-distribution data were converted to $\log_{10}$ (oocyte diameter, µm) to encompass the extensive size range, and plotted as size-frequency polygons.

Responsiveness to 1-Methyladenine

The ovarian hormone 1-methyladenine (1-MA) is an inducer of oocyte final maturation and spawning in

Table 1. *Ctenodiscus crispatus*. Genetic variation, as proportion of polymorphic enzymes, heterozygosity for individual genes (h), and average heterozygosity (H). Heterozygosities calculated after Nelson and Hedgecock (1980). Buffers = I: Tris-citrate, pH 6.3 (Selander *et al.*, 1971); II: lithium hydroxide, pH 8.0 (Ridgway *et al.*, 1970); III: Tris-borate, pH 8.6 (Markert and Faulhaber, 1965). Stains were modified from (A) Shaw and Prasad (1970), (B) Selander *et al.* (1971), and (C) Brewer (1970)

Enzymes	Buffer	Stain	No. of individuals scored	Frequency of heterozygotes (h)
Group I enzymes				
Fumarase (FUM)	III	A	3	0
Glutamate-oxaloacetate transaminase (GOT)	III	B	7	0.143
α-Glycerophosphate dehydrogenase (α-GPDH)	III	A	6	0
Hexokinase (HK)	I	B	18	0.444
Isocitrate dehyrogenase (IDH)	I	B	9	0.111
Lactate dehydrogenase (LDH)	II	A	2	0
Malate dehydrogenase (MDH)	I	B	24	0.083
Phosphoglucose isomerase (PGI)	II	B	45	0.711
Superoxide dismutase (SOD)	III	C	28	0.179
Group II enzymes				$H_I = 0.186 \pm 0.080$
Alkaline phosphatase (ALP)	I	A	6	0.167
Esterase-I (EST-I)	II	A	28	0.143
Esterase-II (EST-II)	II	A	49	0.184
Leucine aminopeptidase (LAP)	II	B	33	0.091
				$H_{II} = 0.146 \pm 0.020$
Proportion of polymorphic enzymes = 0.769; average heterozygosity, $H = 0.174 \pm 0.055$				

all sea stars studied to date (Kanatani, 1969, 1975), including *Ctenodiscus crispatus* (Turner, 1976). To clarify further the reproductive condition of the study population, freshly-collected females were injected with 0.5 ml of 2×10^{-4} *M* 1-MA in filtered artificial seawater. Although 1-MA-induced spawning is dose-dependent (Kanatani, 1969), preliminary experiments in winter and summer showed no enhanced effect of concentrations as high as 5×10^{-3} *M*. The present studies were done over the course of 1 yr, and females were tested at 2.5°, 5.0° and 10.0 °C at all seasons to examine the effects of both season and temperature on the effectiveness of the hormone in inducing spawning and on the latent period (see Turner, 1976). Seawater-injected controls were included in each experiment, and none spawned. Of 47 male specimens injected during 1976-1979, none responded to 1-MA.

Population Size-Frequency Analysis

Large collections of juveniles from August 1978 (n = 97) and March 1979 (n = 160) and of adults from March 1979 (n = 136) were measured. Juveniles were dried at 60° to 65 °C for 3 d and weighed. R and r of adults were measured as previously described. R was converted to dry weight (W) using the relationship $W = 1.558\ R - 2.221$ ($r^2 = 0.994$; $P < 0.001$) determined on a subsample of 35 individuals.

Oxygen Uptake

Rates of oxygen uptake ($\dot{V}_{O_2}$) by intact female mud stars were determined polarographically as described in Shick (1976). Seasonal measurements of $\dot{V}_{O_2}$ were made at the temperature of the seabed at the times of collection, using 10 to 15 specimens ranging from 400 mg to 3.1 g dry wt, and on a group of 5 individuals of average size (about 2 g) in August 1976.

Electrophoresis

Horizontal starch-gel electrophoresis of fresh and frozen tissue homogenates was carried out as described in Shick and Lamb (1977). Preliminary studies revealed no tissue-specific differences in zymogram patterns, and gonads generally gave the best results. Buffers and enzyme stains used are detailed in Table 1. The frequency of heterozygous individuals at each locus (h) and across loci (H) was calculated after Nelson and Hedgecock (1980, p. 252).

Results

Gonad Index and Composition

During the period February through November, 1977, the mean gonad index (GI) varied from 2.99 to 4.98

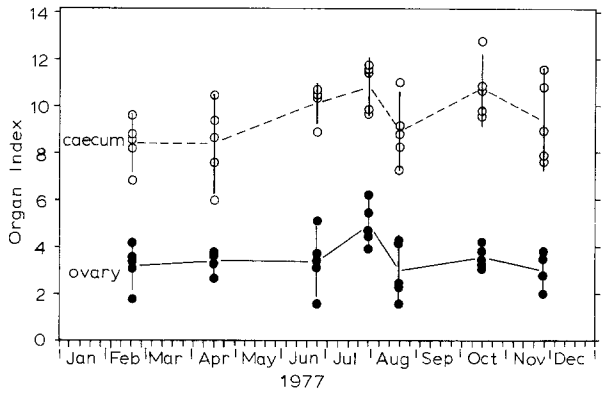

Fig. 1. *Ctenodiscus crispatus.* Monthly mean female gonad and pyloric caecum indices, as % of total body dry wt. Vertical lines represent 95% confidence intervals for 5 individuals per month. July ovary index is significantly different from all others (Duncan's multiple range test, $P < 0.05$)

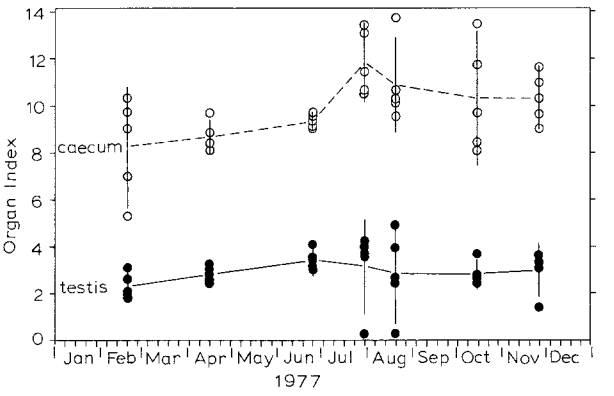

Fig. 2. *Ctenodiscus crispatus.* Monthly mean male gonad and pyloric caecum indices, as % of total body dry wt. Vertical lines represent 95% confidence intervals for 5 individuals per month. Monthly variances for both indices were significantly heterogenous (Bartlett's Box - F, $P < 0.01$), precluding valid comparisons of means by ANOVA

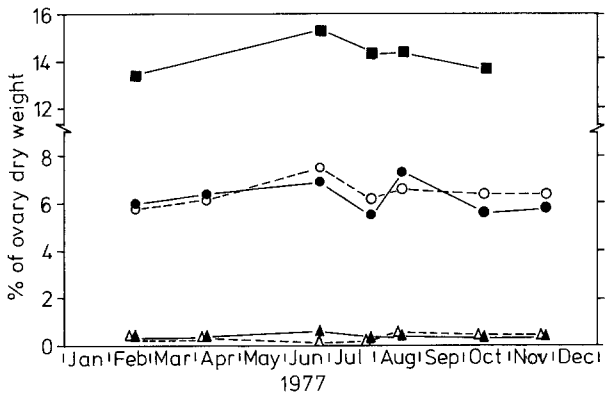

Fig. 3. *Ctenodiscus crispatus.* Monthly values of levels of protein (■), neutral (●) and polar (○) lipid, polysaccharide (▲), and free reducing sugars (△) in ovaries. Each point represents mean of duplicate analyses of averaged (pooled) samples ($n = 5$) from each month's collection

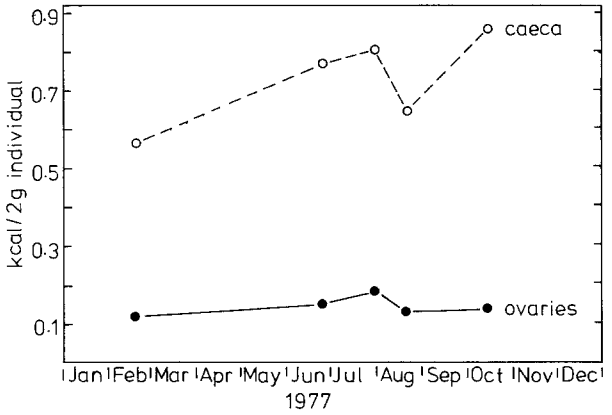

Fig. 4. *Ctenodiscus crispatus.* Total monthly caloric content of ovaries and pyloric caeca of an average 2 g female. Values calculated from data in Figs. 1, 3 and 13

in female *Ctenodiscus crispatus* (Fig. 1) and from 2.28 to 3.42 in males (Fig. 2). The female GI rose significantly ($P < 0.05$; Duncan's multiple range test) only in July, and otherwise remained unchanged throughout the year. The male GI did not show significant change over the period of observation, although the variances of male GI in July and August were significantly greater than those over the remainder of the year (Bartlett's Box $F = 3.234$, $P = 0.004$). Single specimens with spent testes (GI = 0.27 and 0.30, respectively) were seen in both July and August.

No pronounced seasonal variations of any of the biochemical constituents were seen in the ovaries (Fig. 3). Protein concentration was 13.7 to 15.4% of ovary dry weight. The concentration of total lipid was 11.7 to 14.4%, of which 46.7 to 52.2% was neutral lipid. Polysaccharide concentration was 0.48 to 0.80%, and free reducing sugars were 0.06 to 0.49% of dry weight. Owing to both the low amplitude of the ovary index fluctuation and the relative constancy of their biochemical composition, the caloric content of the ovaries of an average 2 g female was remarkably stable at 0.12 to 0.18 kcal (Fig. 4)

No seasonal variation was seen in testis concentrations of neutral or polar lipid, polysaccharide or free reducing sugars (Fig. 5). Total lipid was 11.0 to 11.7% of testis dry weight, with neutral lipid comprising 34.5 to 40.2% of total lipid. Polysaccharide concentration was 0.07 to 0.29%, and that of free reducing sugars was 0.43 to 0.50%. The greater variablility of testis protein concentration (27.9 to 36.1%) is not reflected in other biochemical compounds and is unex-

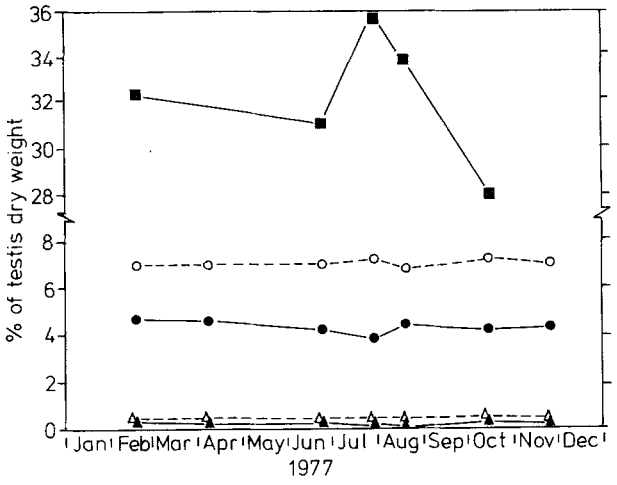

Fig. 5. *Ctenodiscus crispatus.* Monthly values of levels of protein (▪), neutral (•) and polar (○) lipid, polysaccharide (▲), and free reducing sugars (△) in testes. Each point represents mean of duplicate analyses of averaged (pooled) samples (n = 5) from each month's collection

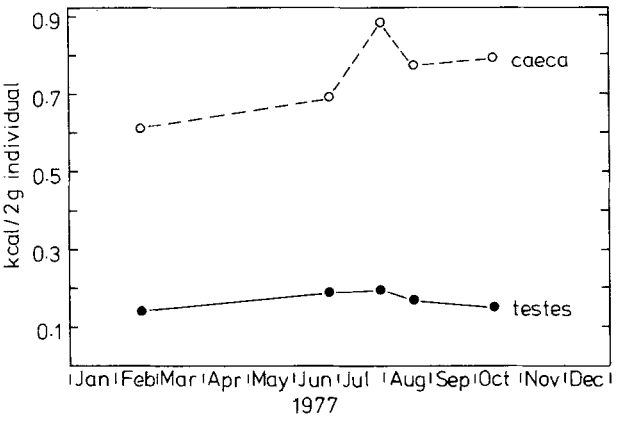

Fig. 6. *Ctenodiscus crispatus.* Total monthly caloric content of testes and pyloric caeca of an average 2 g male. Values calculated from data in Figs. 2, 5 and 14

plained. The testis caloric content of an average 2 g male is again seasonally invariant at 0.16 to 0.20 kcal (Fig. 6).

Gonad Histology

The ovary wall in *Ctenodiscus crispatus* has briefly been described by Walker (1974). It is composed of two concentric sacs separated by the genital coelomic (perihemal) sinus. The outer sac consists of visceral peritoneum, connective tissue, circular muscle fibers and an internal epithelium. The inner sac consists of epithelial cells and longitudinal muscle fibers, the hemal sinus, and the germinal epithelium. The hemal sinus was filled with a granular, PAS-positive coagulated fluid and contained free amoeboid cells. No changes in the width of the hemal sinus nor in the staining characteristics of the contained material were noted over the observation period.

It was not possible to construct stages of ovary development based on qualitative characters as used previously for *Asterias vulgaris* by Lowe (1978), *Leptasterias hexactis* by Chia (1968), and *L. tenera* by Worley *et al.*, (1977). All female *Ctenodiscus crispatus* displayed a wide range of oocyte diameters, ranging from approximately 15 to 400 μm (Fig. 7, Fig. 8). Oocytes of diameter greater than ca. 150 μm showed distinct staining characteristics, being PAS-positive and staining red (as opposed to violet) with PTAH, and of a distinct "moth-eaten" appearance (Fig. 7A), as described for mature oocytes of *Pisaster ochraceus* by Mauzey (1966), *L. hexactis* by Chia (1968), and *A. vulgaris* by Lowe (1978). All oocytes were surrounded by a halo of follicle cells (Fig. 7B). Phagocytes were observed free in the lumen in all months, and were commonly associated with senescent oocytes. Nests of oogonia (Fig. 7B) were seen in specimens from all collections, but decreased in frequency in late autumn and early winter.

Size-frequency polygons of oocyte diameters for individual females are presented in Fig. 8. The same data, with individuals pooled within months, are shown in Fig. 9. Significant differences in size-class distributions of oocytes were observed among individuals in May and July [heterogeneity G-test (Sokal and Rohlf, 1969, Chapter 16) $P < 0.005$], and among months ($P < 0.0005$). Analysis of variance revealed a significant increase in mean oocyte diameter over the period from February (66 μm diameter) through November (100 μm diameter). The frequency of oocytes in the 0 to 50 μm range was lowest in November (9.2%) and greatest in February (37.7%) (Fig. 10), suggesting a mid-winter period of primary oogenesis. Although no significant difference among months was seen in the frequency of mature (PAS-positive) oocytes, their frequency tended to increased with time and to mirror the decline in the frequency of the smallest oocytes (Fig. 10).

Mature oocytes (those showing yolk-specific staining with PAS or PTAH) were abundant throughout the year (Fig. 11), although significant differences in size-class distributions of the ooyctes were observed among collections (heterogeneity G-test, $P < 0.005$). Cumulative frequency plots of the log-diameter distributions on probability paper (Cassie, 1954) were inconclusive, but suggested the existence of four separate populations of mature oocytes during the course of the year. More certain is the disappearance between April and May, and between October and November, of the largest of these oocytes, having attained a maximum diameter > 400 μm (Fig. 11).

Senescent PAS-positive oocytes (characterized by agranular, densely-stained regions, Fig. 7C, were fre-

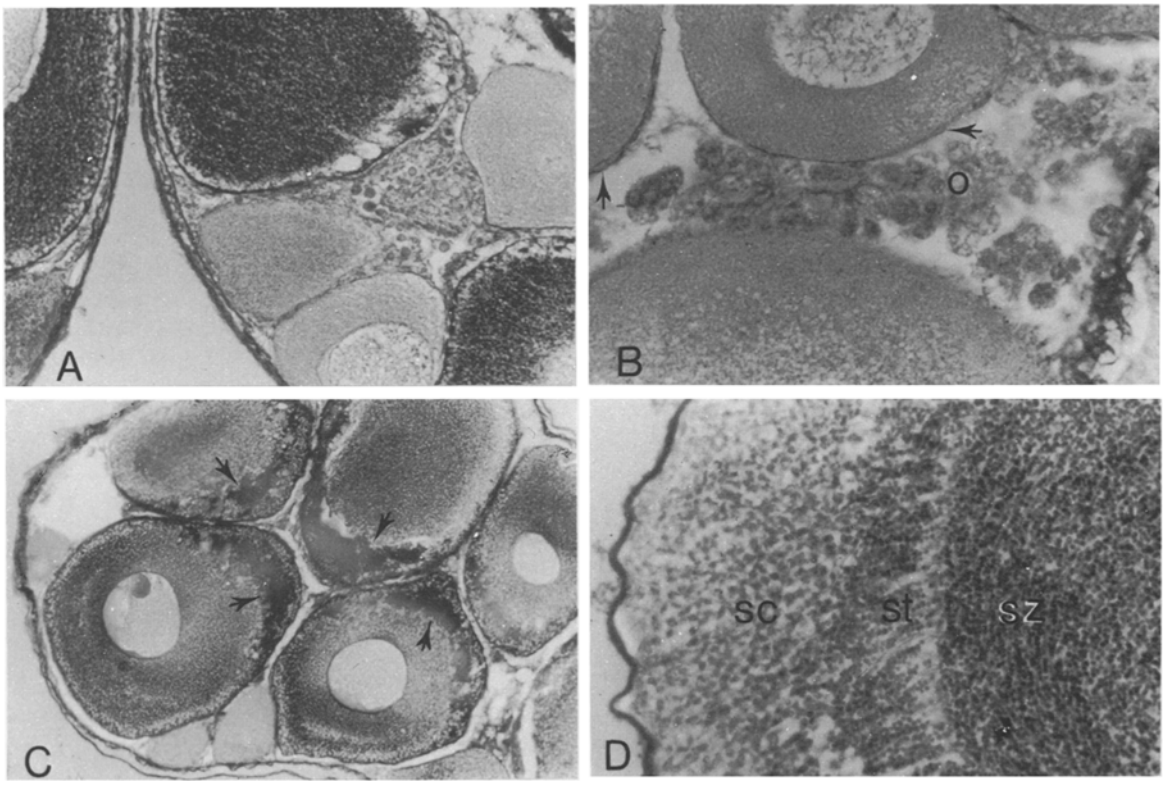

Fig. 7. *Ctenodiscus crispatus.* (A) Section of ovary showing mature, PAS-positive (dark staining) and developing PAS-negative (lighter staining) oocytes; (B) follicle cells (arrows) associated with developing oocytes, and nest of oogonia (o); (C) senescent PAS-positive oocytes, characterized by agranular, densely-staining atritic regions (arrows); (D) section of testis showing spermatogenic columns (sc), spermatids (st), and mature, tailed spermatozoa (sz). All specimens collected November 1977. (A), (C), (D) X 100; (B) X 400

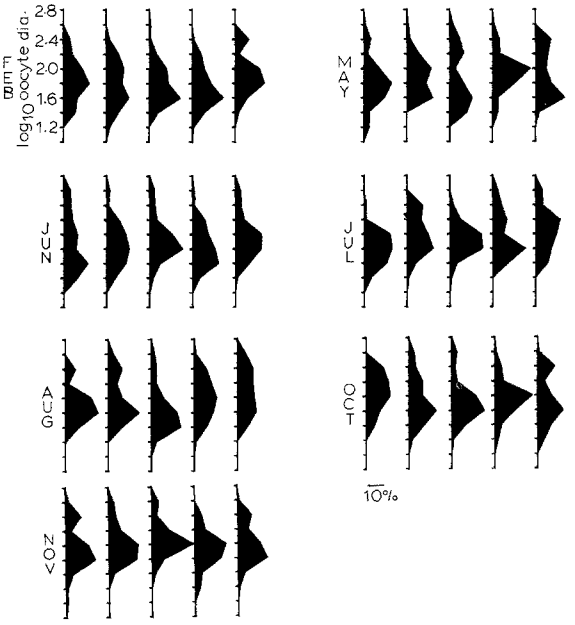

Fig. 8. *Ctenodiscus crispatus.* Monthly size-class distributions in 1977 of oocytes in ovaries of 5 individuals. Significant within-monthly variation in distribution exists in May and July (heterogeneity *G*-test, $P < 0.005$). Variation in distributions between months is significant (heterogeneity *G*-test, $P < 0.0005$). dia: diameter

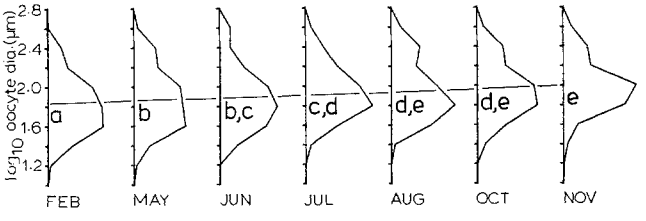

Fig. 9. *Ctenodiscus crispatus.* Size-class distributions in 1977 of oocytes, 5 individuals pooled within each month. Variation in distributions between months is significant (heterogeneity *G*-test, $P < 0.0005$). Horizontal line connects mean monthly values of $\log_{10}$ (oocyte diameter). Mean values labelled with different letters (a–e) are significantly different (Duncan's multiple range test, $P < 0.05$)

quently observed in various stages of atresia. No atritic PAS-negative oocytes were ever seen. Size-frequency distributions of senescent oocytes are superimposed on those of healthy mature oocytes in Fig. 11. No clear seasonal trend in the frequency of these atritic oocytes was observed, although atresia tended to be more frequent in the larger-size oocytes.

The general histology of the testis resembled that in other asteroids (e.g. Cognetti and Delavault, 1960; Pearse, 1965; Lowe, 1978; Walker, 1980). As was the

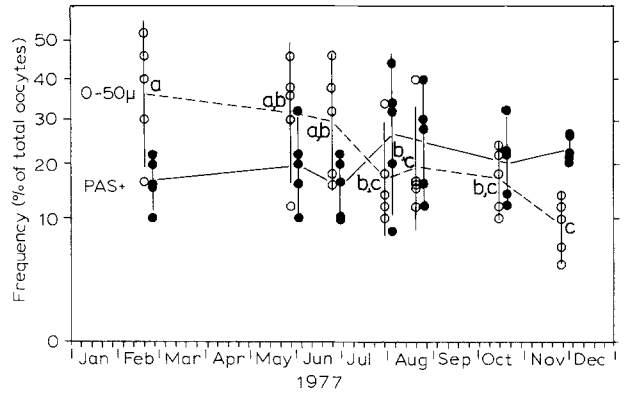

Fig. 10. *Ctenodiscus crispatus.* Monthly frequencies of 0 to 50 μm diameter (○) and PAS-positive (●) oocytes (n = 5 individual ovaries per month). Ordinate was angle-transformed (y = arcsin $\sqrt{p}$) to normalize variances. Vertical lines represent 95% confidence intervals. Mean frequencies of 0 to 50 μm oocytes labelled with different letters (a–c) are significantly different (Duncan's multiple range test, $P < 0.05$). No significant differences among mean frequencies of PAS-positive oocytes were found over the period of observation (ANOVA, $P > 0.10$)

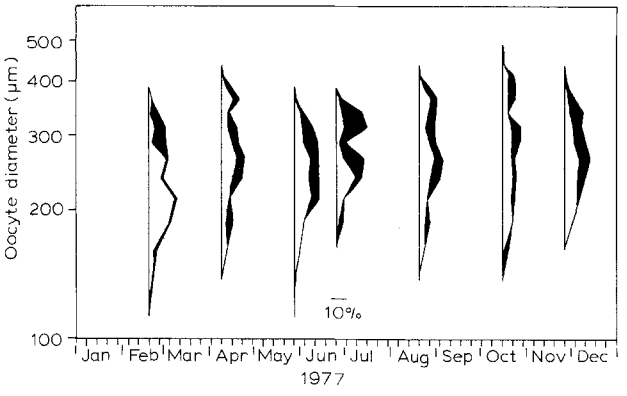

Fig. 11. *Ctenodiscus crispatus.* Monthly size-class distributions of mature yolked (PAS-positive) oocytes, 4 to 5 individuals pooled within collections. Shaded areas represent senescent atritic oocytes. Ordinate log-transformed [$y = \log_{10}$ (oocyte diameter)] to normalize variances. Among-month differences in distributions are significant (heterogeneity G-test, $P < 0.005$)

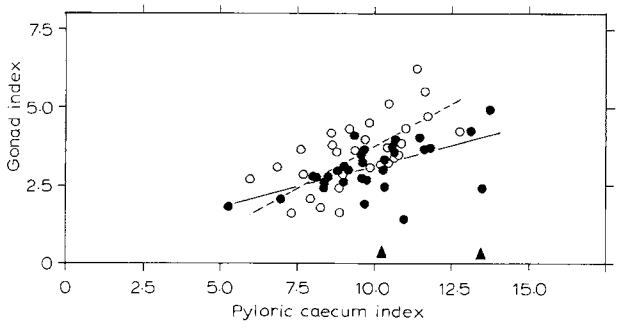

Fig. 12. *Ctenodiscus crispatus.* Correlation of female (○) (n = 35) and male (●) (n = 33) gonad and pyloric caecum indices. For females, $y = 3.51 \pm 0.55$ ($x - 9.56$) $P < 0.001$. For males, $y = 3.07 + 0.27$ ($x - 9.83$), $P < 0.001$. Triangles represent two exceptional males from July and August (see text, "Results") which were excluded from analysis

case in females, no classification of gonad stage based on qualitative characters was possible. The germinal epithelium in all specimens examined was elaborated into spermatogenic columns, with successively distal regions of the columns being comprised of increasingly old cell stages (Fig. 7D). Spermatogonia and mature, tailed sperm (free in the lumen) were numerous in all individuals from all collections. No seasonal trend in the thickness of the germinal epithelium (spermatogenic column height) was seen, and this parameter was highly variable even within individual testes. Phagocytic cells were not observed in the testes.

Pyloric Caecum Index, Composition, and Relation to Reproduction

The pyloric caecum index was slightly more variable than the gonad index, ranging from 8.4 to 10.9% in females and 8.2 to 11.8% in males (Figs. 1 and 2). The female caecum index cycle was bimodal, with maxima in July and October while that in males showed a single peak in July. The August decrease in caecum indices paralleled the decline in gonad indices at that time. Considering all individuals measured, the pyloric caecum index was strongly positively correlated with the gonad index (Fig. 12) in females ($r^2 = 0.64$, $P < 0.001$). With the two exceptional individuals from July and August omitted from analysis, a strong positive correlation was also observed in males ($r^2 = 0.56$, $P < 0.001$).

Caecum levels of storage compounds (protein, neutral lipid, and polysaccharide) showed slight seasonal variability in both sexes (Figs. 13 and 14). In females, protein increased from 26.1% of caecum dry weight in February to a maximum of 31.8% in July, then declined to 29.0% in August. Neutral lipid remained constant at approximately 7.5% from February through July, and became more variable during August through November. Polysaccharide rose from less than 1% in February to a peak of 5.1% in June, declined to 2.6% in July, and rose and remained above 4.5% for the remainder of the sampling period. Polar lipid and free reducing sugars showed little variation. Protein was less variable in males, and both neutral lipid and polysaccharide remained stable at about 7.5 to 8% and 1 to 2%, respectively, from February through July. Both then rose rapidly to 10.4 and 5.1%, respectively, in August, with polysaccharide remaining high and neutral lipid gradually declining through November.

The caloric content of the pyloric caeca of an average 2 g individual showed a similar annual pattern in both sexes (Figs. 4 and 6), increasing from February through July, declining in August, and increasing sharply (females) or slightly (males) in October. The tendency for total caloric content to increase over the observation period was owing both to a similar tendency in caecum index and to an increase in storage components, and may reflect thermal effects on feeding and digestion, since temperature increased steadily during this

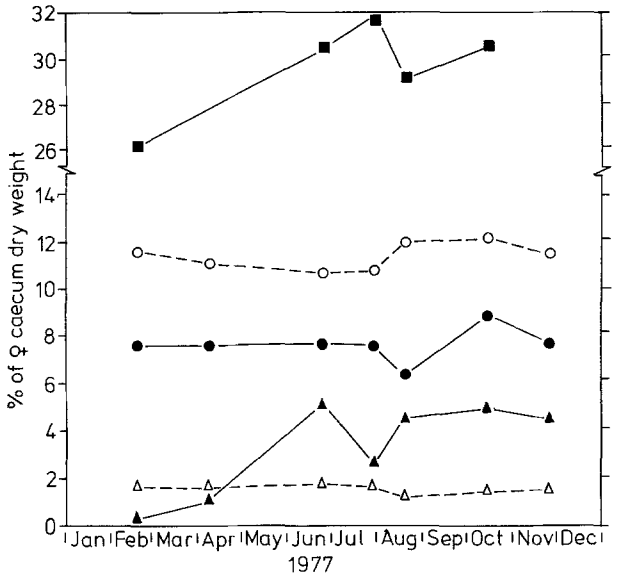

Fig. 13. *Ctenodiscus crispatus*. Monthly values of levels of protein (■), neutral (●) and polar (○) lipid, polysaccharide (▲), and free reducing sugars (△) in female pyloric caeca. Each point represents mean of duplicate analyses of averaged (pooled) samples (n = 5) from each month's collection

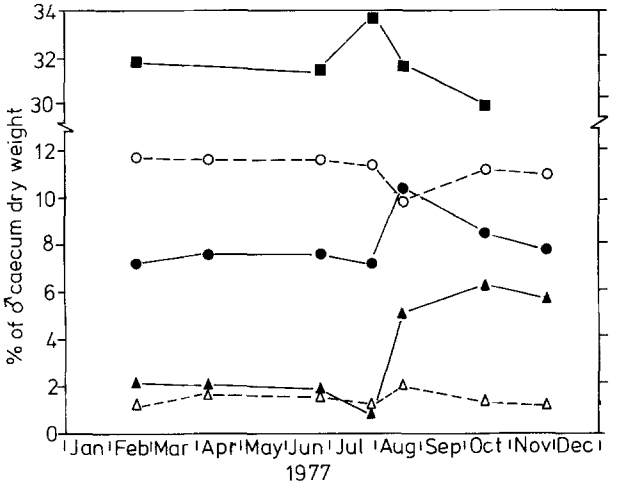

Fig. 14. *Ctenodiscus crispatus*. Monthly values of levels of protein (■), neutral (●) and polar (○) lipid, polysaccharide (▲), and free reducing sugars (△) in male pyloric caeca. Each point represents mean of duplicate analyses of averaged (pooled) samples (n = 5) from each month's collection

period (Table 2, and Edwards, 1980). The August decline in caecum caloric content was largely a function of a reduced caecum index rather than of a variable decrease in individual storage components, implying cell destruction (cf. Lawrence, 1973).

Responsiveness to 1-Methyladenine

The effectiveness of 1-methyladenine in inducing spawning in female *Ctenodiscus crispatus* varied both seasonally and with temperature (Table 2). The greatest response (100% of injected specimens spawning) occurred in early August 1976 at 10 °C, slightly higher than the seabed temperature. No specimens spawned in February or May 1977. During late July to mid-August (the period of greatest sensitivity), the magnitude of the spawning response was directly related to laboratory exposure temperature between 2.5° and 10.0 °C. By late August the percentage of sea stars spawning appeared to decline, although the small sample size makes this uncertain. The length of the latent period (time between injection of 1-MA and initial appearance of shed ova; Turner, 1976) was inversely related to temperature and did not vary seasonally at a given exposure temperature. No control specimens spawned in any of the experiments.

Individual Size-Frequency Analyses

Adult specimens of *Ctenodiscus crispatus* were continuously and normally distributed from a dry weight of 0.4 to 3.4 g (Fig. 15). Plots of cumulative frequency on probability paper (Cassie, 1954) revealed no clear size classes in these data. Juvenile mud stars were con-

Table 2. *Ctenodiscus crispatus*. Effects of season and temperature on responsiveness of females to 1-methyladenine

Date	Seabed Temp (°C)	2.5 °C			5.0 °C			10.0 °C		
		n	No. spawning	Latent period, h (± SD)	n	No. spawning	Latent period, h (± SD)	n	No. spawning	Latent period, h (±SD)
13 Aug. 1976	7	10	4	22.60 (0.61)	10	8	13.82 (0.65)	10	10	8.40 (0.54)
18 Feb. 1977	2	5	0	-	5	0	-	5	0	-
14 Apr. 1977	3.5	5	1	24–27	5	0	-	5	0	-
27 May 1977	4.7	5	0	-	5	0	-	5	0	-
25 June 1977	-	5	0	-	5	0	-	5	2	8.92, 10.92
27 July 1977	6.7	5	0	-	5	2	12.15, 14.03	5	4	8.25 (0.50)
23 Aug. 1977	7.2	3	2	22.0, 22.5	-	-	-	3	1	6.25
13 Oct. 1977	8.0	5	0	-	5	1	36–52	5	0	-

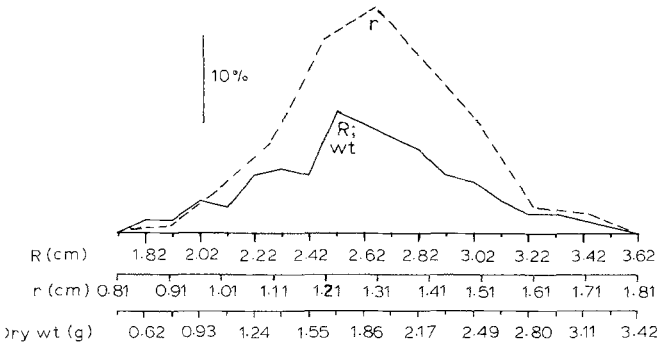

Fig. 15. *Ctenodiscus crispatus.* Size-class distributions of adults in March 1979 (n = 136). Deviation from normality was not significant (Kolmogorov-Smirnov test, $P < 0.95$)

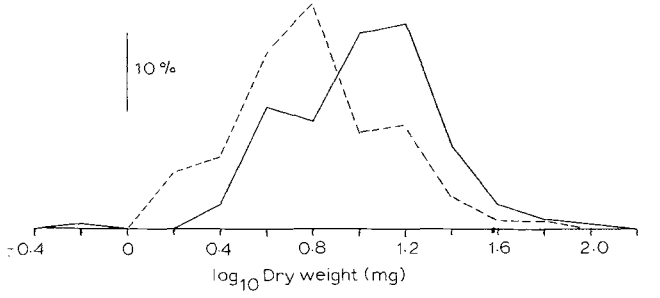

Fig. 16. *Ctenodiscus crispatus.* Size-class distributions of juveniles in August 1978 (- - - -) (n = 97) and March 1979 (——) (n = 160). Deviation from log-normality was not significant [Kolmogorov-Smirnov test, $P < 0.40$ (August); $P < 0.95$ (March)]. Mean size in March 1979 was significantly greater than that in August 1978 (Student's t-test, $P < 0.01$)

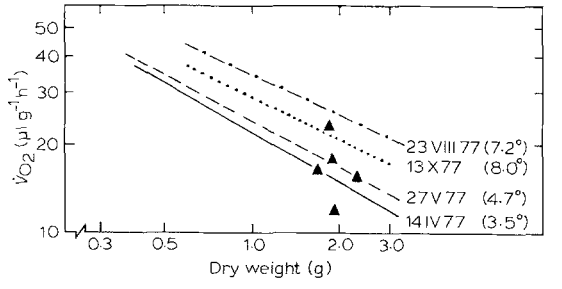

Fig. 17. *Ctenodiscus crispatus.* Seasonal rates of oxygen uptake as a function of body size in females at measured seabed temperatures (°C). Best-fit lines calculated by least-squares regression analysis of 10 to 15 individuals per collection. Triangles are data for 5 individuals collected on 13 August 1976 (seabed temperature 7 °C) and $\dot{V}_{O_2}$ measured at 7.5 °C

tinuously and log-normally distributed in both collections analyzed (Fig. 16). Although no distinct size classes are apparent in either collection, the mean body weight increased significantly from 6 mg in August 1978 to 15 mg in March 1979. Individuals less than 1 mg dry weight were present in both collections.

Oxygen Uptake

Oxygen uptake by female specimens of *Ctenodiscus crispatus* was affected by both season and temperature (Fig. 17). $\dot{V}_{O_2}$ in an average 2 g mud star was 15.0 µl g^{-1} h^{-1} at the seabed temperature of 3.5 °C in April; the rate at the seasonal temperature of 8.0 °C in October was 21.0 µl O_2 g^{-1} h^{-1}, giving a Q_{10} of 2.11. However, $\dot{V}_{O_2}$ in late August (temperature 7.2 °C) was elevated to 25.3 µl g^{-1} h^{-1}, and may be related to the decline in female gonad and pyloric caecum indices at that time. In early August 1976 (temperature 7.5 °C), when organ indices remained at their peak signifying no spawning had yet occurred, the mean $\dot{V}_{O_2}$ for 5 individuals (mean dry wt 1.91 g) was 17.1 µl g^{-1} h^{-1}, so that the increased $\dot{V}_{O_2}$ in late August may reflect an increased energy demand after an increased intensity of reproduction.

Genetic Variation

The population of *Ctenodiscus crispatus* under study is genetically rather variable. Ten of the 13 enzyme-coding genes examined are polymorphic, the frequency of heterozygotes (h) at these loci ranges from 0 to 0.711, and average heterozygosity (H) is 0.174 ± 0.055 (SE), which is at the high end of the heterozygosity spectrum in benthic marine invertebrates (cf. Valentine, 1976, p. 90; and Somero *et al.*, in press). Sample sizes and individual values of h are presented in Table 1. The enzymes studied have been broadly categorized as Group I (central metabolic enzymes) or Group II (enzymes processing food substrates) (Gillespie and Langley, 1974; Nelson and Hedgecock, 1980).

Discussion

Reproduction

Several lines of evidence indicate that reproduction in *Ctenodiscus crispatus* is continuous, with several superimposed periods of increased intensity. We interpret the observed pattern as a manifestation of a continuously available food source (clayey silt, of which 10% of the dry weight is organic) for the adults, and as an adaptation to a seasonally predictable rain of phytoplankton-derived detritus which may be of particular importance to directly-developing juveniles.

The ovary and testis indices of *Ctenodiscus crispatus* display the lowest seasonal amplitude of any asteroid yet examined (reviewed by Lawrence and Lane, in press), and the continuous presence of mature eggs led von Hofsten (1915) and Thorson (1936) to conclude that spawning occurs over a very long period. These are crude measures of reproductive state, however, and Giese and Pearse (1974) recommend the use of additional techniques such as gonad histology.

In *Ctenodiscus crispatus*, proliferating spermatogenic columns, differentiating spermatids, and mature, tailed spermatozoa (Fig. 7D) are present in virtually all males throughout the year. The situation is highly complex, in that the relative extent of proliferation and differentiation varies not only among individuals, but also among columns within a single testis, so that unlike the more nearly synchronous condition described in *Asterias vulgaris* (Lowe, 1978; Walker, 1980), proliferation, differentiation, and maturation of sperm occur continuously and asynchronously within the *C. crispatus* population.

A similar situation exists in the ovaries. Not only is there little variation in the ovary index, but the ovaries in any given month have the full size range of oocytes, from less than 30 to approximately 400 μm in diameter (Figs. 7 and 8). Such a large, yolky egg (neutral lipid comprises up to 52.5% of total ovary lipid, extremely high among asteroids) implies that development is direct in this species (cf. Thorson, 1963; Tyler and Gage, 1980b), a point to be considered below. The variation in oocyte size-frequency distribution is greater among individuals within a month (Fig. 8) than among months when all individuals are pooled (Fig. 9). *Ctenodiscus crispatus* thus belongs to a small group of asteroids exhibiting truly aseasonal, asynchronous oocyte growth and maturation (Table 3), which is distinct from a simple overlapping of generations of oocytes (Pearse, 1965).

Cognetti and Delavault (1962) first drew the distinction between asteroids displaying asynchronous oocyte growth and those showing synchronous growth. Ovaries from representatives of the first category displayed a wide range of oocyte diameters with heterogenous staining properties, attributed to different amounts of yolk. Ovaries from representatives of the second category contained either a single homogenous oocyte population or two readily distinguishable populations, the smaller oocytes being present along the gonad wall. Phagocytic cells were present at all times in ovaries of the first group, as they are in *Ctenodiscus crispatus*, but appeared only for a short time after spawning in ovaries of the second group.

The pooling of 5 individuals within each month to construct a series of oocyte size-frequency polygons (Fig. 9) emphasises the similarity of reproductive condition of the *population* from month to month. The significant increase in the mean size of oocytes throughout the year (Fig. 9) is apparently owing to a decrease in the frequency of the smallest size classes (Fig. 10), implying a mid-winter period of primary oogenesis followed by differential growth rates of the oogonia. There is no significant inverse trend in the frequency of PAS-positive (mature) oocytes (Fig. 10), suggesting their continuous loss during the year, which is supported by the continuous presence of senescent oocytes and phagocytic cells in the ovaries. The difference in frequencies between the smallest and the mature oocytes is well-accounted for by PAS-negative oocytes of intermediate size. Although there is no histological evidence of a massive spawning event or evacuation of the gonads, the loss of mature oocytes is not uniform throughout the year, the largest size class disappearing between April and May, and between October and November (Fig. 11). This disappearance represents release to the environment, since there is no increase in the incidence of atritic oocytes or phagocytes at these times.

The constancy of the gonad biochemical composition again indicates the lack of pronounced seasonality in reproductive condition. The unchanging level of neutral lipid in ovaries of *Ctenodiscus crispatus* is decidedly unlike the pattern found in the seasonal spawners *Odontaster validus* (by Pearse, 1965), *Luidia clathrata* (by Lawrence, 1973), *Echinaster* sp. (by Ferguson, 1975b), *Asterias vulgaris* (by Lowe, 1978), and *A. rubens* (by Oudejans and van der Sluis, 1979), which show marked fluctuations in neutral or total lipid during the reproductive cycle. Because somatic maintenance takes precedence over gonad development in asteroids when food is scarce (Harrold and Pearse, 1980), the continuous presence of mature gonads in *C. crispatus* suggests that the mud stars are equally well-nourished throughout the year. Gonad development is not at the expense of pyloric caecum reserves, since these organ indices and caloric content vary in concert rather than reciprocally (Figs. 1, 2 and 12), and the only changes in caecum-stored polysaccharides and neutral lipid occur in midsummer (Figs. 13 and 14), coincident with or just after the only decline in gonad index. As in most other asteroids (Lawrence and Lane, in press), the pyloric caeca represent a greater caloric reserve than do the gonads (Figs. 4 and 6).

An additional method of assessing reproductive condition in asteroids has been little used except by cell biologists as a tool for obtaining ripe gametes. The ovarian hormone 1-methyladenine causes final oocyte maturation and spawning only of full-grown oocytes (Kanatani, 1969), and the responsiveness of female *Ctenodiscus crispatus* to it varied seasonally (Table 2), although the temperature at which the response occurred in the laboratory did not always coincide with the field temperature. When administered in early August 1976, 1-MA was 40 to 100% effective in eliciting egg release. No injected specimens spawned in February 1977, 20% spawned in April, none in May, 40% in June, 40 to 80% in July, 33 to 67% in late August, and 20% in October, again demonstrating at least some reproductively active individuals during much of the year, with a superimposed increase in reproductive intensity in the population, especially in midsummer. The lack of seasonality in the length of the latent period (Table 2) is indicative of minimal fluctuation in ovarian physiology throughout the year, unlike the situation in the seasonally-spawning *Astropecten aurantiacus* (Kanatani, 1969).

Hofsten (1915) has suggested some seasonality of reproduction in *Ctenodiscus crispatus*, based on his ability to identify distinct "age groups" in some collections. We were unable to discern such groups in adult

Table 3. Broad classification of reproductive periodicity in various sea stars. Crosses indicate parameters used to infer periodicity

Gonad index	Oocyte histology	Testis histology	Caecum measurements	Chemical analysis	Environmental factors considered	Distinctly seasonal	Aseasonal
+	+	+			+	*Asterias amurensis* (Hatanaka and Kosaka, 1958; Kim, 1968)	
+			+	+		*A. rubens* (Vevers, 1949; Jangoux and Vloebergh, 1973; Jangoux and van Impe, 1977; Oudejans and van der Sluis, 1979; Oudejans *et al.*, 1979)	
+	+	+	+	+	+	*A. vulgaris* (Lowe, 1978)	
	+	+				*Asterina gibbosa* (Bruslé, 1969a, b)	
	+	+				*A. pancerii* (Cognetti and Delavault, 1962)	
	+	+				*Astropecten aurantiacus* (Cognetti and Delavault, 1962)	
	+	+				*A. bispinosus* (Cognetti and Delavault, 1962)	
	+	+				*A. irregularis* var. *pentacanthus* (Cognetti and Delavault, 1962)	
	+	+				*A. jonstoni* (Cognetti and Delavault, 1962)	
+			+		+	*Astrostole scabra* (Town, 1980)	
	+	+				*Coscinasterias tenuispina* (Cognetti and Delavault, 1962)	
+			+	+		*Echinaster* sp. (Ferguson, 1975a, b)	
	+	+				*Hacelia attenuata* (Cognetti and Delavault, 1962)	
+	+		+		+	*Leptasterias hexactis* (Chia, 1968; Menge, 1974)	
	+				+	*L. tenera* (Worley *et al.*, 1977)	
+		+	+	+	+	*Luidia clathrata* (Lawrence, 1973; Dehn, 1980)	
	+	+				*Marthasterias glacialis* (Cognetti and Delavault, 1962)	
+	+	+	+	+	+	*Odontaster validus* (Pearse, 1965)	
	+	+				*Ophidiaster ophidianus* (Cognetti and Delavault, 1962)	
+			+	+		*Oreaster (Pentaceros) hedemanni* (Rao, 1966)	
+			+		+	*O. reticulatus* (Scheibling, 1979)	
+	+	+	+	+[a]		*Patiria miniata* (Farmanfarmaian *et al.*, 1958; Nimitz, 1971, 1976)	
	+	+				*Patiriella calcar* (Lawson-Kerr and Anderson, 1978)	
+	+		+		+	*P. regularis* (Crump, 1971)	
+			+			*Pisaster brevispinus* (Farmanfarmaian *et al.*, 1958)	
+			+			*P. giganteus* (Farmanfarmaian *et al.*, 1958; Greenfield *et al.*, 1958)	
+	+	+	+	+		*P. ochraceus* (Farmanfarmaian *et al.*, 1958; Greenfield *et al.*, 1958; Nimitz, 1971, 1976)	
	+	+				*Sclerasterias richardi* (Falconetti *et al.*, 1976, 1977)	
+	+				+	*Stichaster australis* (Barker, 1979)	
	+	+					*Anseropoda membranacea*[b] (Cognetti and Delavault, 1962)
+	+	+	+	+	+		*Ctenodiscus crispatus* (present paper)
	+	+					*Echinaster sepositus*[b] (Cognetti and Delavault, 1960)
	+	+					*Henricia sanguinolenta*[b] (Cognetti and Delavault, 1962)
	+	+					*Patiriella exigua* (Lawson-Kerr and Anderson, 1978)

[a] Qualitative histochemistry only
[b] Females only; males are synchronous and distinctly seasonal

mud stars larger than 400 mg (Fig. 15), perhaps owing to the notorious variability in individual growth rates in asteroids (Nauen and Böhm, 1979). (In *C. crispatus*, this may relate less to competition and unstable trophic conditions, as suggested by Nauen and Böhm for *Asterias vulgaris*, than to intrinsic individual differences, since the population under study is highly variable genetically, as discussed below.) The significant difference in mean size of juvenile *C. crispatus* collected in August 1978 and March 1979 (Fig. 16) probably represents the growth of one cohort, and further implies some seasonality in the intensity of recruitment, although the presence, in both collections, of individuals less than 1 mg again indicates more frequent recruitment.

Taking the combined evidence from the gonad indices, histology and biochemical composition, 1-MA sensitivity, and juvenile size-frequency spectra, we conclude the following pattern of reproduction in *Ctenodiscus crispatus*: At least a few individuals of the population are reproducing at any time during the year.

There is no massive spawning event: we have never seen a spent female, and 1-MA-injected specimens release only a portion of their contained oocytes. Considering the asynchronous oocyte growth and maturation, it may be that any female is capable of releasing a few eggs several times a year. Ultimately, this is likely to be a consequence of the continuously available food supply for adults (Edwards, 1980; Shick *et al.*, in press), which is also manifested in the relative stability of the biochemical composition of the gonads and of the pyloric caecum index. (We note that the organic content of the sediment inhabited by the study population is not only extremely stable but also usually high, being 10% of the dry weight. The latter condition probably does not occur in arctic and deep-sea environments inhabited by this widespread, eurybathic species. Indeed, even much of the organic matter in our sediment samples is of terrestrial origin and thus may be refractory and scarcely available to the mud stars.) Continuous "reproductive readiness" may be important because of the infaunal nature and low vagility of *C. crispatus* (Gislén, 1924; Madsen, 1961a; Shick, 1976; Edwards, 1980; Shick *et al.*, in press), ensuring fertilization during chance encounters between individuals as they move through the sediment, as suggested for a deep-sea gastropod by Rokop (1979). Moreover, the spawned eggs of *C. crispatus* are positively buoyant (own personal observation) because of their very high neutral lipid content, and their release only when a "mate" is present might reduce the loss of unfertilized eggs to the water column. We have no evidence that eggs are brooded, as has been suggested for *C. australis* (Lieberkind, 1926).

At certain times there is an increase in the intensity of reproduction: the loss of the largest oocytes from the ovaries between April and late May (and the increased responsiveness of females to 1-MA in April) precisely coincides with the end of the first of two spring blooms of phytoplankton at this site (Morris and Skea, 1978); likewise, the disappearance of the largest oocytes in October-November is coincident with the termination of the fall bloom, and the significant increase in the ovary (and pyloric caecum) index and responsiveness to 1-MA in July is at the end of the second large spring bloom. The superimposition of this seasonality on the continuous, low level of reproduction may reflect a change in the *quality* of available food, especially for directly-developing juveniles: summer phytoplankton at the study site incorporate proportionally more $^{14}CO_2$ into protein than do winter plankton (Morris and Skea, 1978), and dead phytoplankton settling onto the sediment may provide a richer, less refractory, and more accessible food source or essential nutrients to very small juveniles feeding at the sediment-water interface.

We emphasize that the pattern of reproduction in this population was inferred from several different parameters, no one of which alone would give the complete picture. We agree with Giese and Pearse (1974) that multiple techniques are useful, although such studies are work-intensive and have rarely been carried out (Table 3). The comparative ease of 1-MA administration *in vivo* recommends itself for studies of other sea stars to separate true (physiological) from apparent (histological) reproductive competence (e.g. Farmanfarmaian *et al.*, 1958).

Ctenodiscus crispatus produces relatively few, large, yolky eggs, breeds continuously, and is apparently iteroparous – it therefore falls toward the "*K*" end of the *r*- and *K*-selection continum. Southwood (1977) has argued that *K*-strategies will evolve either when the environment is homogeneous, or when temporal variations in climate and resource availability are predictable. The trophic environment (sediment) of adult mud stars is spatially and temporally homogeneous in particle size spectrum and organic content (Edwards, 1980). The seasonality of primary production (Morris and Skea, 1978) is rather predictable and imposes some seasonality on breeding, but nevertheless is consistent with the development of *K*-strategies.

The gonad index in female *Ctenodiscus crispatus* is low (range 2.99 to 4.98) compared to that in other asteroids (maximum GIs generally range from 10 to 30; Lawrence and Lane, in press), and may indicate a reduced reproductive effort in the mud star [although using gonad weight or volume (or fecundity) as a measure of effort is not always warranted (Menge, 1974; Lowe, 1978)]. Calculation of the actual annual reproductive effort by measuring the difference in energy content between the ripe and post-spawning ovaries, as has been done for *Leptasterias hexactis* (Menge, 1974) and *Asterias vulgaris* (Lowe, 1978), is not possible in *C. crispatus* because of the seasonal constancy of the caloric content of its ovaries (Fig. 4). A less satisfactory comparative measure is to sum the ovary caloric content throughout the year, although this method assumes equal recycling (by phagocytes) and release of gonad energy in the species compared, a criterion unlikely to be met. Monthly data are not available for *L. hexactis*, but in *A. vulgaris* the total energy present in the gonads during a year is approximately 2 kcal g^{-1} dry body weight (calculated from Lowe, 1978). The annual value in *C. crispatus* is 0.82 kcal g^{-1} (missing monthly values were obtained by extrapolating between points in Fig. 4), again suggesting a lower reproductive effort in the mud star, which seems related both to the arctic origin and deposit-feeding lifestyle of this species, as discussed below.

Clarke's (1980) suggestion that continuously low temperatures have a role in the development of *K*-strategies, including low reproductive effort, by reducing individual energy demands and turnover may apply to the stenothermal, boreo-arctic *Ctenodiscus crispatus*. The mud star has a low metabolic rate that is not usually affected by temperature within the normal environmental range (Fig. 17), the Q_{10} for seasonal oxygen uptake between 3.5° and 8.0 °C being 2.11. Indeed, aerobic metabolism appears to be more affected by season than by temperature *per se*: oxygen uptake

by mature females is 20% greater at 7.2 °C in late August than at 8.0 °C in October, probably owing to a late summer increase in reproductive intensity, since $\dot{V}_{O_2}$ values in early August (Fig. 17) are not elevated. The increased $\dot{V}_{O_2}$ in late August immediately follows the period of greatest sensitivity to 1-MA in late July, and coincides with the only notable decline in ovary and pyloric caecum caloric content after their elevation in late July, suggesting an increased energy demand at this time. The variable intensity of reproduction is not directly related to temperature, which increases gradually and continuously from February through November at this depth (Table 2, and Edwards, 1980), but rather seems more closely linked to periodic influxes of phytoplankton-derived detritus, as discussed above. The overall reproductive effort may reflect not only the low temperature experienced by *C. crispatus*, but also the mud star's detrital food source.

The relative unimportance of temperature and the primacy of nutritional factors in affecting reproductive periodicity in this population of *Ctenodiscus crispatus* has relevance to the biology of deep-sea populations of this and other echinoderms, which inhabit an area of constant (low) temperature yet which show some seasonality of reproduction. Considerable data exist on reproductive periodicity in deep-sea ophiuroids (Schoener, 1968; Rokop, 1974; Lightfoot *et al.*, 1979; Tyler and Gage, 1979, 1980a, b). To summarize these briefly, the synchronous, seasonal reproduction and high fecundity in *Ophiura ljungmani* is attributed to its origin on the upper continental slope and only recent invasion of the deep sea, and to the need to synchronize release of its presumably planktotrophic larvae with the seasonal presence of sinking planktonic food. *Ophiomusium lymani* and *Ophiacantha bidentata* are more typical deep-sea species, having lower fecundities and direct, or an abbreviated larval, development, and showing asynchronous year-round reproduction which nevertheless varies in intensity. Deep-sea porcellanasterid sea stars also continuously produce few, large eggs and have a non-planktonic development (Madsen, 1961 b, pp 181, 202), and the presence in single populations both of individuals having "ripe" and "unripe" gonads (Madsen, 1961 b, p. 181) suggests asynchronous reproduction. These species fit the case described by Rex *et al.* (1979) for the abyssal gastropod *Benthonella tenella*: "If seasonality in reproduction exists at these depths, it seems limited to changes in the rate of continuous reproduction." The latter pattern is common in deep-sea invertebrates, and it seems likely that, due to the low concentration and refractory nature of organic detritus in deep-sea sediments, adult deposit feeders extract it at a constant (low) rate, which is manifested in continuous production of gametes (Rex *et al.*, 1979; also Chia, 1974). Tyler and Gage (1979) further suggest that such continuous, low recruitment to an energy-poor environment maximizes initial survivorship of juveniles. Thus, the periodic occurrence of richer trophic conditions afforded by settling plankton may be less important to adult deposit feeders such as *C. crispatus* and other soft-bottom asteroids which ingest large amounts of bulk sediment, than to directly-developing juveniles feeding at the sediment-water interface.

Genetic Variation

Ingestion of bulk sediment by deep-sea benthos, and by *Ctenodiscus crispatus*, provides a stable source of nutrition which is manifested not only in aseasonal reproduction but perhaps also in the genetic structure of populations. The population of *C. crispatus* under study is genetically extremely variable, despite Chia's (1974) prediction that low fecundity by itself should restrict such variation. Of the 13 enzyme-coding genes studied, 10 are polymorphic, and the average frequency of heterozygous individuals (H) is 0.174. The stability of trophic resources has been advanced by Valentine (1976) and Ayala and Valentine (1978) as a partial explanation for the large amount of genetic variability in many deep-sea invertebrates, which may follow a spatially "coarse grain" strategy in their utilization of the temporally "fine-grained" environment, individuals of a population perhaps being habitat and food specialists, a tendency grown out of competition for limited, but stable, resources (Valentine, 1976, p. 91).

This "trophic resource stability hypothesis" was originally framed with little evidence or consideration of the foods actually eaten by the species for which genetic data were being generated. For example, 8 species of deep-sea asteroids collectively studied by Ayala *et al.* (1975) and by Murphy *et al.* (1976) have high levels of heterozygosity (ca. 9 to 20%). Rather than being trophic specialists, as the hypothesis predicts, however, they appear to be generalists: at least 5 of the 8 genera represented are classified as omnivores and also have large amounts of sediment in their diets (Carey, 1972). Even in environments where primary production is strongly seasonal, organic detritus provides a storage function during periods of low productivity (Darnell, 1967). Indeed, the near ubiquity of organic detritus (both particulate and dissolved) in the diets of aquatic consumers, especially those classified as filter-feeders and grazers and therefore presumed by the trophic stability hypothesis to be heavily dependent on seasonally-available resources, poses a threat to the fundamental tenet of the hypothesis *sensu stricto*.

Spatial and temporal heterogeneity of the physical environment have also been put forward as causes of genetic variability (for reviews see: Selander, 1976; Soulé, 1976; Nelson and Hedgecock, 1980). Owing to its infaunal existence at a water depth of 70 m, the population of *Ctenodiscus crispatus* under study is buffered from the strong seasonality of temperature which prevails in shallower water at the same latitude, so the impact of this temporal component

of environmental heterogeneity on the population may be slight. However, the sediment is patchy with respect to primary amine and hydrogen sulfide concentrations (Shick *et al.*, in press), probably stemming from local variations in bioturbation by the dense deposit-feeding community at the study site (Edwards, 1980), and the environment may therefore be perceived as coarse-grained by a small sea star with limited mobility. Further, the abundance of putative (chemo) sensory structures in the cribriform organs of *C. crispatus* (Edwards, 1980) and the mud star's distinctive behavioral response to hydrogen sulfide (Shick, 1976) imply an ability to discriminate among habitat patches within its small sphere of activity (cf. Jumars, 1976).

Following Gillespie and Langley (1974), Nelson and Hedgecock (1980), as part of their hybrid "environmental heterogeneity – trophic diversity" model, have broadly categorized enzymes as being either central metabolic enzymes (Group I) or enzymes processing externally derived (food) substrates (Group II). High heterozygosity across Group I enzyme-coding genes should be positively correlated with variability in physical habitat parameters and with small size and low individual vagility (see also Selander and Kaufman, 1973), while high heterozygosity among Group II enzymes should relate to the breadth of the diet.

In *Ctenodiscus crispatus*, Group I heterozygosity (H) is 0.186 ± 0.080 SE, and H in Group II is 0.146 ± 0.020. The high levels of polymorphism and individual heterozygosity in this species appear to be well accounted for by the Nelson and Hedgecock hypothesis: the small mud star has limited vagility and inhabits a spatially (and mildly temporally) heterogeneous environment, yet it is also a trophic generalist, subsisting on a stable supply of organically complex detritus and on macrofauna in its habitat sediment. The high heterozygosities (average 0.123) in omnivorous deep-sea asteroids (Ayala *et al.*, 1975; Murphy *et al.*, 1976) may be similarly interpretable, particularly when compared with the very low heterozygosities (0.021 and 0.011) in the shallow-water, vagile carnivores, *Asterias forbesi* and *A. vulgaris* (Schopf and Murphy, 1973). No quantitative genetic data are available for the deep-sea mud-eating Porcellanasteridae, but Madsen (1961a, p. 54) notes that morphological polymorphism is pronounced in this group and that "a quite remarkable infraspecific variation . . . has been found in some of the large samples of single populations."

The study population of *Ctenodiscus crispastus* is also extremely large, which itself may contribute to genetic variability (Soulé, 1976; Siebenaller, 1978). Examination of other, truly deep-sea populations of the eurybathic *C. crispatus* would retain the criteria of trophic generalism (Carey, 1972) and large population size (Alton, 1966), while further reducing seasonality, and thus perhaps clarify the relative contributions of these factors to genetic structure and reproductive periodicity in marine invertebrate populations.

Acknowledgements. This study has benefited from the help of numerous people, whose cooperation we greatly appreciate: J. H. Dearborn, K. C. Edwards, R. G. and B. D. Gustafson, E. F. Lowe, and other friends occasionally pressed into service, for shipboard assistance; the Ira C. Darling Center of the University of Maine for the use of the R. V. "Lee"; and boat captains F. Bowers and M. Dunn for their numerous assistances; H. H. DeWitt and D. Schnitker for the loan of collecting gear; M. S. Tyler for help with histological techniques; J. A. Dykens for some chemical analyses; D. Hedgecock, J. M. Lawrence, D. M. Lowe, K. Nelson, J. S. Pearse, and G. N. Somero for advance copies of manuscripts and/or helpful discussions; and B. L. Bayne and J. H. Dearborn for critically reading the manuscript. The research was supported by U.S. National Science Foundation Grant PCM-7681467 (Regulatory Biology) to J. M. Shick and J. H. Dearborn.

Literature Cited

Alton, M. S.: Bathymetric distribution of sea stars (Asteroidea) off the northern Oregon coast. J. Fish. Res. Bd Can. *23*, 1673–1714 (1966)

Ayala, F. J. and J. W. Valentine: Genetic variation and resource stability in marine invertebrates. *In*: Marine organisms: genetics, ecology, and evolution, pp 23–51. Ed. by B. Battaglia and J. A. Beardmore. New York: Plenum Press 1978

Ayala, F. J., J. W. Valentine, D. Hedgecock and L. G. Barr: Deep-sea asteroids: high genetic variability in a stable environment. Evolution, Lawrence, Kansas *29*, 203–212 (1975)

Barker, M. F.: Breeding and recruitment in a population of the New Zealand starfish *Stichaster australis* (Verrill). J. exp. mar. Biol. Ecol. *41*, 195–211 (1979)

Boolootian, R. A.: Reproductive physiology. *In*: Physiology of Echinodermata, pp 561–613. Ed. by R. A. Boolootian. New York: John Wiley & Sons 1966

Brewer, G. J.: An introduction to isozyme techniques, 186 pp. New York: Academic Press 1970

Brody, S.: Bioenergetics and growth, 1023 pp. New York: Hafner Publishing Company 1945

Bruslé, J.: Les cycles génitaux d'*Asterina gibbosa* P. Cah. Biol. mar. *10*, 271–287 (1969a)

Bruslé, J.: Sexualité d'*Asterina gibbosa*, astéride hermaphrodite, des côtes de Marseille. Mar. Biol. *3*, 276–281 (1969b)

Carey, A. G., Jr.: Food sources of sublittoral, bathyal and abyssal asteroids in the northeast Pacific Ocean. Ophelia *10*, 35–47 (1972)

Cassie, R. M.: Some uses of probability paper in the analysis of size frequency distributions. Aust. J. mar. Freshwat. Res. *5*, 513–522 (1954)

Chia, F.-S.: Some observations on the development and cyclic changes of the oocytes in a brooding starfish, *Leptasterias hexactis*. J. Zool, Lond. *154*, 453–461 (1968)

Chia, F.-S.: Classification and adaptive significance of developmental patterns in marine invertebrates. Thalassia jugosl. *10*, 121–130 (1974)

Clarke, A.: A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. Biol. J. Linn. Soc. *14*, 77–92 (1980)

Cognetti, G. et R. Delavault: Récherches sur la sexualité d'*Echinaster sepositus* (echinoderme, astéride). Etude des glandes genitales chez les animaux des côtes de Livourne. Cah. Biol. mar. *1*, 421–432 (1960)

Cognetti, G. et R. Delavault: La sexualité des astérides. Cah. Biol. mar. *3*, 157–182 (1962)

Crump, R. G.: Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill), a common New Zealand asteroid. J. exp. mar. Biol. Ecol. *7*, 137-162 (1971)

Darnell, R. M.: Organic detritus in relation to the estuarine ecosystem. *In*: Estuaries, pp 376-382. Ed. by G. H. Lauff. Washington D. C.: American Association for the Advancement of Science 1967. (Publs Am. Ass. Advmt Sci. No. 83)

Dehn, P. F.: The annual reproductive cycle of two populations of *Luidia clathrata* (Asteroidea). I. Organ indices and occurrence of larvae. *In*: Echinoderms: present and past, pp 361–367. Ed. by M. Jangoux. Rotterdam: A. A. Balkema 1980

Edwards, K. C.: Morphology and feeding biology of *Ctenodiscus crispatus* (Echinodermata: Asteroidea), 223 pp. Master of Science thesis, University of Maine, Orono 1980

Falconetti, C., D. Fredj-Reygrobellet et G. Fredj: Sexualité et fissiparité concomitantes chez l'astérie *Sclerasterias richardi*: premières données. Mar. Biol. *34*, 247-257 (1976)

Falconetti, C., D. Fredj-Reygrobellet et G. Fredj: Induction de l'émission des gamètes et premiers stades du developpement larvaire chez l'astérie fissipare *Sclerasterias richardi*. Mar. Biol. *39*, 171-178 (1977)

Farmanfarmaian, A., A. C. Giese, R. A. Boolootian and J. Bennett: Annual reproductive cycles in four species of West Coast starfishes. J. exp. Zool. *138*, 355-367 (1958)

Ferguson, J. C.: The role of free amino acids in nitrogen storage during the annual cycle of a starfish. Comp. Biochem. Physiol. *51A*, 341-350 (1975a)

Ferguson, J. C.: Fatty acid and carbohydrate storage in the annual reproductive cycle of *Echinaster*. Comp. Biochem. Physiol. *52A*, 585-590 (1975b)

Giese, A. C.: On the chemical constitution of some echinoderms. *In*: Physiology of Echinodermata, pp 757-796. Ed. by R. A. Boolootian. New York: John Wiley & Sons 1966

Giese, A. C. and J. S. Pearse: Introduction: general principles. *In*: Reproduction of marine invertebrates. Vol. 1. Acoelomate and pseudocoelomate metazoans, pp 1-49. New York: Academic Press 1974

Gillespie, J. H. and C. H. Langley: A general model to account for enzyme variation in natural populations. Genetics, Austin, Tex. *76*, 837-848 (1974)

Gislén, T.: Echinoderm studies. Zool. Bidr. Upps. *9*, 1-316 (1924)

Greenfield, L., A. C. Giese, A. Farmanfarmaian and R. A. Boolootian: Cyclic biochemical changes in several echinoderms. J. exp. Zool. *139*, 507-524 (1958)

Harrold, C. and J. S. Pearse: Allocation of pyloric caecum reserves in fed and starved sea stars, *Pisaster giganteus* (Stimpson): somatic maintenance comes before reproduction. J. exp. mar. Biol. Ecol. *48*, 169-183 (1980)

Hatanaka, M. and M. Kosaka: Biological studies on the populations of the starfish, *Asterias amurensis*, in Sendai Bay. Tohoku J. agric. Res. *9*, 159-178 (1958)

Hofsten, N., von: Die Echinodermen des Eisfjordes. Zoologische Ergebnisse der Schwedische Expedition nach Spitzbergen 1908. Teil II - 2. K. svenska VetenskAkad. Handl. *54* (2), 1-282 (1915)

Holland, D. L. and P. A. Gabbott: A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. J. mar. biol. Ass. U.K. *51*, 659-668 (1971)

Holland, D. L. and J. Hannant: Addendum to a micro-analytical scheme for the biochemical analysis of marine invertebrate larvae. J. mar. biol. Ass. U.K. *53*, 833-838 (1973)

Humason, G. L.: Animal tissue techniques, 641 pp. San Francisco: W. H. Freeman 1972

Itzhaki, R. F. and D. M. Gill: A micro-biuret method for estimating proteins. Analyt. Biochem. *9*, 401-410 (1964)

Jangoux, M. and E. van Impe: The annual pyloric cycle of *Asterias rubens* L. (Echinodermata: Asteroidea). J. exp. mar. Biol. Ecol. *30*, 165-184 (1977)

Jangoux, M. et M. Vloebergh: Contribution à l'étude du cycle annuel de reproduction d'une population d'*Asterias rubens* (Echinodermata: Asteroidea) du littoral belge. Neth. J. Sea Res. *6*, 389-408 (1973)

Jumars, P. A.: Deep-sea species diversity: does it have a characteristic scale? J. mar. Res. *34*, 217-246 (1976)

Kanatani, H.: Induction of spawning and oocyte maturation by L-methyladenine in starfishes. Expl Cell Res. *57*, 333-337 (1969)

Kanatani, H.: Maturation-inducing substances in asteroid and echinoid oocytes. Am. Zool. *15*, 493-505 (1975)

Kim, Y. S.: Histological observations on the annual change in the gonad of the starfish, *Asterias amurensis* Lütken. Bull. Fac. Fish. Hokkaido Univ. *19*, 97-108 (1968)

Lawrence, J. M.: Level, content, and caloric equivalents of the lipid, carbohydrate, and protein in the body components of *Luidia clathrata* (Echinodermata: Asteroidea: Platyasterida) in Tampa Bay. J. exp. mar. Biol. Ecol. *11*, 263-274 (1973)

Lawrence, J. M. and J. E. M. Lane: The utilization of nutrients by post-metamorphic echinoderms. *In*: Echinoderm nutrition, Ed. by M. Jangoux and J. M. Lawrence. Rotterdam: Balkema Books (In press)

Lawson-Kerr, C. and D. T. Anderson: Reproduction, spawning and development of the starfish *Patiriella exigua* (Lamarck) (Asteroidea: Asterinidae) and some comparisons with *P. calcar* (Lamarck). Aust. J. mar. Freshwat. Res. *29*, 45-53 (1978)

Lieberkind, I.: *Ctenodiscus australis* Lütken. A brood-protecting asteroid. Vidensk. Meddr dansk naturh. Foren. (Ser 8) *82*, 183-196 (1926)

Lightfoot, R. H., P. A. Tyler and J. D. Gage: Seasonal reproduction in deep-sea bivalves and brittlestars. Deep-Sea Res. *26A*, 967-973 (1979)

Lowe, E. F.: Relationships between biochemical and caloric composition and reproductive cycle in *Asterias vulgaris* (Echinodermata: Asteroidea) from the Gulf of Maine, 95 pp. Ph. D. dissertation, University of Maine, Orono 1978

Luna, L. G.: Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd ed. 258 pp. New York: McGraw-Hill 1968

Madsen, F. J.: The Porcellanasteridae. A monographic revision of an abyssal group of sea-stars. Galathea Rep. *4*, 33-174, 13 plates (1961a)

Madsen, F. J.: On the zoogeography and origin of the abyssal fauna in view of the knowledge of the Porcellanasteridae. Galathea Rep. *4*, 177-218 (1961b)

Markert, C. L. and I. Faulhaber: Lactate dehydrogenase isozyme patterns of fish. J. exp. Zool. *159*, 319-332 (1965)

Mauzey, K. P.: Feeding behavior and reproductive cycles in *Pisaster ochraceus*. Biol. Bull. mar. biol. Lab., Woods Hole *131*, 127-144 (1966)

Menge, B. A.: Effects of wave action and competition on brooding and reproductive effort in a rocky intertidal starfish, *Leptasterias hexactis*. Ecology *55*, 84-93 (1974)

Morris, I. and W. Skea: Products of photosynthesis in natural populations of marine phytoplankton from the Gulf of Maine. Mar. Biol. *47*, 303-312 (1978)

Murphy, L. S., G. T. Rowe and R. L. Haedrich: Genetic variability in deep-sea echinoderms. Deep-Sea Res. *23*, 339-348 (1976)

Nauen, C. E. and L. Böhm: Skeletal growth in the echinoderm *Asterias rubens* L. (Asteroidea, Echinodermata) estimated by ^{45}Ca-labelling. J. exp. mar. Biol. Ecol. *38*, 261-269 (1979)

Nelson, K. and D. Hedgecock: Enzyme polymorphism and adaptive strategy in the decapod Crustacea. Am. Nat. *116*, 238-280 (1980)

Nimitz, Sister M. Aquinas: Histochemical study of gut nutrient reserves in relation to reproduction and nutrition in the sea stars, *Pisaster ochraceus* and *P. miniata*. Biol. Bull. mar. biol. Lab., Woods Hole *140*, 461-481 (1971)

Nimitz, Sister M. Aquinas: Histochemical changes in gonadal nutrient reserves correlated with nutrition in the sea stars,

Pisaster ochraceus and *Patiria miniata*. Biol. Bull. mar. biol. Lab., Woods Hole *151*, 357-369 (1976)

Oudejans, R.C.H.M. and I. van der Sluis: Changes in the biochemical composition of the ovaries of the seastar *Asterias rubens* during its annual reproductive cycle. Mar. Biol. *50*, 255-261 (1979)

Oudejans, R.C.H.M., I. van der Sluis and A. J. van der Plas: Changes in the biochemical composition of the pyloric caeca of female seastars, *Asterias rubens*, during their annual reproductive cycle. Mar. Biol. *53*, 231-238 (1979)

Pearse, J. S.: Reproductive periodicities in several contrasting populations of *Odontaster validus* Koehler, a common antarctic asteroid. Antarctic Res. Ser. *5*, 39-85 (1965)

Rao, K. S.: Reproductive and nutritional cycles of *Oreaster hedemanni* Lütken. J. mar. biol. Ass. India *8*, 254-272 (1966)

Rex, M. A., C. A. Van Ummersen and R. D. Turner: Reproductive pattern in the abyssal snail *Benthonella tenella* (Jeffereys). *In*: Reproductive ecology of marine invertebrates, pp 173-188. Ed. by S. E. Stancyk. Columbia: University of South Carolina Press 1979. (Belle W. Baruch Library in Marine Science, Symp. Ser. No. 9)

Ridgway, G. J., S. W. Sherburne and R. D. Lewis: Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. *99*, 147-151 (1970)

Rokop, F. J.: Reproductive patterns in the deep-sea benthos. Science, N. Y. *186*, 743-745 (1974)

Rokop, F. J.: Year-round reproduction in the deep-sea bivalve molluscs. *In*: Reproductive ecology of marine invertebrates, pp 189-198. Ed. by S. E. Stancyk. Columbia: University of South Carolina Press 1979. (Belle W. Baruch Library in Marine Science, Symp. Ser. No. 9)

Scheibling, R. E.: The ecology of *Oreaster reticulatus* (L.) (Echinodermata: Asteroidea) in the Caribbean, 361 pp. Ph. D. dissertation, McGill University, Montreal, Canada 1979

Schoener, A.: Evidence for reproductive periodicity in the deep sea. Ecology *49*, 81-87 (1968)

Schopf, T.J.M. and L. S. Murphy: Protein polymorphism of the hybridizing sea stars *Asterias forbesi* and *Asterias vulgaris* and implications for their evolution. Biol. Bull. mar. biol. Lab., Woods Hole *145*, 589-597 (1973)

Selander, R. K.: Genic variation in natural populations. *In*: Molecular evolution, pp 21-45. Ed. by F. J. Ayala. Sunderland, Massachusetts: Sinauer Associates 1976

Selander, R. K. and D. W. Kaufman: Genic variability and strategies of adaptation in animals. Proc. natn. Acad. Sci. U.S.A. *70*, 1875-1877 (1973)

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson and J. B. Gentry: Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Tex. Publs (Stud. Genet. No. 7103) *6*, 49-90 (1971)

Shaw, C. R. and R. Prasad: Starch gel electrophoresis of enzymes – a compilation of recipes. Biochem. Genet. *4*, 297–320 (1970)

Shick, J. M.: Physiological and behavioral responses to hypoxia and hydrogen sulfide in the infaunal asteroid *Ctenodiscus crispatus*. Mar. Biol. *37*, 279-289 (1976)

Shick, J. M., K. C. Edwards and J. H. Dearborn: Physiological ecology of the deposit-feeding sea star *Ctenodiscus crispatus*: ciliated surfaces and animal-sediment interactions. Mar. Ecol. Prog. Ser. (In press)

Shick, J. M. and A. N. Lamb: Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. Biol. Bull. mar. biol. Lab., Woods Hole *153*, 604-617 (1977)

Siebenaller, J. F.: Genetic variation in deep-sea invertebrate populations: the bathyal gastropod *Bathybembix bairdii*. Mar. Biol. *47*, 265-275 (1978)

Sokal, R. R. and F. J. Rohlf: Biometry. The principles and practice of statistics in biological research, 776 pp. San Francisco: W. H. Freeman & Co 1969

Somero, G. N., J. F. Siebenaller, and P. W. Hochachka: Physiological and biochemical adaptations of deep-sea animals. *In*: The sea, Vol. 8. Ed. by G. T. Rowe. New York: Wiley-Interscience (In press)

Soulé, M.: Allozyme variation: Its determinants in space and time. *In*: Molecular evolution, pp 60-77. Ed. by F. J. Ayala. Sunderland, Massachusetts: Sinauer Associates 1976

Southwood, T.R.E.: Habitat, the templet for ecological strategies? J. Anim. Ecol. *46*, 337-365 (1977)

Thorson, G.: The larval development, growth and metabolism of arctic marine bottom invertebrates compared with those of other seas. Meddr Grønland *100*, 1-55 (1936)

Town, J. C.: Movement, morphology, reproductive periodicity, and some factors affecting gonad production in the seastar *Astrostole scabra* (Hutton). J. exp. mar. Biol. Ecol. *44*, 111-132 (1980)

Turner, R. L.: Sexual difference in latent period of spawning following injection of the hormone 1-methyladenine in *Echinaster* (Echinodermata: Asteroidea). Gen. comp. Endocr. *28*, 109-112 (1976)

Tyler, P. A. and J. D. Gage: Reproductive ecology of deep sea ophiuroids from the Rockall Trough. *In*: Cyclic phenomena in marine plants and animals, pp 215-222. Ed. by E. Naylor and R. G. Hartnoll. Oxford: Pergamon Press 1979

Tyler, P. A. and J. D. Gage: Reproduction and growth of the deep-sea brittlestar *Ophiura ljungmani* (Lyman). Oceanol. Acta *3*, 177-185 (1980a)

Tyler, P. A. and J. D. Gage: Reproductive patterns in deep sea ophiuroids from the North East Atlantic. *In*: Echinoderms: present and past, pp 417-421. Ed. by M. Jangoux. Rotterdam: A. A. Balkema 1980b

Valentine, J. W.: Genetic strategies of adaptation. *In*: Molecular evolution, pp 78-94. Ed. by F. J. Ayala. Sunderland, Massachusetts: Sinauer Associates 1976

Vevers, H. G.: The biology of *Asterias rubens* L.: growth and reproduction. J. mar. biol. Ass. U.K. *28*, 165-187 (1949)

Walker, C. W.: Histology and ultrastructure of the gonad of *Ctenodiscus crispatus* (Asteroidea). Am. Zool. *14*, p. 1264 (1974)

Walker, C. W.: Spermatogenic columns, somatic cells and the microenvironment of germinal cells in the testes of seastars. J. Morph. *166*, 81-107 (1980)

Worley, E. K., D. R. Franz and G. Hendler: Seasonal patterns of gametogenesis in a North Atlantic brooding asteroid, *Leptasterias tenera*. Biol. Bull. mar. biol. Lab., Woods Hole *153*, 237-253 (1977)

Date of final manuscript acceptance: January 29, 1981.
Communicated by J. Mauchline, Oban