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REPRODUCTIVE AND NUTRITIONAL CYCLES OF *OREASTER HEDEMANNI* LÜTKEN*

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INTRODUCTION

It is known that in the gonads biochemical synthesis takes place at the time of gametogenesis, involving large amounts of organic materials to be utilized in the formation of gametes, for example, protein and lipids in the eggs and desoxyribonucleic acid in sperms (Giese, 1959a). To meet these demands reserves stored in other organs reach the gonads preparatory to gametogenesis. The problem of the relationship between the reproductive and nutritional cycles is an important one and has received some attention in recent years. This aspect has been studied by some workers, in starfish (Farmanfarmaian *et al.*, 1958, Greenfield *et al.*, 1958, Pearse 1965), in oysters (Mitchell 1916, Russell 1923, Masumoto *et al.*, 1934), in chitons (Giese and Araki 1962, Tucker and Giese 1962) and in barnacles (Barnes *et al.*, 1963). The data of Farmanfarmaian *et al.* (*loc. cit.*) and Greenfield *et al.*, (*loc. cit.*) show differences in regard to the time taken for and also in the extent of accumulation of organic materials in storage organs even in closely related species of *Pisaster* under the order Forcipulata. Therefore, it has been considered worthwhile taking up the present investigation in *Oreaster hedemanni* Lütken, an asteroid belonging to a different order Phanerozoa, for comparison. Quantitative changes in lipids, protein and glycogen in the gonads of *O. hedemanni* have also been studied to find out the extent of accumulation during maturation.

MATERIAL AND METHODS

The starfish *Oreaster hedemanni* were collected during 1963-1965 from Mandapam coast off the Gulf of Mannar where they occur in shallow waters upto depths of three fathoms. Random samples of ten to twelve specimens of starfish were collected fresh in successive months. The starfish ranged between 100 gms. and 250 gms. in weight.

For studying the reproductive cycles of the starfish the gonad index method of Farmanfarmaian *et al.*, (1958) was adopted with slight modification. The value obtained when the weight of the gonad is divided by the total weight of the starfish and multiplied by 100 has been taken as the gonad index. The sex and stages of maturity of the starfish were noted by microscopic examination of teased bits of gonads. Four stages have been distinguished i.e., (1) immature with little or inactive gametogenetic activity in gonads (2) maturing with active gametogenetic activity in gonads (3) mature with ripe gametes and (4) spent with the gonads shrunken after discharge of gametes.

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The changes in size of pyloric caecae in relation to reproductive stages were estimated by determining hepatic index of each specimen following Farmanfarmaian *et al.*, (1958).

For drying the gonads and pyloric caecae and estimating the lipid and protein contents of tissues the methods of Giese *et al.* (1959), and Steyermark (1961) were used. The glycogen content of tissues was determined by estimating the sugar content with the methods of Good, Krammer and Somogyi (1933) and Hawk *et al.*, (1954). The glycogen content was calculated from the sugar value on multiplying it by the factor 1.11.

Observations

Storage organs and reproductive cycle :

The pyloric caecae are paired structures arising from the pyloric stomach and lying in each of the arms suspended by two longitudinal mesenteries. The caecae have longitudinal ducts with lateral racemose branches leading into follicles which contain lipids, protein and glycogen in storage cells (Anderson, 1953).

The present studies indicate that there is an annual reproductive cycle in the female and male *O. hedemanni* (Rao 1965a, b). The gonads of the starfish are in an immature condition in May and June and in maturing condition in July and August. The gonads attain maturity by October, spawning occurs in December and the gonads enter and remain in a resting phase until June of the following year.

Hepatic indices :

The mean hepatic indices of immature males in May and June are 4.55 and 4.35 respectively (Fig. 1). The mean index decreases to 3.99 in July when they

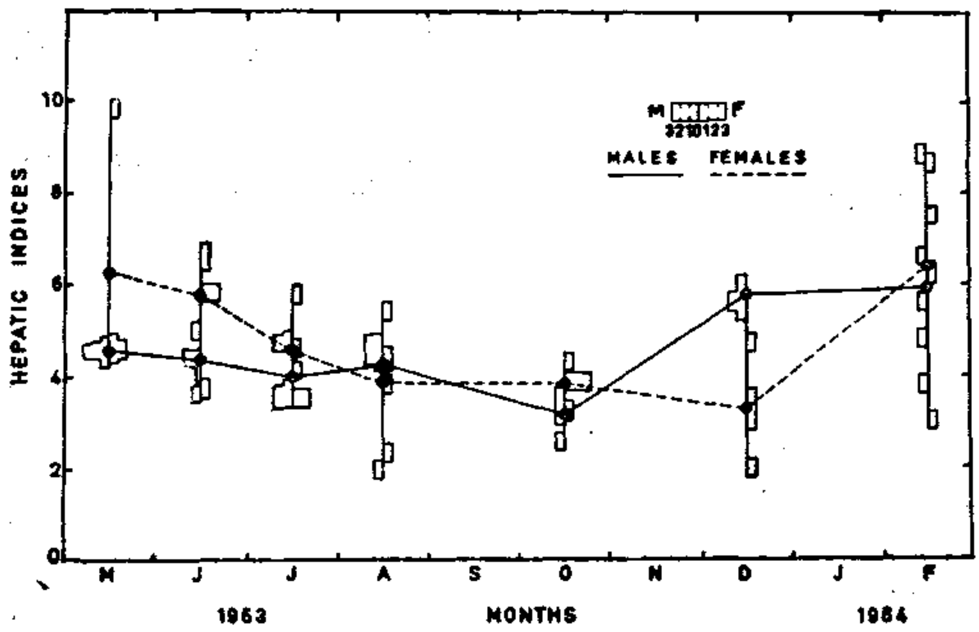

Fig. 1. Hepatic indices of male and female *Oreaster hedemanni* with mean values during 1963-64. Numbers at the right hand top corner indicate numbers of individuals analysed.

are maturing. It shows a slight increase to 4.24 in August. This is followed by a decrease to 3.16 in October by which time the starfish are mature. After spawning in December, the index increases to 5.73 and remains high at 5.92 in spent ones in February of the following year.

In females the hepatic index decreases from 6.22 in May to 5.73 in June and still further progressively to 4.58 and 3.84 in July and August in maturing ones, reaching a value of 3.82 in mature females by October. In spawning females it is 3.25 in December but in spent ones 6.34 in February.

An increase in hepatic index in *O. hedemanni* after spawning period and a decrease in the same during the period of maturation of gonads are suggestive of at first active accumulation and subsequently the utilization of reserve organic materials in the pyloric caecae in the respective periods, as observed by Farmanfarmaian *et al.* (1958) in other starfish namely *Pisaster ochraceus* and *P. brevispinus.*

In contrast to *O. hedemanni*, in the starfish *Patiria miniata* which has been observed to breed throughout the year, the pyloric caecae have not shown seasonal quantitative changes to denote sharp phases of accumulation and depletion of the reserves, these apparently taking place at close intervals all through the life of the organism after the attainment of first sexual maturity.

In *Odontaster validus* of McMurdo Station in the Antarctic no well-defined changes occur in the size of pyloric caecae during the annual reproductive cycle, nutrients being probably continually channelled into the gonads from the food taken (Pearse, 1965). But in *Odontaster validus* at Cape Evans north of McMurdo Station there is parallel growth of the pyloric caecae and gonads during the annual reproductive cycle. The marked increase in the size of the pyloric caecae of this population in the pre-spawning period has been attributed to accumulation of large amounts of organic reserves, due to intensive feeding; the decrease in the size of the pyloric caecae in the winter has been considered to be due to utilization of the reserves for gametogenetic activity.

Gonad indices:

The mean gonad index increases from a low value of 0.33 in immature males in May through June and July to 1.48 in maturing males in August (Fig. 2). The

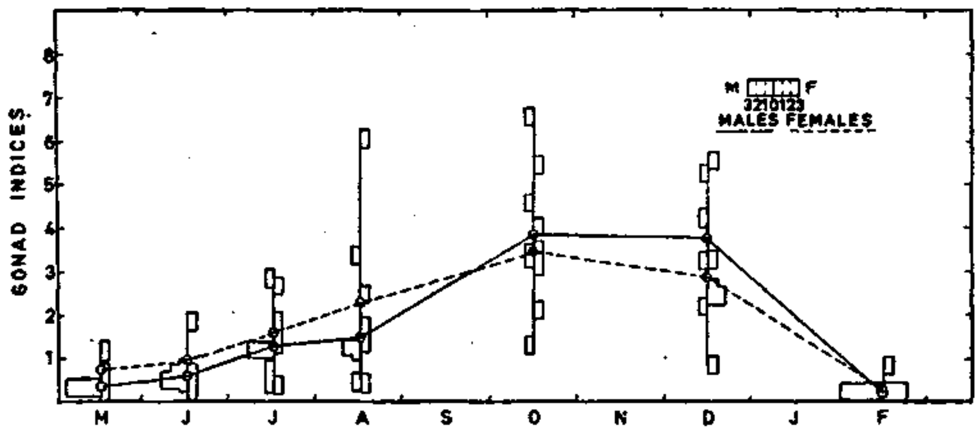

FIG. 2. Gonad indices of male and female *O. hedemanni* with mean values during 1963-64.

index increases to 3.80 in mature males in October, shows a slight decrease to 3.74 in spawning males in December and reaches a value of 0.23 in spent ones in February.

In the case of females also a similar trend is noticed. The index is low in May, 0.71 but it increases to 0.95 in June and 1.60 in July. The mean index increases to 2.29 in August and reaches a maximum value of 3.43 in October when the gonads are mature. It shows a fall to 2.87 in December when spawning occurs and to a very low value of 0.30 in February in spent females.

The studies on stages of maturity and gonad indices have been extended to another year 1964-65 (Table I). During this year also the gonad indices of the starfish of both sexes show very similar seasonal changes as in the previous year.

TABLE I

Seasonal changes in gonad indices of *Oreaster hedemanni* in 1964-65

Stage of maturity of starfish	Months	Mean gonad index of males with S.D.	Mean gonad index of females with S.D.
immature ..	June '64	0.44±0.02	0.63±0.03
maturing ..	July	1.21±0.23	1.36±0.27
maturing ..	August	1.47±0.28	1.95±0.23
mature ..	October	4.07±0.35	5.25±0.29
spawning ..	December	1.75±0.87	2.06±0.81
spent ..	February '65	0.19±0.01	0.31±0.01
spent ..	April	0.17±0.002	0.28±0.002

The annual reproductive cycle of *O. hedemanni* resembles that of *P. ochraceus*. There is a difference in the period of commencement of gametogenesis in June in the former species and about October in the latter. Similarly, the initiation of spawning activity is different in the two species being in December in one and in April in the other. In *P. brevispinus* also there is a similar reproductive cycle with gametogenesis commencing in October-November and spawning in April. In *Odontaster validus* of the Antarctic also there appears to be an annual reproductive cycle with the gonads maturing between November and April and spawning taking place between April and September (Pearse, 1965).

In contrast to *O. hedemanni* some species of echinoderms of Madras coast have been observed to breed throughout the year. In the sea urchin *Salmacis bicolor* mature individuals have been observed throughout the year (Aiyar, 1935). In another sea urchin of the same coast *Stomopneustus variolaris* the period of active breeding is noted to be during October to December although mature gametes in the gonad have been found all round the year (Giese *et al.*, 1964).

The reproductive periodicity of echinoderms has been considered to be influenced by factors like availability of food, variations in the environmental temperatures and day-length (Giese, 1959 a, b). It has been noted that the gonads of the sea urchin *Strongylocentrotus purpuratus* do not attain sexual maturity if the animal is starved but that large amounts of lipids, protein, glycogen and nucleic acids accumulate in the gonads under normal conditions (Giese, 1959 a). He is of opinion that the availability of organic materials is an essential pre-requisite for the growth and maturation of gonads. Feder (1956) has noted that the gonads of *P. ochraceus* do not attain maturity if the starfish are starved before accumulation of reserve materials in the pyloric caecae. Since the accumulation of reserve organic materials in pyloric caecae precedes the maturation of gonads during the annual reproductive cycle in *Pisaster ochraceus* and *P. brevispinus*, and these materials are subsequently used up during the period of maturation of the gonads, it has been suggested that the nutritional stage of the starfish determines their reproductive periodicity (Farmanfarmaian *et al.*, 1958, Greenfield *et al.*, 1958).

O. hedemanni also shows an increase in the size of pyloric caecae in the sexually inactive period followed by a decrease in the same as the gonads progressively undergo maturation.

Lipids, protein and glycogen in pyloric caecae in relation to reproductive cycle.

The work of Ferguson (1964 a) shows that the pyloric caecae are the only important storage organs in starfish and other sites like coelomic fluid and body wall are not of much significance. Hence, the pyloric caecae alone have been studied for the changes in the organic reserves lipids, protein and glycogen in *O. hedemanni*.

1. *Lipids in pyloric caecae :*

The lipid contents of pyloric caecae of male *O. hedemanni* (Fig. 3) are higher in the immature and maturing stages per unit weight than in mature stages. The contents in pyloric caecae of immature ones range between 21.18% and 29.73% dry weight in May and June. They range between 25.44% and 39.14% in maturing males in August. There is a decrease in the lipid content in pyloric caecae to 22.63% in mature males in October. It shows an increase to 39.04% in spent males.

In females the lipid contents of the pyloric caecae (Fig. 3) in immature stages in May and June range between 21.00% and 48.4% dry weight. The contents show a slight decrease to 29.26% and 30.63% respectively in maturing females in July and August, and a marked decrease in October to 17.67% in mature ones. The content shows next an increase to 30.23% in February in spent females.

There is no well-defined decrease in reserve lipids in per cent dry weight between the immature and maturing stages but there is a decrease in the total content following decrease in size of the pyloric caecae (Table II). As the gonads reach the mature stage, the pyloric caecae decrease in size further and the lipid content also shows a marked decrease per unit weight. Between December and February the lipid contents again reach high levels between 30% and 39% dry weight showing active accumulation.

The decrease in the lipids in the pyloric caecae taking place simultaneously with the increase of the same in the gonads (as shown in a later section) is suggestive of their being transferred from pyloric caecae to gonads during maturation.

The total lipid contents of pyloric caecae of *O. hedemanni* having more or less the same weights, but in different reproductive stages, were estimated to find out the extent of decrease of reserve lipids during maturation of gonads (Table II). A comparison of the decrease in the total lipid content of pyloric caecae with the increase in the total lipid content of the gonads between the immature and mature stages shows that upto about one-third of the total content of the pyloric caecae is

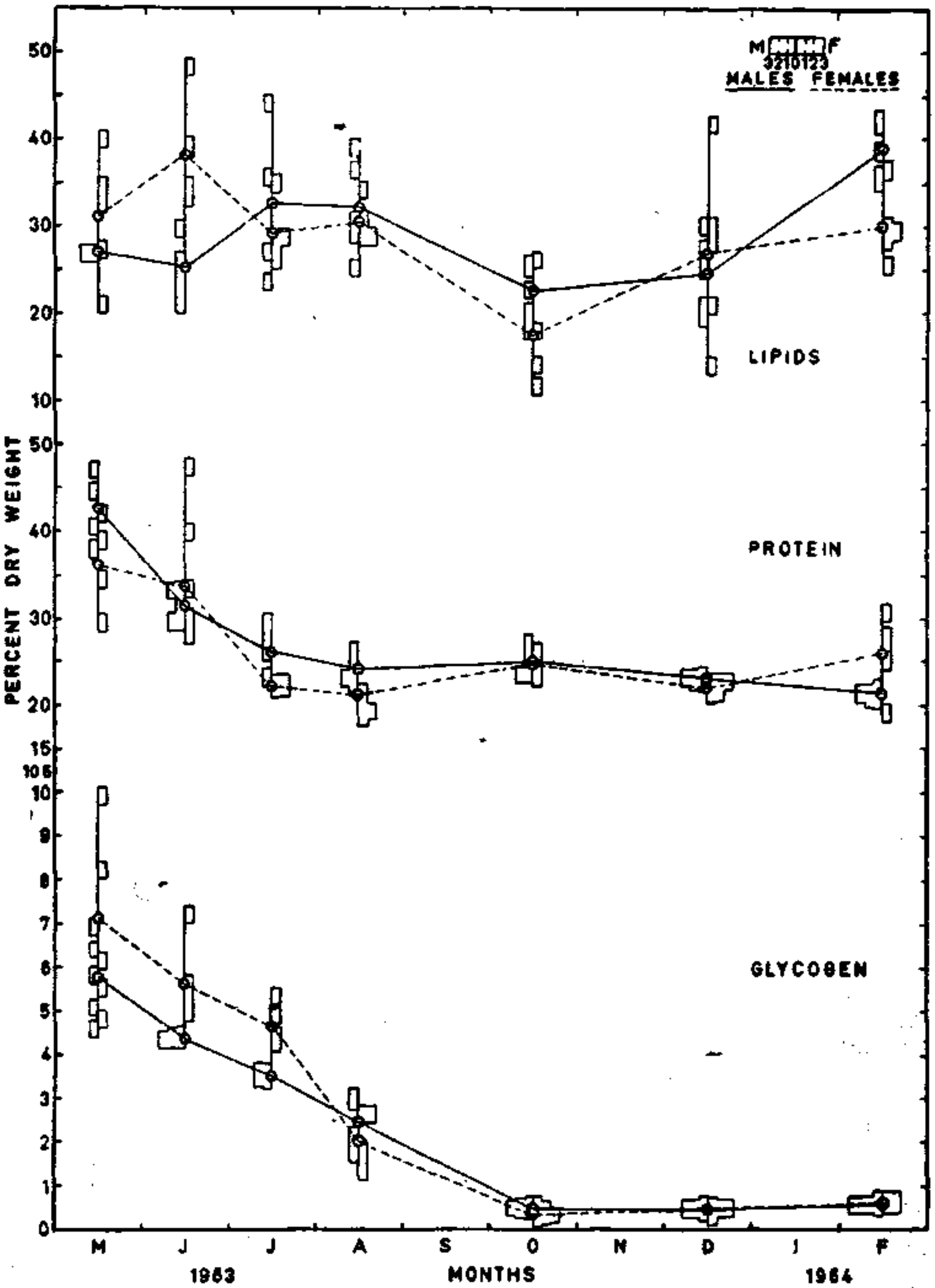

FIG. 3. Percentage contents of lipids, protein and glycogen of pyloric caecae of male and female *O. hedemanni* with mean values during the annual reproductive cycle. Numbers at the right hand top corner indicate numbers of individuals analysed.

TABLE II

Changes in total lipid content of pyloric caecae and gonads of *Oreaster hedemanni* in relation to maturation of reproductive organs (each value mean of 4 individuals ranging in weight between 175 gms. and 250 gms.).

Stage of maturity of starfish	Total lipid content of pyloric caecae of males (mg.)	Total lipid content of testes of males (mg.)	Total lipid content of pyloric caecae of females (mg.)	Total lipid content of ovaries of females (mg.)
immature	728.90 ±55.40	5.99 ±2.00	897.92 ±91.61	16.17 ±5.90
maturing	602.45 ±102.98	130.80 ±37.84	600.60 ±45.91	80.00 ±22.14
mature	299.35 ±19.13	123.06 ±42.87	288.55 ±35.55	164.78 ±41.74
spent	939.62 ±15.97	13.62 ±2.51	822.03 ±92.05	9.87 ±2.21

transferred to mature gonads. The rest of the lipids lost by the pyloric caecae of immature *Oreaster* may be considered to be utilized for the energy requirements of the starfish including those for maturation of the reproductive organs.

The pyloric caecae of *O. hedemanni* show differences in the maximum levels of lipids compared to *Pisaster* spp. Lipids in the former species (males) amount to a maximum of 32.76% dry wt. in the maturing stage and decrease to 22.63% in the mature ones (mean values); in the respective stages of *P. ochraceus* the maximum lipid contents are 37.3% and 22.2% (vide Fig. 2, Greenfield *et al.*, 1958). In that species lipids reach a maximum of 50% dry wt. during active growth of the digestive gland. In *P. giganteus* a maximum of 75% has been observed in rare instances by the same authors. The maximum of lipid contents in pyloric caecae of the individual *O. hedemanni* observed in the present work were 44. 29% and 35.05% dry weight in maturing males and females respectively; the corresponding values in the mature stages for the respective sexes in *P. ochraceus* were 25.90% and 26.29%. The orders Phanerozoa and Forcipulata which include *Oreaster* and *Pisaster* respectively are different from each other from the evolutionary point of view, the former being primitive and lower in rank than the latter (Hyman, 1955) but the differences in the extent of accumulation of lipids do not seem to depend on the evolutionary order of the members, for in the species under the same genus viz. *P. giganteus* and *P. ochraceus* such differences have also been met with.

In members of other taxonomic groups also, quantitative changes of lipids in storage organs in relation to reproductive cycle are seen. In the chiton *Katherina* the observations of Lawrence *et al.* (1965) indicate inverse relationship between the weight of the digestive gland and gonad cycles suggesting that the nutritional demands of the gonadal growth might result in the reduction in the size of the gland. However, Giese and Araki (1962) observed no well-defined changes in the percentage contents of the different organic materials including lipids in the digestive gland during maturation. A similar relationship between the body tissues and the ovary has been noted in the barnacle *Balanus balanus* by Barnes *et al.* (1963).

Katherina, *Balanus* and *Oreaster* differ in the maximum levels to which lipids increase. Lipids are present to the maximum level of 11.5% dry wt. in the digestive gland of *K. tunicata* (Giese and Araki 1962), 29.38% in the body of *Balanus* and 48.4% in *O. hedemanni*. In the growth of gonads, which is preceded by accumulation of reserves in the storage organs lipids appear to be more important than glycogen in *Oreaster* and *Balanus* compared to *Katherina*.

2. *Protein in pyloric caecae :*

The protein content of the pyloric caecae of male *O. hedemanni* is the highest in the immature stage in May amounting to a value of 42.75% dry wt. (Fig. 3). It decreases to 31.56% as the gonads increase in size with the commencement of gametogenetic activity. There is a further decrease to 26.23% and 24.3% in the maturing males in July and August and to 25.09% in the mature ones in October. In the spent males it is lowest being 21.48% in February.

In females also the protein content of pyloric caecae shows decrease during the maturation of gonads (Fig. 3). The content of the caecae is highest in immature females in May when it is 36.35% dry wt. and decreases to 33.91% in June with commencement of gametogenesis. It shows a further decrease to 22.31% and 21.03% in maturing females in July and August. The content shows a slight increase to 24.79% in starfish in the mature stage in October but this may be due to individual variations in the protein content of the samples examined. The content shows an increase to 26.04% in spent females in February.

Thus the protein content of the pyloric caecae in both sexes of *O. hedemanni* progressively decreases during the period between June and October when the gonads are maturing. After spawning, the protein content of the spent male starfish does not show an increase per unit weight in February. But the pyloric caecae show a marked increase in size between October and February with a rise in hepatic index from 3.16 to 5.92 in males and from 3.82 to 6.34 in females which indicates an increase in the total protein content of the pyloric caecae (Table III) after spawning.

TABLE III

Changes in total protein content of pyloric caecae and gonads of *Oreaster hedemanni* in relation to maturation of reproductive organs (each value mean of 4 individuals ranging in weight between 175 gms. and 250 gms.)

Stage of maturity of starfish	Total protein content of pyloric caecae of males (mg.)	Total protein content of testes of males (mg.)	Total protein content of pyloric caecae of females (mg.)	Total protein content of ovaries of females (mg.)
immature	960.91 ±20.85	36.76 ±7.82	823.95 ±73.79	86.21 ±16.08
maturing	839.27 ±44.95	222.70 ±91.56	426.42 ±32.57	170.30 ±39.08
mature	334.42 ±56.78	850.95 ±174.78	336.90 ±11.18	1009.23 ±74.69
spent	595.62 ±45.93	35.66 ±1.35	693.83 ±78.15	23.75 ±6.92

The decrease occurring in the total protein content of the pyloric caecae is much lower compared to the increase in the same of the testes and ovaries between the two gonadal stages. This shows that proteins additional to those already present in the pyloric caecae in the immature stage, accumulate in the gonads as they mature. These additional proteins have to be obtained from protein precursors, the free amino acids derived from food and distributed to the various organs through the perivisceral fluid.

In *P. ochraceus* the mean protein content in different reproductive stages is constant at 30% with the observed values varying between 15% and 45% dry wt. in almost all stages of immature and mature starfish. The wide variations may be due to the splitting of the proteins in the organs and releasing of the derived amino acids into the perivisceral coelomic fluid for utilization by other organs including gonads. This release of the amino acids from the pyloric caecae appears to be followed by reaccumulation of fresh protein out of the amino acids derived from food.

In comparison to *Oreaster*, in *Katherina* the quantitative levels of proteins in the digestive gland of spent and ripe males do not show well-defined differences per unit weight, being 25.4% and 30.2% dry wt. respectively; those in the spent and ripe females are 30.2% and 26.3% dry wt. (Giese and Araki, 1962). However, a decrease in the total protein content of the digestive gland is indicated by a decrease in the total size of the digestive gland (Lawrence *et al.*, 1965).

In *Balanus balanus* proteins in connective tissues of the body have been noted to decrease from 28.12% dry wt. in the immature stage to 26.88% dry wt. in the mature stage and this has been considered to be due to their transfer after degradation to maturing gonads (Barnes *et al.*, 1963); further the total protein of the body of *Balanus* shows a decrease to less than half its level in the immature stage as the gonads mature showing a heavy demand on protein reserves in the body of the barnacle correlated with reproductive activity.

3. *Glycogen in pyloric caecae :*

In males in May to July when they are in immature or maturing stages the glycogen content of pyloric caecae has been found to range between 3.45%-6.44% dry wt.; in August when they are still in maturing stages there is a further decrease to 1.74%-3.08%. In October when the individuals are in mature condition the glycogen contents are 0.45%-0.54% and the values show a slight increase to 0.52%-0.69% in spent ones (Fig. 3).

In females in the immature stages found in May and June the glycogen content of pyloric caecae ranges between 4.82%-9.93%; in maturing stages in July and August the content shows decreased values 1.54%-5.36%. The very low values 0.24%-0.54% have been observed in October in mature ones. A slight increase in glycogen content is seen in spent ones i.e. 0.56%-0.66%, in February.

The above account shows that the glycogen content in percentage dry weight of the pyloric caecae is highest in May and decreases towards October. After spawning, when the size of pyloric caecae and hepatic indices have increased in February, the glycogen content of the caecae also increases which is indicative of reaccumulation of glycogen.

The levels of glycogen in pyloric caecae of starfish in February are lower compared to those in May suggesting delayed accumulation of glycogen to maximum levels unlike lipids which reach to the maximum level by February after spawning in December. The reason for delayed accumulation of glycogen is not clear. It is known that in mammals carbohydrate metabolism is largely influenced by hormonal secretions (Fruton and Simmonds, 1962) but the nature of these if any, exercising similar control on glycogen uptake in starfishes is not known.

The quantitative changes in glycogen in the pyloric caecae of *Oreaster* show a trend similar to that of *Pisaster ochraceus.* This indicates similarity generally in accumulation and utilization of glycogen between the two species.

The data on changes in the total glycogen content of the pyloric caecae and the gonads of *Oreaster* in relation to reproductive stages given in Table IV show that very little amount of glycogen lost by the pyloric caecae accumulates in gonads on maturation. The glycogen content of the testes and ovaries of the starfish increases from low values to a maximum of 15.43 mg. and 12.26 mg. By contrast, the pyloric caecae show a decrease in total content from 108.1 mg. to 8.75 mg. in mature male starfish and from 236.8 mg. to 6.53 mg. in female starfish ; thus in *Oreaster* large amounts of glycogen are utilized for energy requirements and only a small amount accumulates in gonads. It is known that glycogen is a reserve for immediate use by tissues (Giese, 1966).

TABLE IV

Changes in total glycogen content of pyloric caecae and gonads of *Oreaster hedemanni* in relation to maturation of reproductive organs (each value mean of 4 individuals ranging in weight between 175 gms. and 250 gms.).

Stage of maturity of starfish	Total glycogen content of pyloric caecae of males (mg.)	Total glycogen content of testes of males (mg.)	Total glycogen content of pyloric caecae of females (mg.)	Total glycogen content of ovaries of females (mg.)
immature	155.43 ±40.76	0.99 ±0.03	170.40 ±45.99	2.26 ±0.23
maturing	42.57 ±7.17	4.06 ±1.47	37.76 ±3.89	3.16 ±0.49
mature	7.34 ±0.94	10.48 ±2.36	6.00 ±0.13	10.75 ±0.93
spent	14.01 ±1.41	0.51 ±0.02	15.14 ±1.79	0.43 ±0.14

Glycogen amounts to a maximum of 9.93% dry wt. in pyloric caecae of *Oreaster* in the immature stage and shows a decrease to 0.24% with the maturation of gonads. In *B. balanus* glycogen amounts to about 11.25% dry wt. in immature stage and decreases to 4.8% in the mature stage (Barnes *et al.*, 1963). In *Katherina* in the digestive gland it is low in spent stage being 0.59% dry wt. and decreases to 0.41% in the mature stage, thus showing not very significant percentage change, but the gland itself being reduced in size there is evidence to show that considerable quantity of glycogen is used up during the maturing stages.

Accumulation of organic materials in gonads in relation to reproductive cycle.

1. *Lipids :*

The lipid content of male *O. hedemanni* (Fig. 4) increases from a low level of 6.5% dry wt. in immature ones in May to 24.93% in maturing ones in August. It, however, shows a decrease to 7.32% in mature testes in October and again an increase to 17.76% in February in spent testes.

The ovaries of *O. hedemanni* show similar quantitative changes in their lipid content in relation to the gonadal stages in maturation. There is an increase of lipids from 7.33% in immature stages in May to 18.52% in maturing stage in August and a decrease to 7.65% in the mature stage in October. In the spent ones in February there is an increase of lipids to 37.67%.

O. hedemanni is similar to *P. ochraceus* in regard to the quantitative changes in lipids. In the latter species the lipid contents of testes and ovaries increase from 6% and 9% dry wt. in immature stages to 31% and 19% in maturing stage but decrease to 3% and 7% in the mature stage (Greenfield *et al.*, 1958). But in *P. ochraceus* the lipid content of some maturing ovaries has been observed to be as high as 62% dry wt. compared to a maximum of only 22.73% dry wt. observed in the ovaries of the same stage in *O. hedemanni.* It appears that there is less intensive accumulation of lipids in the latter species.

The factors for the decrease in lipid contents of the gonads between the maturing and mature stages are not known. From a similar decrease in the percentage content of glycogen in the ovaries of *B. balanus*, Barnes *et al.*, (1963) have stated that glycogen is utilized for the respiration of the gonads. In the testes and ovaries of *O. hedemanni*, between the maturing and mature stages, though the lipid content by percentage dry weight decreases the total content of the lipids shows an increase with the increase in size of the gonads (Table II). This shows that the rate of synthesis of lipids is not proportionate to the rate of increase in the size of the gonads.

No marked differences are seen between the two sexes in the lipid contents of mature gonads of *O. hedemanni.* In *Odontaster validus* the mature ovaries have been reported to contain more lipid (21.16%) than mature testes (12.08%) (Pearse, 1965). While in *O. hedemanni* the lipids increase in percentage content from immature to maturing stages with a sudden decrease in the mature ones, in *B. balanus* there is a steady increase in lipid content from immature stage (18.5% dry wt.) to the mature stage (31% dry wt.) (Barnes *et al.* 1963). Among the chitons in *Katherina tunicata* the increase in the lipid contents from immature to mature stages is slight being from 11.3% to 12.3% dry wt. in testes and from 19.5% to 23% in ovaries (Giese and Araki 1962) ; in *Cryptochiton stelleri* the increase is fairly marked from immature to mature stages in the case of ovaries being 16% to 31% dry wt. but low in testes being from 6.11 to 11% dry wt. (Tucker and Giese, 1962). Of the Echinoids, *Strongylocentrotus* shows a slight increase from 15% and 17% in immature testes and ovaries to 22% and 20% in the mature ones (Giese *et al.*, 1959) ; in *Allocentrotus* the increase in the respective phases is from 12·63% and 12.83% in immature testes and ovaries to 14.32% and 15.83% in mature ones (Giese, 1961).

The lipid content of ova removed from gonads has been observed to increase from 6.28%dry wt. in immature stage to 22.34% in maturing stage and decrease to 7.23% in mature ova. These changes in the ova in different reproductive stages are similar to those in the entire homogenized gonads including the interfollicular tissues and ova. The purpose of accumulation of lipids in ova of fish has been pointed out to be their being oxidized for the growth of embryo until commencement of feeding

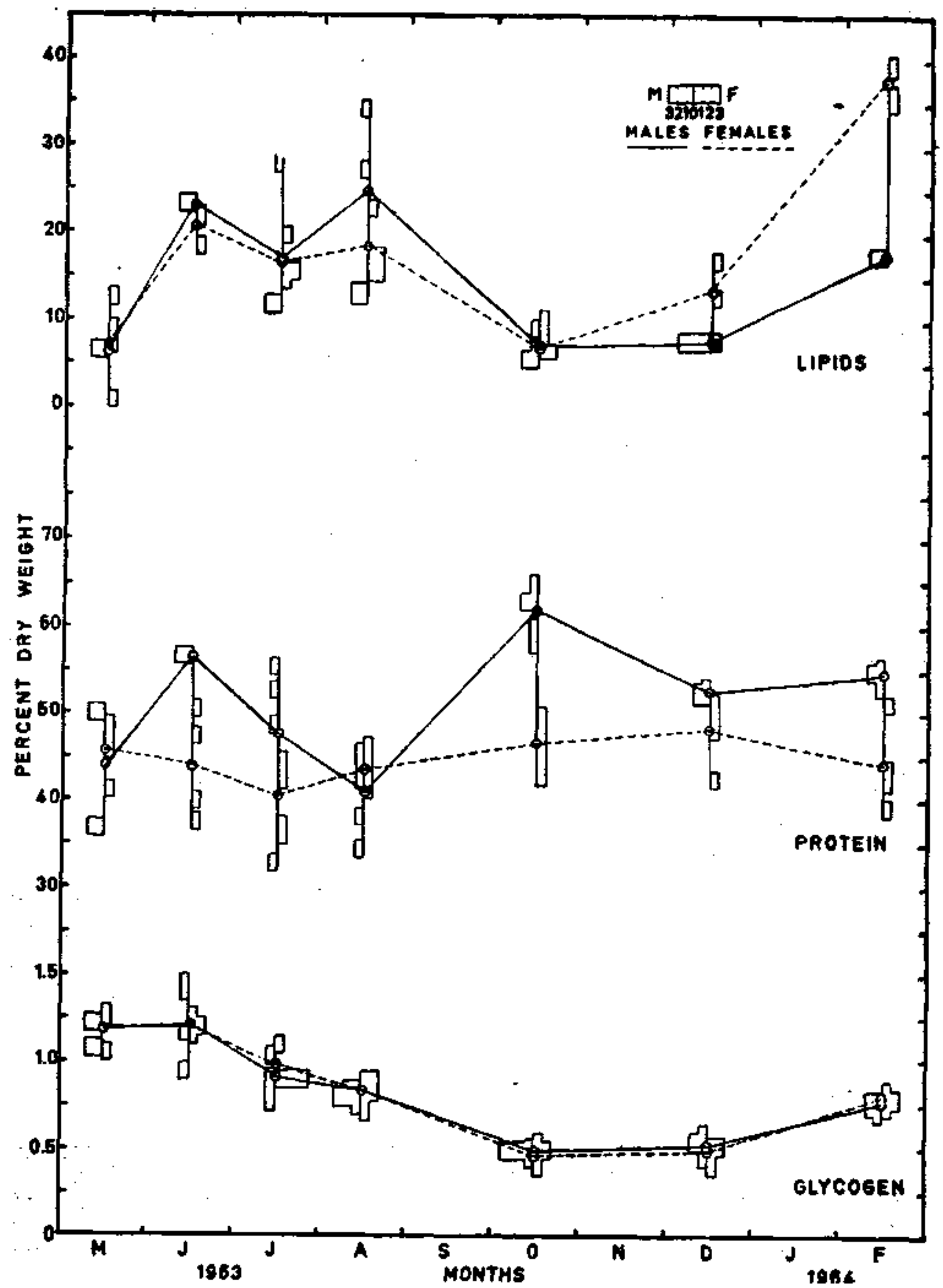

FIG. 4. Percentage contents of lipids, protein and glycogen of gonads of male and female *O. hedemanni* with mean values during the annual reproductive cycle.

in the larvae (Smith, 1952). Lipids in the mature testes and ovaries of *Oreaster hedemanni* may be utilized to meet the energy requirements of the gamates on spawning and for the growth of the embryo in the case of ova.

2. *Protein :*

The protein content of the gonads of *O. hedemanni* (Fig. 4) shows no well-defined changes in relation to maturation stages. In testes it increases from 44.02% dry wt. in the immature stage in May to 47.52% in maturing stage in July and decreases to 41.15% in testes of the same stage in August. The content shows an increase to 62.29% in mature testes in October and decreases to 54.53% in spent testes in February.

The protein content of ovaries decreases from 45.83% dry wt. in immature stage in May to 43.71% dry wt. in maturing stage in August ; it increases slightly to 46.56% in mature stage in October and decreases to 44.18% in spent ovaries in February.

In the absence of well-defined changes in the protein content of the reproductive organs in per cent dry weight during maturation, *O. hedemanni* resembles *Pisaster ochraceus* in which the protein content of testes and ovaries varies between 30% and 55% dry wt. in the immature and mature stages (Greenfield *et al.*, 1958). However, an increase in protein content of testes and ovaries from 36.5% and 32.0% dry wt. in the immature stage to 40% and 43% in the mature stage has been noted in *Strongylocentrotus purpuratus* (Giese *et al.*, 1959). By contrast, the protein content of gonads of *Allocentrotus fragilis* does not show cyclical quantitative changes as the reproductive organs attain maturity, the contents in spent testes and ovaries being 36.10% and 20.87% and in the mature gonads 31.32% in the two sexes (Giese, 1961). The starfish *Oreaster hedemanni* resembles *Allocentrotus* and *Pisaster ochraceus* in the protein content of the gonads not showing well-defined changes on maturation which is indicative of absence of active and steady accumulation of protein leading to an increase in percentage content by dry weight. However, the total content of protein as in the case of lipids increases as the gonads mature and increase in weight (Table III).

A comparison of the quantitative variations in protein of gonads during maturation of *O. hedemanni* with members of other taxonomic group shows the following differences. In contrast to *O. hedemanni*, the protein contents of gonads of *Katherina tunicata* (Giese and Araki, 1962) and *Balanus balanus* and *B. balanoides* (Barnes *et al.*, 1963) increase in percentage dry weight as the reproductive organs attain maturity. The protein contents of the testes and ovaries of *Katherina* increase from 21.2% and 20.1% dry wt. in the spent stage to 44.0% and 42.6% in mature stages. In *Balanus balanus* and *B. balanoides* the protein contents of ovaries increase from 42.5% and 40.63% dry wt. to 55% and 56.25% respectively.

The protein levels in both the immature and mature stages of *O. hedemanni* are in general high ranging in individual females from 35.92% to 51.48% dry wt. and in individual males from 32.93% to 65.37%. These high values obtained throughout the period of observation indicate that proteins are important organic constituents of gonads at all stages of sexual maturity unlike lipids which attain maximum levels in the maturing stages only.

The mean protein content of immature ova was 43.45% dry wt., maturing ova 43.63% and mature ova 44.38%. Protein in the mature ova of *O. hedemanni* appears to be important reserve organic materials and are probably utilized as a source of energy during embryonic development after fertilization as noted (Smith, 1947) in *Salmo*.

3. *Glycogen :*

In May the glycogen contents of the testes in immature stage are 1.08%-1.24% dry wt. (Fig. 4). In August they show a decrease to 0.75%-0.88% in maturing testes ; the glycogen content of the mature testes decreases still further to 0.45%-0.52%. In February glycogen of the testes in spent stage shows increase to 0.71%-0.78% dry wt.

The glycogen content of ovaries of female *O. hedemanni* may be seen from fig. 4 to show trends similar to those of males, with higher levels in immature gonads per cent dry weight in May and a decrease in the content in August and October as the gonads mature. The higher glycogen content of the gonads of *O. hedemanni* in per cent dry weight in May indicates accumulation of glycogen in the gonads in the immature stage. The progressive decrease in the glycogen content of gonads of both sexes between the maturing and mature stages when the gonad indices show increased values may be significant.

Changes in the glycogen content of gonads, similar to those in *O. hedemanni* have been noted in the ovaries of *Balanus balanus* (Barnes *et al.*, 1963) and the authors have stated that gonadal glycogen is utilized in relation to respiration of the ovary. It is possible that glycogen in the gonads of *O. hedemanni* is similarly utilized in relation to respiration of reproductive organs. It is also possible that gonadal glycogen is utilized after conversion into glucose for the synthesis of lipids or protein in maturing gonads of *O. hedemanni*. Barnes *et al.* (*loc. cit.*) stated that there may not be transformation of glycogen in ovary into lipids or protein in *B. balanus*. In view of the absence of evidence further studies are required to determine whether the decrease in the glycogen content of gonads of *Oreaster hedemanni* during maturation is due to utilization in relation to respiration or in relation to synthesis of lipids or protein in maturing gonads.

The glycogen content of ova in different stages of maturation in ovaries of *O. hedemanni* showed quantitative changes similar to those in the entire ovaries. The glycogen content of immature ova is 1.20% dry wt. and decreases to 0.80% in maturing ova and 0·47% in mature ova. These observations suggest that there is progressive decrease of glycogen in the ova in per cent dry weight correlated with maturation due to a decrease in the rate of synthesis in per cent dry weight in the developing ova. Smith (1952) has shown that carbohydrate amounts to 1.84 mg./gm. wet weight in the eggs of *Salmo irideus* and decreases to 1.27 mg/gm. wet weight, on the 68th day on development and it has been stated that carbohydrate is utilized for the energy needs of the developing embryo. The glycogen accumulating in the ova of *Oreaster hedemanni* may be similarly utilized during the early development of the egg after fertilization.

Though the glycogen content of gonads in per cent dry weight decreases during maturation, the total glycogen content of the gonads increases (Table 4). This is due to the large increase in the weight of the gonads as indicated by the gonad indices rising from 0.33 and 0.71 in immature males and females to 7.32 and 3.43 in mature ones. The total glycogen content of the testes of *O. hedemanni* increases from 1.38 mg. in immature stage to 6.44 mg. in the mature stage and that of ovaries from 2.11 mg. in the immature stage to 9,71 mg. in the mature stage. It is worth noting that the increase in the total glycogen content of gonads of both sexes is low unlike that of lipids and protein.

In conclusion it may be stated that a study of the total quantitative changes of lipids, protein and glycogen in gonads show that all the three organic constituents increase progressively from immature stage onwards and reach the maximum levels in the mature stage. From the point of view of relative abundance of the organic constituents in mature gonads protein ranks the highest, lipids come next and glycogen the lowest.

Discussion

In *Oreaster hedemanni* the fluctuations in gonad indices of both sexes show an increase during maturation with a fall following spawning. A steady increase in gonad index during the maturation period to reach a maximum value followed by a fall in the same does not always indicate spawning. In *Stomopneustus variolaris* the gonad indices have been found to steadily rise from July to April and the decrease in the following month (Giese *et al.*, 1964) but gravid individuals with reproductive elements have been found to occur in almost all the months of the year. This indicates a general build up of the gonads in the course of a year with intermittent spawning. The drop in gonad indices about May probably indicates peak spawning about this period. That there is a single spawning period in *O. hedemanni* in a year is confirmed by the microscopic examination of the gonads through all the stages in the course of a year. In most of the starfish so far studied there appear to be definite reproductive cycles marked by a single spawning period in each. *Pisaster ochraceus, P. giganteus, P. brevispinus* (Farmanfarmaian *et al.*, 1958) *Odontaster validus* (Pearse, 1965) and *Asterias rubens* (Chadwick, 1923) have each a distinct spawning period as *O. hedemanni* but *Patiria miniata* has been stated to spawn at any time during the course of the year (Farmanfarmaian *et al., loc. cit.*).

It is suggestive from the observations recorded in the foregoing study that at least one of the causal factors influencing breeding periodicity and the associated changes in the gonads may be nutritional reserves. For the growth and maturation of gonads, there is the need for large amounts of organic materials which come from either the storage organs if such organs exist as in the case of starfishes, or they are derived from absorbed food through the intestinal walls and directly accumulated in the gonads as in the sea urchins. The experimental studies of Ferguson (1964a, b) show that the pyloric caecae of starfish are the main storage organs of reserve organic materials which are released by diffusion by these organs into the perivisceral coelomic fluid for utilization by other organs including gonads. The pyloric caecae of *O. hedemanni* undergo well-defined seasonal changes in relation to the annual reproductive cycle. The increase and decrease of the size of pyloric caecae are accompanied by fluctuations in gonad indices in an inverse order. The increase in size of pyloric caecae from the lowest to the highest level takes place in a very short period between October and February. The process of accumulation of organic reserves is more rapid in *O. hedemanni* compared to *Pisaster ochraceus* in which the reserve organic materials accumulate to the maximum levels in ten months. The rapid accumulation of nutritional reserves in *O. hedemanni* may be related to the ready availability of food in abundance during the period or the general prevalence of high temperatures of environmental waters which may promote a rapid accumulation of the organic reserves. It is clear that environmental factors influence and modify the pattern of accumulation of nutritional reserves even in closely allied species. The work of Pearse (1965) on *Odontaster validus* shows that the availability of food influences the accumulation of organic reserves.

Of the three major constituents in the pyloric caecae of *Pisaster* lipids appear to be the more important in view of the accumulation in comparatively larger quantities (being 50% dry wt. in *P. ochraceus* and 75% dry wt. in *P. giganteus*) and the decrease in the content during the annual reproductive cycle. It is suggestive that lipids of pyloric caecae contribute to energy economy during gonadal changes preparatory to spawning in the starfish. The lipid content of pyloric caecae of *O. hedemanni* also shows a marked fall during maturation of gonads. The protein of pyloric caecae is not utilized to the same extent in all starfish. In contrast to *Pisaster ochraceus* in *O. hedemanni* there is a fall of protein values per unit weight of the pyloric caecae between the immature and mature stages which is probably accounted for by transfer to gonads. Glycogen occurs in small quantity compared to lipids and protein in all the starfish but it also shows a progressive decrease in content with the maturation of gonads. However, in *O. hedemanni* glycogen of the pyloric caecae shows an increase only in the immature stage and not in the spent stage.

The lipid contents of the storage organs of Asteroids so far studied are much higher compared to those in the barnacle *Balanus balanus* (29.38%) and much more so than inthe chitons *Katherina* (11.5%) and *Mopalia* (13.6%). The well-defined differences in the levels of lipids of storage organs of the above species belonging to different phyla viz. Echinodermata, Arthropoda and Mollusca suggest that there may be distinct biochemical potentials i.e. distinct differences in the levels upto which synthesis and accumulation of reserve organic materials takes place in members of different taxonomic groups as proposed by Slobodkin (1961). He considered that different taxonomic groups may differ broadly in caloric content, such differences being correlated with biochemical characteristics. All organisms do not necessarily maintain the same store of identical biochemical components in different phyla. This is suggestive of caloric requirements being met by different organic constituents in members of different taxonomic groups. This is supported by the data at present available on the lipid, protein and glycogen contents in storage organs of marine invertebrates.

For the utilization of reserve lipids, protein and glycogen in pyloric caecae of starfish for accumulation in gonads a process of degradation of the materials may be necessary. This process appears to take place with the commencement of gametogenesis and maturation of gonads.

The lipids, from the changes in the contents of gonads of *O. hedemanni* appear to play an important role in gametogenesis. The data obtained in the present study shows an initial increase in lipid content of gonads in both sexes upto the maturing stage which represents accumulation for subsequent utilization in relation to growth of the maturing organs. The protein contents of testes and ovaries of *O. hedemanni* do not undergo well-defined quantitative changes in per cent dry weight during maturation. Glycogen occurs only in very small quantity in the gonads of *O. hedemanni* as in *P. ochraceus*.

The patterns of biochemical changes noted in the gonads of *O. hedemanni* are found in starfish so far studied. Other animal groups show different patterns in the nature of the constituents and their accumulation. *Strongylocentrotus* (Giese *et al*., 1958), *Katherina, Mopalia* (Giese and Araki, 1962). *Cryptochiton* (Tucker and

Giese, 1966) and *Balanus* (Barnes *et al.*, 1963) show an increase in the lipid and protein contents of gonads from a low level to a higher level during maturation. A careful study of the work done so far on species belonging to different taxonomic groups shows that there is similarity generally in accumulation of organic materials among members of the same class but when members of different classes or phyla are compared well-defined differences are observed in this respect. These findings support Slobodkin's hypothesis (1961) that in regard to accumulation of organic materials in relation to maturation of gonads the various animal species may show differences correlated with their taxonomic position.

SUMMARY

The nutritive cycles of the starfish *Oreaster hedemanni* have been studied by examination of the storage organs in the course of a year.

The hepatic indices of the starfish show high values in the spent and immature stages and low values during maturation of the gonads.

In the pyloric caecae lipids and protein are in larger amounts compared to glycogen. The total contents of lipids and protein are higher in the immature stages due to accumulation. A depletion of lipids, protein and glycogen in the pyloric caecae appear to be correlated with the gonadial activity preparatory to spawning.

The changes in gonadial activity have been estimated by determining gonad indices which show an inverse relationship to hepatic indices. The reproductive cycle extends from June to May. The gonads attain maturity by October and spawning occurs in December.

Correlated with these changes the lipid content in percentage dry weight of testes and ovaries shows an increase between the immature and maturing stages and a fall in values on attaining maturity. The total protein content of the gonads shows fluctuations which are inversely correlated with changes in the pyloric caecae. Although the total protein content shows marked fluctuations the changes in the content per unit weight are not marked. Glycogen is present in small quantities at the commencement of gonadial activity. Its percentage decreases during maturation but the total content increases during the period.

The extent of accumulation of the organic materials in storage organs and gonads in *O. hedemanni* is compared with that in members of other groups.

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