

## The annual reproductive cycle of the holothurian *Aslia lefevrei* (Dendrochirota: Echinodermata) \*

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### Abstract

The dendrochirote holothurian *Aslia lefevrei* (Barrois) is locally and densely aggregated ( $> 70$  individuals  $m^{-2}$ ) in Galway Bay, on the west coast of Ireland. It has a discrete, relatively short breeding period from the end of February to the beginning of April, with peak activity in March. The sexes are separate and the single gonad, consisting of numerous tubules (100 to 800), lies in the anterior coelom. Both gonad index values and histological observations indicate that gametogenesis resumes in June/July after a brief post-spawned resting period. Little oocyte growth and sperm production occurs over the warm summer months when the holothurian is actively feeding. Gonad index values, however, increase rapidly during this period and reach high values by early winter without concomitant increases in maturity indices. This is subsequently followed by slight reductions in the gonad indices, before reaching peak values in February. It is suggested that this is probably due to the initial build-up and subsequent assimilation of storage products in the gonad, the development of gametogenic products and the frequent assimilation thereafter of unwanted ova. Spawning generally takes between 5 and 60 min, with the entire contents of the gonad rarely being shed in a single release. During this process, the holothurian raises its anterior end and, with the extended tentacles close together, emits a stream of gametes from the genital pore in the mid-dorsal line. Laboratory experiments and general observations indicate that increased temperature and low light levels may influence spawning behaviour.

### Introduction

In recent years, the physical prominence of many holothurians in benthic communities has generated increased

interest in their reproductive habits (Tanaka, 1958; Krishnaswamy and Krishnan, 1967; Krishnan, 1968; Rutherford, 1973; Green, 1978; Engstrom, 1980, 1982; Conand, 1981, 1982; Tyler and Gage, 1983). Although a wide range of reproductive strategies exist (Hyman, 1955), species found in temperate shallow waters generally display a discrete, short, breeding period. Such is the case with the dendrochirote holothurian *Aslia lefevrei* (Barrois, 1882), which is locally and densely aggregated ( $> 70$  individuals  $m^{-2}$ ) in Galway Bay, on the west coast of Ireland (Fig. 1). Distributed from the Orkneys to the Mediterranean coast of Spain, the holothurian occupies rock crevices from the low-water mark to ca. 20 m. Details of its systematic position, feeding behaviour and general biology are included in the recent works of Costelloe and Keegan (1984 a, b). Some of the more common synonyms listed for this species are *Cucumaria normani* (Pace), *C. lefevrei* (Barrois) and *Ludwigia lefevrei* (Barrois).

Other than Orton's (1914) and Mortensen's (1927) references to the spawning of *Aslia lefevrei* during March, little or no information is available on the reproductive cycle of this species. This account reports on an investigation of the gonadal cycle in *A. lefevrei*, using histological techniques and gonad indices against a background of hydrographic data.

### Materials and methods

The gonadal cycle of *Aslia lefevrei* (Barrois) was monitored at 4 to 6 wk intervals from February 1979 to August 1980. On each occasion, 15 individuals were collected from the Blackrock Reef (54°14'5"N; 9°7'0"W) in Galway Bay (Fig. 1) at a depth of 15 m. Specimens were subsequently dissected and the gonads and remaining body dried separately at 60°C. Only those individuals greater than 5 cm in length were taken for gonad evaluation. Gonad indices were calculated from the formula  $\text{dry gonad wt} \times 100/\text{dry body wt}$  (Tanaka, 1958). Before drying, three

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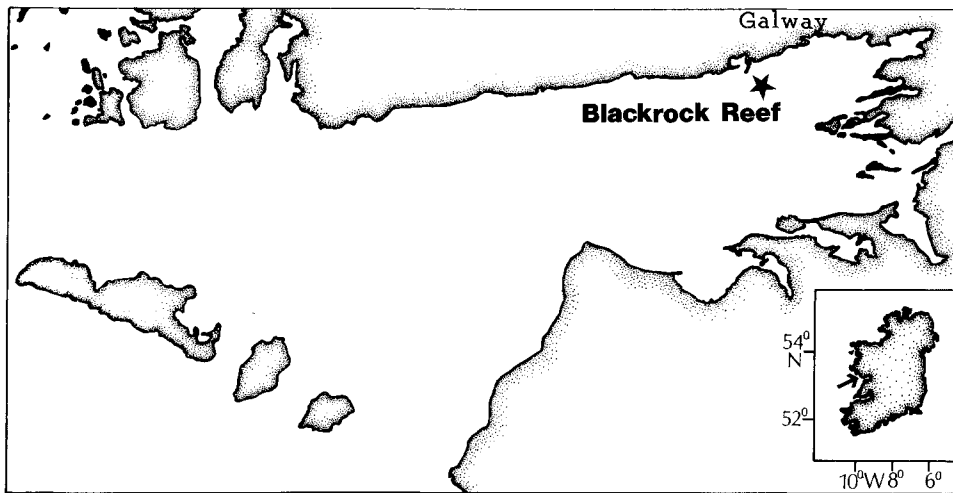


Fig. 1. Blackrock Reef on west coast of Ireland

individual gonadal tubules were removed from each individual and fixed in either neutral buffered formalin or Bouin's fluid. Tissue dehydration was effected in graded alcohols, tissues being subsequently cleared in either xylene or toluene. Both paraffin wax and JB-4 (Polyscience Inc.) tissue-embedding techniques were employed. Paraffin-embedded tissues were impregnated in 54° to 56°C mp paraffin wax in a vacuum oven set at 58°C. JB-4-embedded tissues were taken from 70% alcohol and infiltrated with resin. Infiltration was completed within 3 h at 4°C and the resin was polymerized at room temperature by the addition of a catalyst. Sections were cut on a rotary microtome at 4 to 8 µm for paraffin and 2 to 3 µm for JB-4-embedded tissues. Preparations were stained with Ehrlich's haematoxylin and eosin, or alcian blue 8GX, followed by Mayer's haematoxylin. Sections were examined under a light microscope to assess the stage of maturity and to allow size-frequency counts to be made of the oocytes. Measurements of fifty or more oocytes were taken from each female and the results grouped into 20 µm size-classes. Oocyte measurements were taken across the greatest axis and only from those cut across the nucleus.

Each holothurian was assigned a "stage of maturity" from I to V, according to the method of Tanaka (1958) and Green (1978). Recognition of the stages of maturity is based upon the overall appearance of the gonads and the state of development of their component germ cells.

Maturity indices (MI) were calculated separately for the males and the females in each sample. The maturity index is based on that of Yoshida (1952) and is calculated on the numbers of each stage present in the monthly samples, according to the following formula:

$$MI = \frac{1(\text{no. of individuals in Stage 1}) + 2(\text{no. of individuals in Stage 2}) \dots n(\text{no. of individuals in Stage } n)}{\text{total number of individuals staged that month}}$$

Laboratory experiments were carried out on individuals in flow-through aquaria systems in relatively undisturbed conditions. Temperature-shock treatments were

effected by introducing warmed sea-water into these aquaria, thereby allowing a rapid increase in temperature. These experiments were carried out in conjunction with control tests during both daylight hours and times of low light to complete darkness.

Hydrographic conditions at the collection site (temperature, salinity and dissolved oxygen at surface and bottom (15 m)) were monitored every 4 to 6 wk throughout the study.

## Observations

### Anatomy of the reproductive system

*Aslia lefevrei* is dioecious, as are the majority of the holothuroids. The single gonad is located in the anterior of the coelom and consists of numerous tubules. There is no sexual dimorphism, and sex is only readily determined by the appearance of the gonad at a late stage of development. In immature individuals, sex can only be recognized from histological preparations. The mature gonad may occupy much of the anterior mid-dorsal mesentery and may even extend into the anterior introvert.

Analyses of the ratio of males to females within the population at the Blackrock Reef show a slightly greater number of males. Of the 225 individuals taken for gonad index evaluation, 118 (46.3%) were female and 137 (53.7%) were male. A  $\chi^2$ -test was performed to assess the probability of these values representing an even male-to-female ratio. As the computed  $\chi^2$  value (1.41) is well below the tabulated value of 3.84 ( $P > 0.05$ ), it is concluded that the sex ratio obtained does not depart significantly from a 1:1 ratio.

The gonadal tubules of *Aslia lefevrei* are relatively small, and of uniform length and width in the same individual. Within the species, there is great variation in the number of these tubules which, during the course of the present work, was found to vary from 100 to 800. Individual gonadal tubule size and the number of tubules, however, are not necessarily greater in large individuals.

The tubules of *A. lefevrei* open into a hollow gonadal base, which continues anteriorly as the gonoduct. This opens onto the body surface through a gonopore which is situated in the mid-dorsal line between the tentacles.

In the immature condition, the female gonad is a yellow colour. Typically, at sexual maturity, it is very voluminous and changes to a transparent beige colour. At this stage, the eggs can be seen quite clearly through the gonadal wall. During the non-breeding season, the testis differs little in size or coloration from the ovary. As development proceeds, the testis retains the yellow coloration and increases in size to fill much of the coelomic cavity.

The wall of the gonad (ovary and testis) consists of a coelomic epithelium, a layer of connective tissue and an inner germinal layer. Circular muscle cells are sometimes present in the connective tissue adjacent to the external epithelium. Both the coelomic epithelial layer and the inner germinal layer exhibit great variation in thickness, depending on the stage of maturity of the gonad.

### Gametogenesis

Although the gametogenic cycle of *Aslia lefevrei* is a continuous process, it can be divided into five arbitrary stages, i.e., recovery stage, growth stage, mature stage, shedding stage and resting or post-spawned stage. These equate with the divisions of Tanaka (1958) for *Stichopus japonicus* Selenka, Green (1978) for *Leptosynapta tenuis* (Ayres), Engstrom (1980) for *Holothuria mexicana* Ludwig and *H. floridana* Pourtales, and Conand (1981, 1982) for *Thelenota ananas* (Jaeger), *H. nobilis* (Selenka), *H. fusco-gilva* (Cherbonnier) and *Actinopyga echinites* (Jaeger). The gametogenetic stages of male and female *Aslia lefevrei* are, for the most part, synchronous.

### Oogenesis

#### Recovery stage

Recovery of the female gonad is marked by a thickening of the external epithelium and the germinal layer, which may develop large extensions into the lumen of the tubules (Fig. 2A). Distinct oogonia are impossible to identify with any precision. Developing oocytes are arranged in a single layer (Fig. 2B); they are surrounded by a thin follicular epithelium and small cells described as accessory cells in *Ypsilothuria talismani* Perrier (Tyler and Gage, 1983). The primary oocytes measure 20 to 40  $\mu\text{m}$  in diameter and 60 to 70% of their internal volume is occupied by a large nucleus, with between one and five small nucleoli resting on the inner side of the nuclear membrane.

#### Growth stage

Growth is characterised by very active production of gametes and much nuclear activity. The early oocytes have

a distinct basophilic PAS-negative cytoplasm (Fig. 2C, D). As vitellogenesis proceeds, the cytoplasmic basophilia decreases and PAS-positive granules fill the entire cytoplasm. The chromatin content of the nucleus also undergoes many transformations during meiotic prophase stages of leptonema and pachynema, which results in the chromatin content becoming clearly visible in the translucent nucleoplasm (Fig. 2E). Measuring between 40 and 220  $\mu\text{m}$  at the growth stage, the developing ova occupy much of the now spacious tubule lumina, but still retain the follicular membrane adjoining them to the germinal epithelium.

#### Mature stage

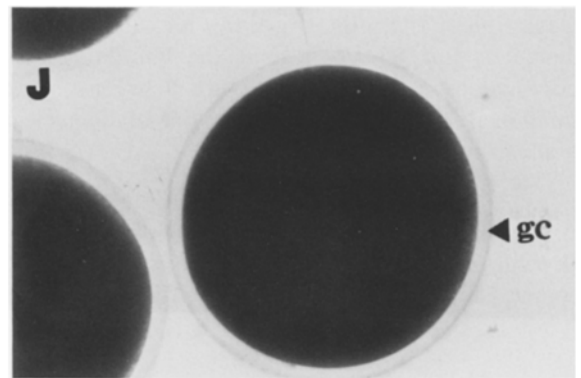
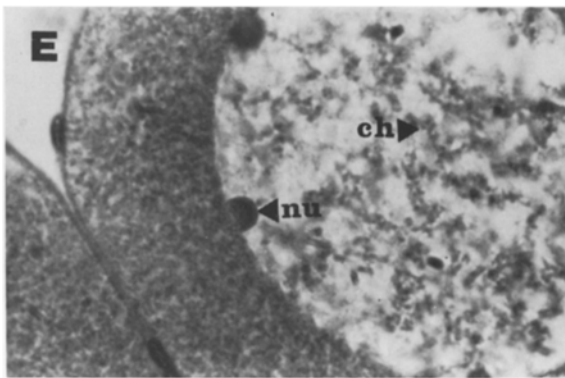
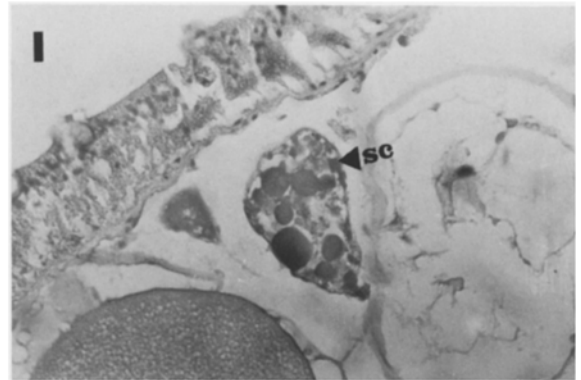
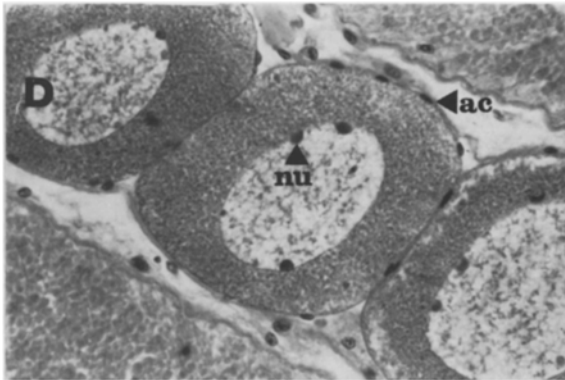
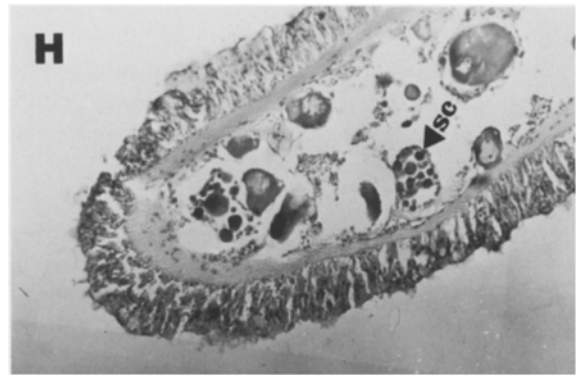
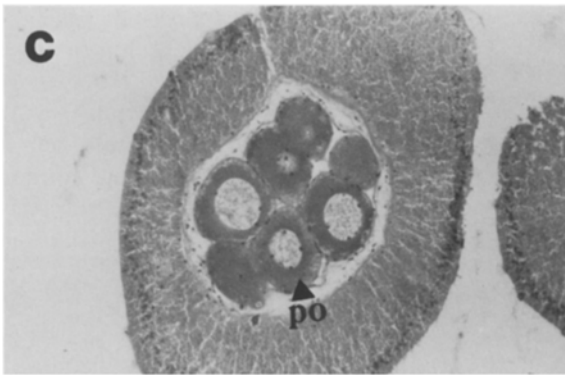
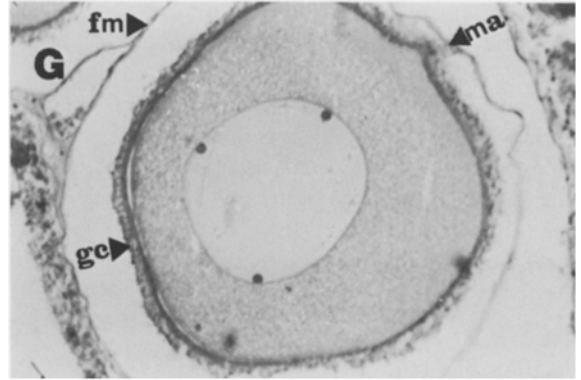
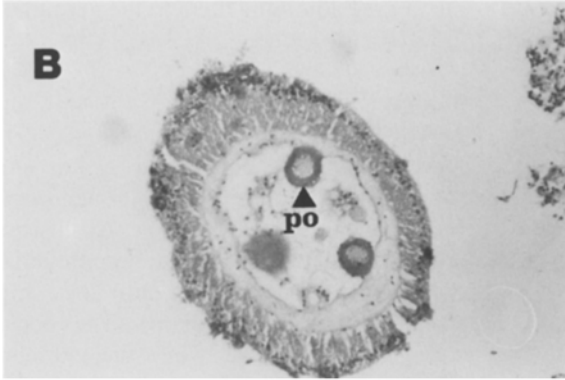
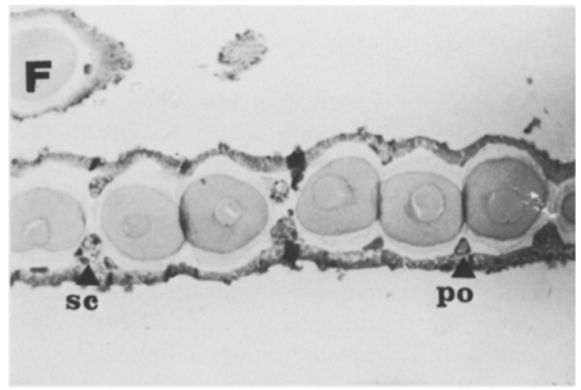
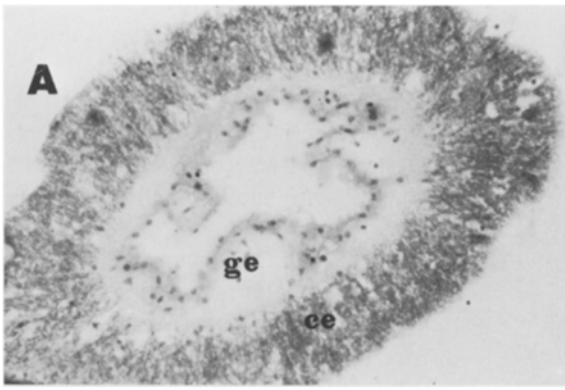
Development of oogonia becomes less active at the mature stage, but may continue even though oocytes fill the entire lumen of the gonadal tubules. At this time, the germinal layer and outer epidermic become much reduced and oocytes continue to grow until they approach shedding size (250 to 340  $\mu\text{m}$  diam). The oocyte cytoplasm is strongly PAS-positive, while the granular appearance of the nucleoplasm changes to a homogeneous opaque region with 1 to 5 distinct nucleoli resting on the nuclear membrane. At a late developmental stage, many ova, which are loosely attached to the inner germinal layer by the follicular membrane, are phagocytosed and broken-down to form enclosed spherule cells (Fig. 2F). These spherules often contain numerous eosinophilic, basophilic and opaque-looking globules. The precise nature of the presumed nutrient assimilation within and transfer from these spherule cells is uncertain.

#### Shedding stage

The gonadal wall becomes very thin and the mature ova are easily visible as spherical bodies lying in the lumina of the tubules. Each oocyte is attached to the inside surface of the follicular membrane by a micropyle appendage (Fig. 2G). Within the follicular membrane and surrounding the egg, a thin jelly coat develops which is strongly PAS-positive. Just before spawning, the mature eggs become free of the follicular membrane and move about freely in the lumen of the tubules. At this stage, they measure between 250 and 340  $\mu\text{m}$  in diameter, with a 10 to 20  $\mu\text{m}$ -thick gelatinous coat. No further changes were observed in the morphology of the oocytes before their release. Kume and Dan (1968) consider that maturation divisions take place during spawning and fertilization in holothurians.

#### Post-spawned stage

Individual *Aslia lefevrei* rarely shed the entire contents of their gonads during spawning. The post-spawned stage is categorized by the presence of a spacious lumen with



numerous large ova scattered about undergoing various stages of resorption. The residual ova are phagocytosed and form spherule cells (Fig. 2 H, I).

Most of the stages in the female gonadal cycle contain some post-pachytene primary oocytes at least 20 to 30  $\mu\text{m}$  in diameter.

### Spermatogenesis

#### Recovery stage

As in oogenesis, the recovery stage is marked by a thickening of the outer epithelium and inner germinal layer (Fig. 3 A, B). Indistinct spermatogonia, measuring approximately 10  $\mu\text{m}$  in diameter, appear on the inner side of the germinal layer. Atwood (1974) suggests that morphogenesis of the holothurian flagellum and associated projections of the distal centriole have not yet been initiated at the spermatogonial stage.

#### Growth stage

Growth of the testis is characterised by very active spermatogenesis. A row of primary spermatocytes line the inner side of the germinal layer (Fig. 3 C, D) and range from 6 to 7  $\mu\text{m}$  in diameter. Spermatogonia and early spermatocytes develop and are considered to be in the pachytene/early diplotene stages of meiosis (Atwood, 1974). The germinal layer develops long extensions into the lumen of the tubules which greatly increases the surface area for spermatocyte production (Fig. 3 E, F). This layer also becomes much reduced in thickness, allowing a large lumen to appear. Early spermatids measure from 3 to 4  $\mu\text{m}$  in diameter and often lie free of, but close to, the spermatocyte layer. During the process of spermatogenesis, the nucleus becomes relatively circular as the chromatin condenses to the final state observed in late spermatids. At this stage, the first indication of the flagellum appears and mature spermatozoa are produced.

#### Mature stage

The mature stage is characterised by a reduction in thickness of the gonad wall, regression of the germinal layer projections and production of spermatozoa (Fig. 3 G, H). The process of spermatogenesis continues until the lumen of the gonad becomes packed with mature spermatozoa.

#### Shedding stage

Active spermatozoa occupy the entire lumen of the gonadal tubules (Fig. 3 I). The gonadal wall becomes thin and transparent. In squash preparations of shedding-stage gonadal tubules, sperm were observed moving about using their long flagella in a whip-like motion for propulsion.

#### Post-spawned stage

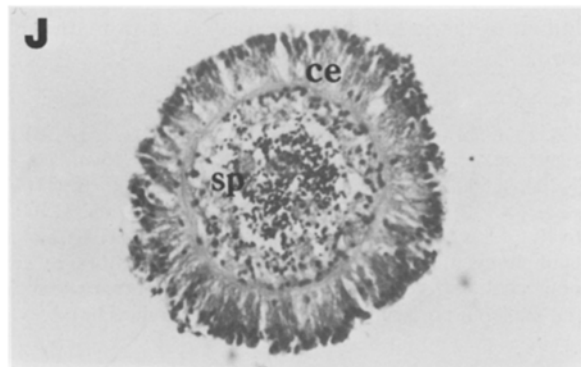
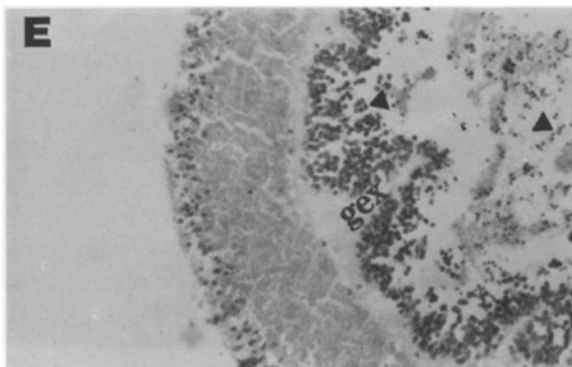
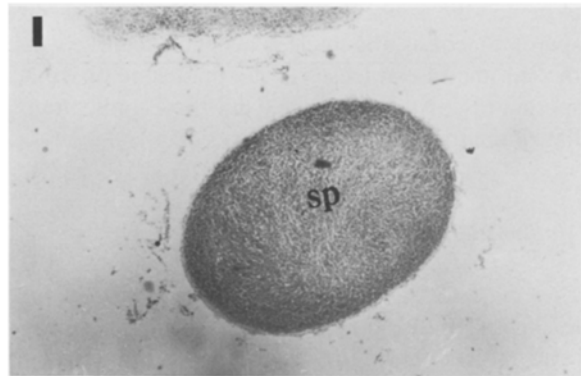
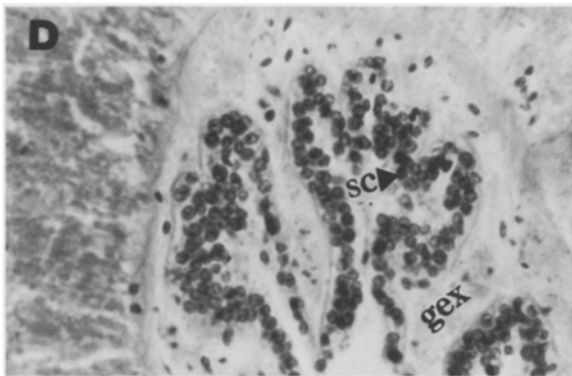
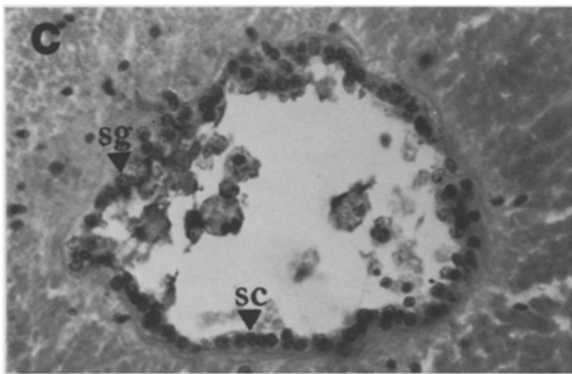
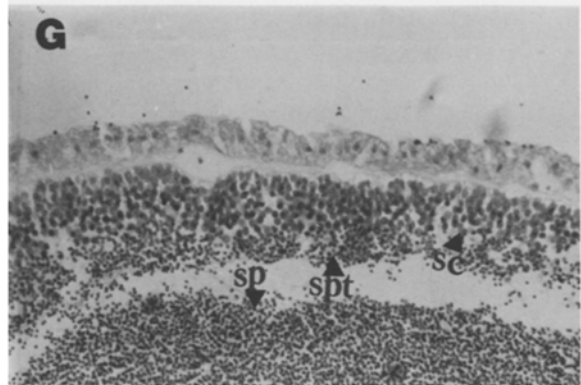
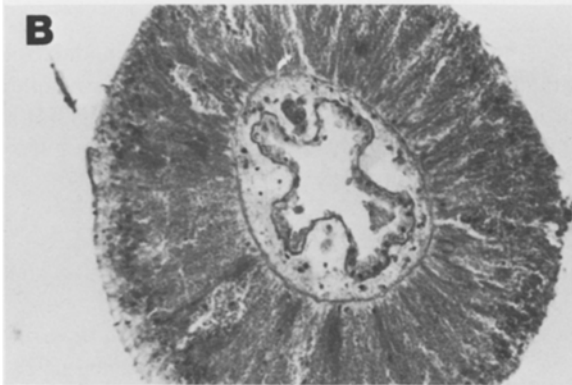
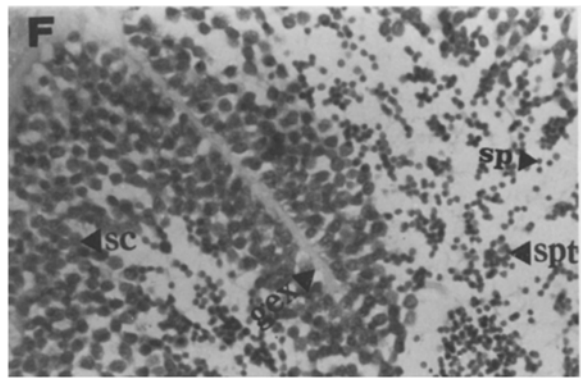
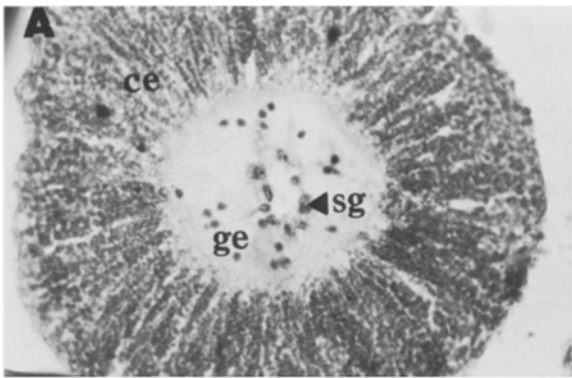
As in the female, the entire gonadal contents are rarely shed in one season and relict sperm often remain in the lumen of the spent tubules (Fig. 3 J). These may be phagocytosed or remain in the tubules until the next spawning.

### Reproductive cycle

The reproductive cycle of *Aslia lefevrei* was monitored over a 19 mo period from February 1979 to August 1980. Gonad index values showed an approximate three-fold increase in the gonad-to-body dry weight over time, with males generally maintaining slightly higher values (Fig. 4 D). After a peak of 18% for males and 17% for females in February 1979, the indices regressed to lows of 6.3 and 6.6%, respectively, in May. Then, after an initial resting period, the male index increased to 18% in November 1979. This increase was not gradual, and was characterised by an initial rapid weight accumulation in June and July, a period of slow growth in August and September, and a final rapid increase up to November. Similarly, the females, after a more extended rest period, underwent a rapid increase in weight during July and August (1979), a decline to September, followed by a slight rise in values to October, and rapid growth up to November (1979). The male and female gonad indices, after a slight decrease, were maintained at a high 15 to 18% value from November 1979 to March 1980.

Inspection of maturity charts (Fig. 5) and indices (Fig. 6) coupled with monthly oocyte-diameter values (Fig. 7; Table 1) give a more detailed picture of the seasonal gonadal development. After the characteristically long post-spawned stage in both sexes, which may extend over a 4 mo period, the recovery stage is indicated in histological preparations and by an increase in the gonad index at the beginning of August (1979). Young oocytes developed in the gonads and measured, on average, 71  $\mu\text{m}$  in diameter at this time (Table 1). During the post-spawning stage, nutritive phagocytes help to break down the large unspawned ova. This activity of the phagocytes may explain the decrease in the average oocyte diameter from the end of June to the beginning of August. Gonad indices also showed a decrease in values after an initial recovery stage in August. The sporadic fluctuations in the gonad index were not reflected in the increasing maturity index values (Fig. 6), and may be explained by nutrient

Fig. 2. *Aslia lefevrei*. Female gametogenic stages. (A, B) recovery stage ( $\times 80$  and  $\times 40$ , respectively); (C–E) growth stage ( $\times 80$ ,  $\times 200$ ,  $\times 500$ , respectively); (F) mature stage ( $\times 20$ ); (G) shedding stage ( $\times 150$ ); (H, I) post-spawned stage ( $\times 50$  and  $\times 100$ , respectively); (J) spawned eggs ( $\times 90$ ). ac: accessory cells; ce: coelomic epithelium; ch: chromatin; fm: follicular membrane; gc: gelatinous coat; ge: germinal epithelium; ma: micropyle appendage; nu: nucleoli; po: primary oocytes; sc: spherule cells



build-up in the gonad walls and subsequent assimilation during gametogenesis and/or assimilation of unwanted ova. The recovery stage of the male extends over an approximate 2 mo period from September to October, but may begin as early as July (Fig. 5). The maturity chart shows a much shorter recovery stage for the female. This however, is probably less than accurate, as the recovery stage and post-spawned stage often overlap, each showing characteristics of the other. Mean oocyte diameter ranged between 75 and 110  $\mu\text{m}$  during the recovery stage (Table 1). Both growing and mature stages extend over 2 mo and can take over 4 mo for the population at large. From October to February, the female ova showed a gradual increase in mean diameter size from 111.5 to 235  $\mu\text{m}$ . Over this period, the male produces large quanti-

ties of spermatozoa. The gonad index (Fig. 4D) showed a rapid increase up to November, and was followed by a slight decline in values until February. As previously suggested, the reduction in value may be due to nutritional assimilation in the gonad and, in the female, the resorption of unwanted ova. Shedding-stage gonads were found over a 4 mo period within the population, with peak values during the month of March and extending into April.

Spawning

*Aslia lefevrei* was first induced to spawn in aquaria at the beginning of February 1980. Spawning took place in the evening, at night, or in the early morning, with the males generally preceding the females. Prior to actual gamete release, *A. lefevrei* extends its tentacles and remains motionless for a period ranging from 5 to 30 min. The holothurian then raises its anterior end vertically and moves in a "cobra-like" fashion, with the extended tentacles close together. A stream of milky sperm or eggs is released from

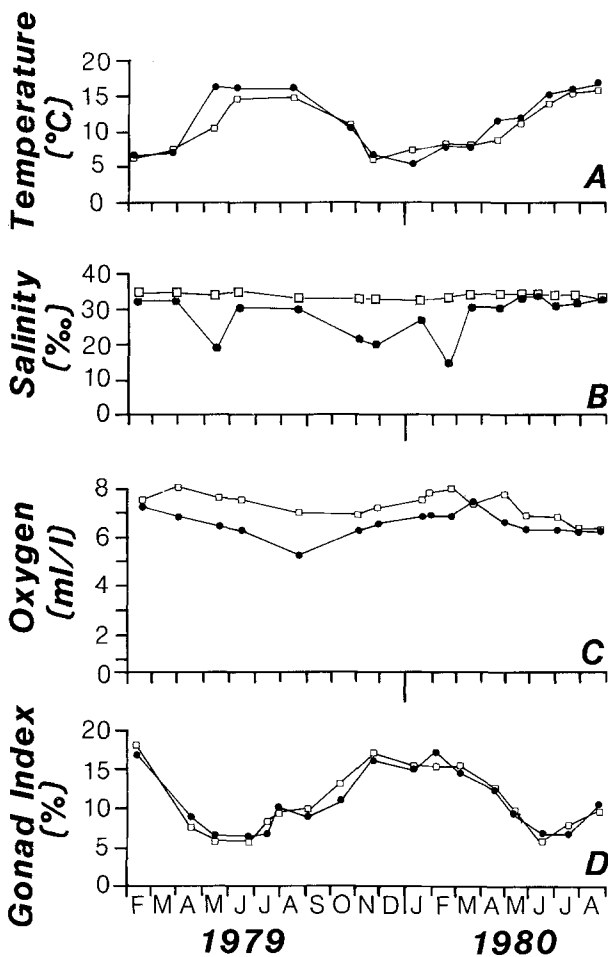


Fig. 4. Temperature (A), salinity (B), and oxygen level (C) of surface (●) and bottom (□) waters at Blackrock Reef. (D) *Aslia lefevrei*, female (●) and male (□) gonad indices (means)

Fig. 3. *Aslia lefevrei*. Male gametogenic stages. (A, B) recovery stage ( $\times 100$ ); (C–F) growth stage ( $\times 500$ ,  $\times 300$ ,  $\times 150$ ,  $\times 500$ , respectively); (G, H) mature stage ( $\times 200$  and  $\times 50$ , respectively); (I) shedding stage ( $\times 40$ ); (J) post-spawned stage ( $\times 40$ ). ce: coelomic epithelium; ge: germinal epithelium; gex: germinal epithelium extension; sc: spermatoocytes; sg: spermatogonia; sp: spermatozoa; spt: spermatids

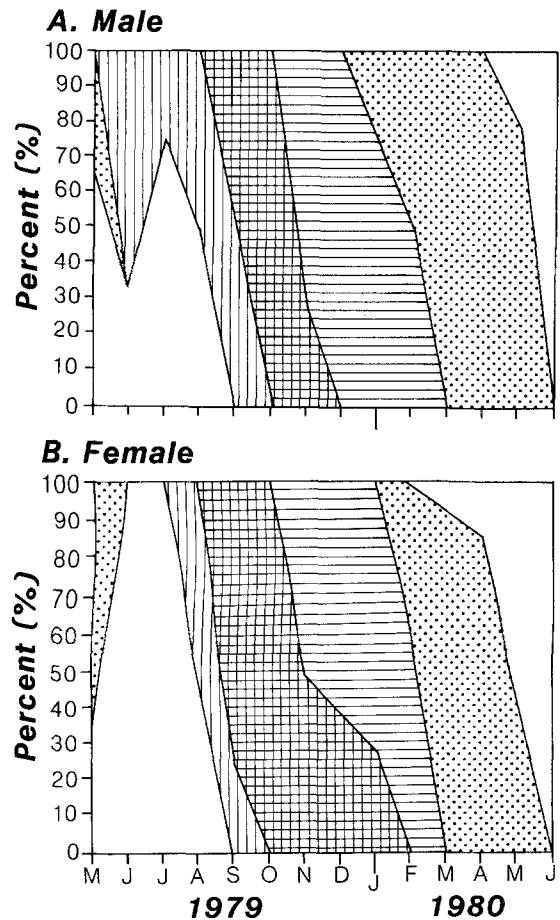
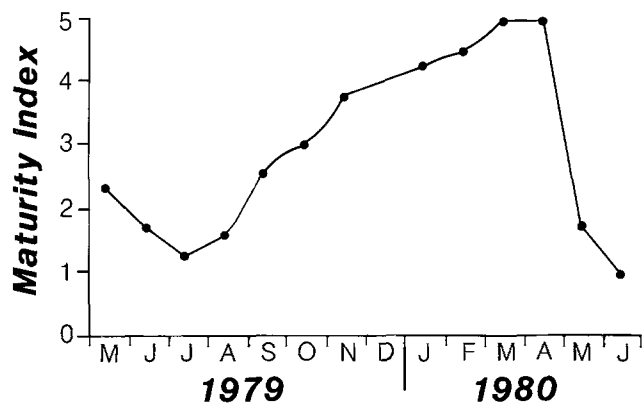


Fig. 5. *Aslia lefevrei*. Male and female maturity charts

### A. Male



### B. Female

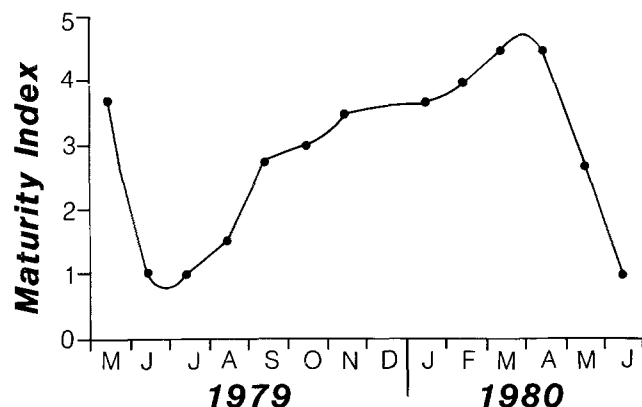


Fig. 6. *Aslia lefevrei*. Male and female maturity indices

the gonopore, which is located between the tentacles in the mid-dorsal line. The liberation of the sperm and eggs is often quite forceful and their dispersal is facilitated by a wafting motion of the tentacles. As shown by Costelloe and Keegan (1984b), freshly spawned eggs do not adhere to the sticky tentacle tips of *A. lefevrei*, and are positively buoyant in the water column. Spawning generally lasts from 5 to 60 min. From histological evidence, however, it would appear that the entire contents of the gonad are rarely shed in one spawning.

Initially, individuals had to be subjected to temperature increases of 6°C or more from an ambient 6°C in February, 1979, before spawning commenced. The same upper limit, i.e., ca. 12°C, was found to hold in April when ambient temperature had increased to 10°C. Irrespective of when temperature increases were initiated or the duration of exposure, spawning only occurred during conditions of low light or complete darkness.

From histological evidence, gonad index values and the spawning behaviour in the laboratory, it appears that *Aslia lefevrei* spawns over a 2 mo period, beginning at the end of February, peaking during mid-March and tailing off in early April. These observations are further strengthened by the occurrence of pentactula larvae of *A. lefevrei* on settlement panels at the study location in late March

and early April (Costelloe, 1983). The eggs, before release, are generally spherical in shape and are surrounded by a striated gelatinous coat. They measure from 250 to 340 µm in diameter, with the gelatinous coat varying from 10 to 20 µm in thickness. After release, they are often flattened along the animal-vegetal axis and measure from 400 to 650 µm on the long and from 400 to 600 µm across the short axis, with a 30 to 40 µm gelatinous coat (Fig. 2J).

#### Physical factors

Surface and bottom readings were taken at the collection site for salinity, dissolved oxygen and temperature over the study period. Surface salinities were rather erratic throughout the year, ranging from 13 to 33.5‰, while those on the bottom (15 m) remained between 32 to 34‰ (Fig. 4b). Annual dissolved oxygen values showed a reverse pattern to that of temperature. The highest oxygen concentrations on the surface and the bottom (15 m) (7.5 and 8.0 ml l<sup>-1</sup>, respectively) occurred during the cold reproductive months, while the lowest (4.5 and 6.0 ml l<sup>-1</sup>, respectively) occurred during the warm months (Fig. 4C). Surface water temperatures ranged from a high of 16°C in the summer to a low of 6°C in the winter, while that of the bottom (15 m) ranged from a mid-summer high of 15°C to a winter low of 6°C (Fig. 4A). Low temperatures combined with a relatively high oxygen concentration were features of the highly productive gametogenic stages.

#### Discussion

Although *Aslia lefevrei* is dioecious, there is no sexual dimorphism and sex is only readily determined from squash mounts, histological preparations and/or the condition of the gonad at a late stage of development. Similarly, Colwin (1948) found no correlation between sex and size, colour, general appearance or behaviour of the dendrochirote *Thyone briareus* (Lesseur). Within the *A. lefevrei* population at Blackrock Reef, no evidence was found for either simultaneous or sequential hermaphroditism. Individuals generally initiated new sex-cell growth for the coming year prior to the release of the mature gametes. This would indicate that there is no change in sexuality from year to year. Tanaka (1958) reported similar primary oocytes in the shedding-stage gonadal tubules of *Stichopus japonicus*.

The morphology and ultrastructure of the gonad of *Aslia lefevrei* resembles closely that of other holothurian species (Atwood, 1974; Green, 1978; Engstrom, 1980). Its gonadal tubules were found to vary greatly in number (100 to 800) and this variation was not related to the size of the individual. Orton (1914) discovered a similar range in tubule numbers for *A. lefevrei* and found that individual tubules can measure up to 3 cm in length and 0.1 cm in diameter. In addition to the quadripartite condition described for the gonad wall of *A. lefevrei* and many other



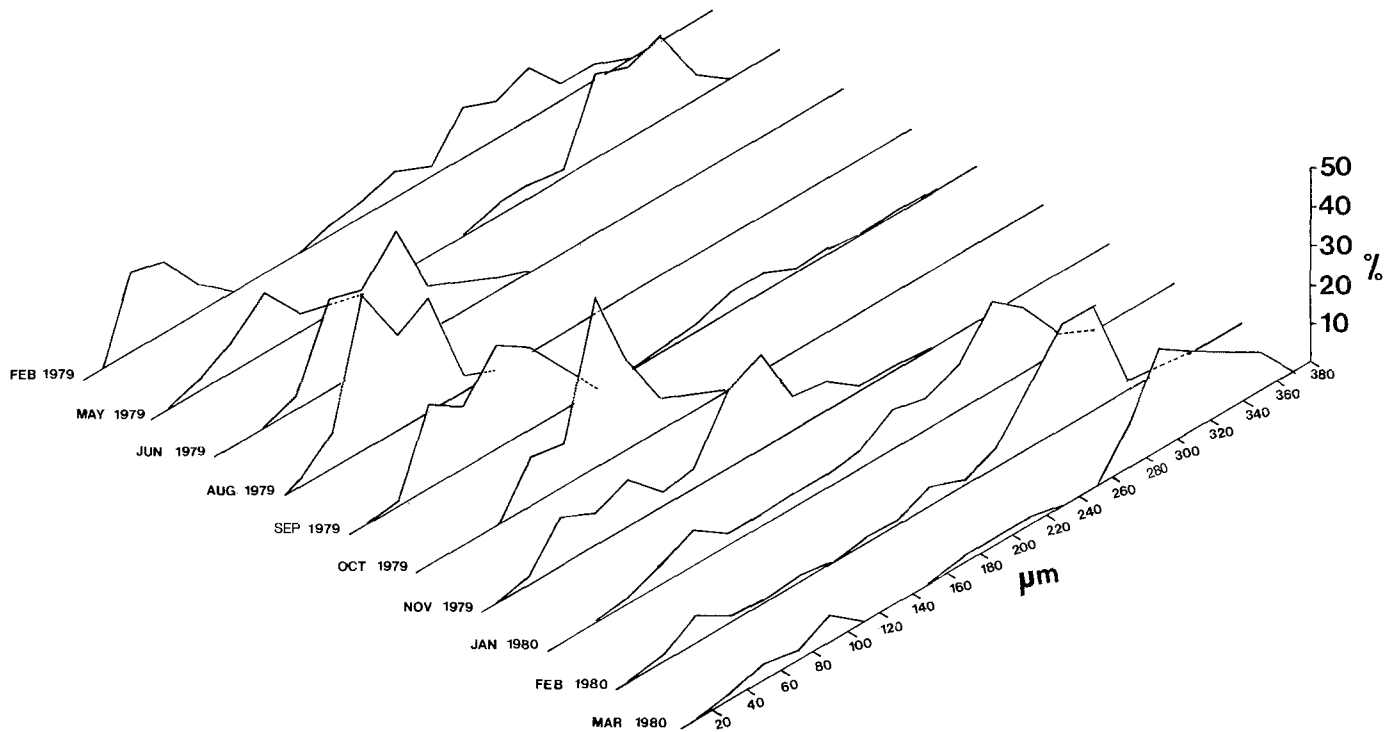


Fig. 7. *Aslia lefevrei*. Histograms of oocyte size-frequencies grouped in 20 µm size-classes

Table 1. *Aslia lefevrei*. Total number of oocytes (N). Mean diameters ( $\bar{x}$ ) and standard deviations (SD) are shown for each cohort in each sample

Date	Cohort	N	$\bar{x}$ (µm)	± SD (µm)
2 Feb. 1979	1	55	235	41
	2	15	44	9.5
30 May 1979	1	66	295	25.5
	2	21	65	19.5
28 June 1979	2	63	103	27.5
8 Aug. 1979	2	56	71	22
13 Sep. 1979	2	132	103	57.5
16 Oct. 1979	2	155	111.5	22.5
12 Nov. 1979	2	103	135	50.5
9 Jan. 1970	2	197	225.5	48
10 Feb. 1980	2	98	235	32.5
	3	15	60	24
15 Mar. 1980	2	89	269.5	30.5
	3	21	71.5	21.5

holothurian species (Green, 1978; Engstrom, 1982), Tyler and Gage (1983) indicated the presence of an amorphous cellular layer surrounding the gonad tubules of the deep-sea holothurian *Ypsilothuria talismani*. They suggested that this layer may contain a protein-carbohydrate complex used as a storage material. Histological sections of the gonad wall of *A. lefevrei* give no indication of a similar amorphous layer surrounding the tubules. As

gametogenesis progresses, however, both the germinal layer and the coelomic epithelium become greatly reduced in thickness. This gradual reduction in tissue occurs during the cooler winter months when the holothurian both reduces its feeding activity (Costelloe and Keegan, 1984 b) and is undergoing greatest gametogenic activity. In addition to an input to the energetics of gamete production, the gonad wall may have an additional function as a storage site of reserves for general metabolic activity. Smith (1981), working with *Neopentadactyla mixta* (Östergren), found a similar reduction of tissues in the gonad and showed a substantial loss in gonad weight over the winter period with a concomitant loss of lipid.

Tanaka (1958) reported a 50-fold increase in the gonad index of *Stichopus japonicus*, while Krishnaswamy and Krishnan (1967) and Rutherford (1973) found a ten- and eight-fold increase in the gonad indices of *Holothuria scabra* Jaeger and *Cucumaria pseudocurata* Cowles, respectively. The smaller three-fold increase in the index values of *Aslia lefevrei* may be due to the presence of residual sperm and ova in the gonad tubules after spawning and the subsequent rapid increase in volume of the gonad walls. This would give relatively high gonad dry weights, thereby maintaining low ratios between body and gonad dry weights.

During the post-spawned stage, nutritive phagocytes help to break down the residual sperm and ova in the gonads. Engstrom (1982) found similar assimilation processes in *Holothuria floridana* and *H. mexicana*. Pearse (1969) suggests that the phagocytic cells also have a role in destroying gametogenetic cells that form out of season and facilitate nutrient transfer in the echinoids *Prionocidaris*

*baculosa* (Lamarck) and *Lovenia elongata* (Gray). This activity may explain the decrease in the average oocyte diameter at the end of June for *Aslia lefevrei*. Gonad indices also showed a decrease after an initial recovery in August. In many urchins, an initial increase in the gonad index values results from nutrient accumulation, and gamete production begins only after adequate nutrient reserves have been established (Pearse, 1969; Gonor, 1973). The fluctuations in the gonad index values of *A. lefevrei* are not reflected in the maturity index values and may well be explained by nutrient production and assimilation in the gonad during gametogenesis.

The reproductive activities of echinoderms are regulated through endogenous pathways that are, in turn, effected by exogenous environmental factors. Environmental stimuli that may influence sexual cycles directly or indirectly include parameters such as changing temperature, salinity, photoperiod, food and habitat availability, current regime, and juvenile recruitment and survival. Annual reproductive cycles and their controlling factors have been investigated for many holothurian species (Tanaka, 1958; Krishnaswamy and Krishnan, 1967; Green, 1978; Engstrom, 1980; Conand, 1981, 1982). Low temperatures combined with relatively high oxygen concentrations occur during the highly productive gametogenic stages in *Aslia lefevrei*. Oocyte size-diameters showed little growth in the warm summer months when *A. lefevrei* feeds continuously over the 24 h period (Costelloe, 1983). However, gonad indices showed greatest increase over this period reaching a peak in November 1980 at a time when the holothuroid reduces its feeding activity (Costelloe and Keegan, 1984 b). This would suggest that *A. lefevrei* undergoes greatest nutrient build-up over the warmer months and relies on these food reserves for general metabolism and gametogenic activity during the winter. This is further indicated by the resorption of many ova, the reduction in volume of the gonad wall and the drop in gonad index values after November without apparent influence on the maturity indices.

For the most part, the gametogenic stages of *Aslia lefevrei* are synchronous, with spawning occurring in male and female individuals from the end of February to the beginning of April. Engstrom (1980), however, reported different gametogenic stages in different gonadal tubules of *Holothuria mexicana* and *H. floridana*, despite the occurrence of a discrete spawning period during the warm months.

The mechanism of spawning has been described for many holothurians with planktotrophic eggs, and varies from a passive slow release from the genital pore (Engstrom, 1980) to a forceful release of eggs from the genital papilla (Nyholm, 1952). Mosher (1982) reported that *Holothuria mexicana* may also raise its anterior end vertically to assist gamete release and some individuals, especially females, weave and writhe to assist spawning. *Aslia lefevrei* was also seen to raise its anterior end vertically during spawning but, unlike the spawning behaviour described by Colwin (1948) and Chia and Bucha-

nan (1969) for other dendrochirote species, it keeps its large dendritic tentacles close together during spawning. In addition, it wafts the tentacles and anterior region to and fro, thereby assisting gamete dispersion.

A variety of environmental factors, including temperature (Tanaka, 1958), light (Conand, 1982), salinity (Krishnaswamy and Krishnan, 1967), current and crowded conditions (Engstrom, 1980), have been reported to influence holothurian spawning. Evidence would seem to indicate that the spawning of *Aslia lefevrei* is influenced by both temperature and light intensity. Laboratory experiments suggest that low light conditions and shock-increase in sea-water temperatures stimulate spawning. Whether or not sudden changes in temperature or certain temperature conditions are required for spawning is uncertain. Ambient temperature changes at the study location did not show any sudden changes in values over the peak spawning period, but did show an increase in values from the earlier cooler winter months over this period. Engstrom (1982) was of the opinion that spawning of *Cucumaria lubrica* Clark at dusk or in the dark may be a response to minimise predation effects on released gametes. Although no evidence was found, this may also be the case for *A. lefevrei*.

Fertilization of released eggs, which measure from 400 to 650  $\mu\text{m}$  on the long and from 400 to 600  $\mu\text{m}$  across the short axis, with a 30 to 40  $\mu\text{m}$  gelatinous coat, occurs in the water column and the embryos undergo indirect development. Recent work by Rutherford (1973), Green (1978), Engstrom (1980) and Tyler and Gage (1983) have discussed at length the implications of egg size and reproductive strategies for many holothurians. In the case of *Aslia lefevrei*, the significance of oocyte diameter in relation to reproductive strategy will be discussed in detail in a further paper.

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