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The annual reproductive cycle of *Achaetobonellia maculata* Fisher (Echiura : Bonellidae)

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Abstract. This study is the first to detail the annual reproductive cycle of any echiuran. Here the annual reproductive cycle of *Achaetobonellia maculata* Fisher is described. Oocytes first appear in the coelomic fluid in late spring or early summer. During fall and winter, gametes production and differentiation continue. Differentiation of gametes lasts four to six months. Spawning occurs in spring. Since the males are the permanent residents in the gonoduct of the female, the fertilization is internal in Bonellidae. Temperature of the sea water probably is the most important exogenous factor controlling the reproductive cycle. Individuals reach sexual maturity when they are one year old.

Keywords. Reproductive cycle ; spawning ; gonoduct ; accessory cells ; oocyte.

1. Introduction

Little is known about the annual reproductive cycle in Echiura. Although some investigators (Hiraiwa and Kawamura 1936 ; Newby 1940) have reported *Urechis caupo* to be fertile throughout the year (with the exception of one or two months in the summer) this appears to be based solely on general observations but no firm data exist.

A general description of reproduction of Echiura has been provided by Gould-Somero (1975), and Singhal and DattaGupta (1982). Gould-Somero (1975) mentioned that the fertilization is internal in Bonellidae, but the males are already permanent residents in the male-sac of the female gonoduct. In other Echiurans fertilization is external but we do not know what (if any) factors ensure simultaneous spawning by males and females in neighbouring burrows. Partially or completely spawned-out *U. caupo* has been collected in late summer (Ricketts and Calvin 1962 ; Gould 1967), and animals will sometimes spawn in the laboratory if the water temperature is raised above 15° C. Therefore, temperature may be a factor. Pilger (1977) studied the annual reproductive cycle in *Listriolobus pelodes*. He found that ovulation lasts three to five months and spawning takes place in spring. Singhal and DattaGupta (1982) reported that oocytes were present

in the coelom of *Achaetobonellia maculata* and *Acanthobonellia vulgaris* for about nine months in a year. This study included a determination of the size at which *A. maculata* becomes sexually mature, the structure of the gonads, the development of the reproductive cells in the coelom and the time, durations and geographical variation in spawning.

2. Materials and methods

A. maculata is a common species of the Pirotan Island, Gulf of Kutch (DattaGupta and Singhal 1978). Since its discovery by Fisher (1953) from the central lagoon of Onotoa, Gilbert Island, the species has not been reported from anywhere except from the aforesaid locality. The population of *A. maculata* from Pirotan Island was studied for its reproductive cycle during 1976 and 1978. Specimens were collected and fixed every month using the method described by Singhal and DattaGupta (1980). Gonads were fixed in Gilson's fluid sectioned at 8 μ and stained with Delafield's hematoxylin and eosin.

In *A. maculata* the young oocytes are associated with a complex of accessory cells during the first stage of development. Later they lose these cells and continue to develop without them. Oocytes with accessory cells will be referred to as "stage I" and those without them as "stage II". To determine the cycle, a sample of coelomic fluid was withdrawn with a syringe from each of five females for each month. Twenty oocytes of each stage were measured for their diameter in each female making a total of 100 for each stage per month. The means of stage I and stage II were adjusted according to the percentage contribution of each stage to the coelomic gamete population. This was accomplished by calculating the mean weighted diameter for each month using the formula :

PSIn $\bar{X}$ SIn + PSIIn $\bar{X}$ SIIn = mean weighted diameter where PSIn and PSIIn are the percentage of stage I and stage II oocytes respectively in the coelom of five female *A. maculata*, during the month n. The values $\bar{X}$SIn and $\bar{X}$SIIn are the mean diameters for the stage I and stage II oocytes during the same month.

As another measure of reproductive periodicity, the concentration of stage I and stage II oocytes within the coelom was determined. To accomplish this, a small sample of coelomic fluid was removed. After fixation and during storage, individual *A. maculata* tend to lose coelomic fluid by diffusion through the body wall and appear deflated although the actual volume it can contain remains unchanged. The net result of this is that the coelomic cells are more concentrated than under normal conditions. To remedy this situation, the individuals were "inflated" with 70% isopropyl alcohol until they reached a subjectively determined uniform tension. Each specimen was shaken to mix the coelomic cells before removal of the sample. The sample then was diluted by an equal volume of 70% isopropyl alcohol and the concentration of each gamete type was determined using a hemocytometer. Ten values were obtained from each of the five females every month from September 1976 to December 1978.

The reproductive cycles were analyzed with several environmental parameters using a multiple regression analysis program. This program is part of the Statistical Analysis System (SAS) and was developed by Barr *et al* (1976). Also from

SAS, Backward Elimination and Maximum R^2 Improvement variable selection procedures were used to determine which, if any, of the parameters contribute significantly to the cycle. Coelomic gamete concentration and weighted gamete diameter are handled separately as the dependent variables. The independent variables include DDT, cadmium, organic nitrogen, sulphide, and bottom temperature.

3. Results

The differentiation of gametes consists of three distinct phases. The gametes begin their development while attached to the gonads. They are in the second phase when they break loose and continue their growth floating freely within the coelom. Finally mature gametes are collected and stored in the gonoducts until spawning. The structure of the gonads of *A. maculata* has already been described (Singhal and DattaGupta 1982).

3.1. *Size at sexual maturity*

The smallest sexually mature female specimen found weighted 2·0 g and measured 25 mm long from mouth to anus. In a sample of 100 female individuals, none weighing less than 2·0 g was sexually mature. Since the male is a permanent resident of the male-sac of the gonoduct of the female, the weight of the female specimen also includes the weight of the male and it cannot be determined as to what is the smallest size and weight of the male for sexual maturity.

3.2. *Coelomic oocyte diameters*

The mean diameters of stage I and stage II coelomic oocytes are shown in figure 1. Each point represents the mean of 100 measurements and the solid bar equals one standard deviation. The smallest stage I oocytes are present in the coelom during the summer months. These cells are 5–7 μm in diameter. The mean diameter of these oocytes begins to increase during the early fall and by November has reached 22 μm. Since this is a mean value, it does not indicate the upper size limit of stage I oocytes. The actual size of an oocyte when it loses its accessory cells is 40 to 42 μm in live material. A mean diameter of app. 22 μm is maintained until late spring when it begins to decrease. This decrease is due to the transformation of large stage I oocytes into stage II.

Stage II oocytes appear in November at the time when the stage I oocytes first reach their maximum mean diameter. The mean size of stage II oocytes increases through the spring. By June, all of the stage II oocytes have been collected from the coelom by the gonostome and accumulated in the egg-sac of the gonoduct. The actual size of an oocyte when it is removed from the coelom is 60–62 μm stage II oocytes are not present in the coelom again until fall.

Also shown in figure 1 is the weighted average diameter of stage I and stage II oocytes combined. An annual cycle clearly is seen in this representation. Small oocytes first appear in the summer. Most of their growth takes place during the fall and winter months.

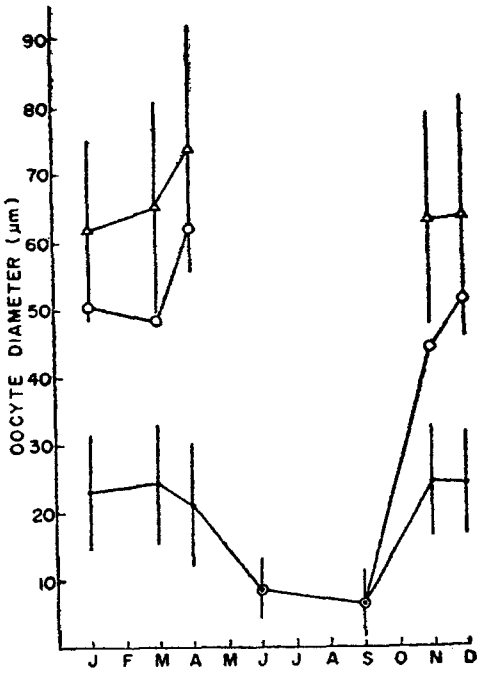

Figure 1. The mean diameter of coelomic oocytes in *A. maculata*. The bar equals ± 1 standard deviation. ● = Stage I oocytes ; △ = Stage II oocytes ; O = weighted mean diameter of stage I and stage II oocytes combined.

The diameters of fixed oocytes differ from those of live oocytes. This was tested by measuring the diameters of 100 live oocytes and 100 fixed oocytes from the same individual and comparing their means. The results show that there is less than a 3% increase in the average diameter after fixation. Since relative values are more important than absolute values, the increase is considered insignificant.

3.3. *Coelomic oocyte concentration*

Figure 2 indicates the concentration of coelomic oocytes. During spring and first half of the summer stage I oocytes are at their lowest concentration (2·2-3·5/mm^3). In specimens collected in July and August, stage I oocytes concentration begins to increase. By September, the concentration has increased four-fold. By November, stage I oocytes reach their highest concentration and become stage II oocytes by the loss of the accessory cells. As more and more stage I oocytes reach this point their concentration slowly decreases, reaching the lowest level again in summer. Oocytes first appear in November and rapidly reach maximum concentration. From December through spring their concentration declines steadily as they are accumulated in the gonoduct. By June, stage II oocytes are not present in the coelomic phase at all.

These data illustrate the same reproductive cycle as do the oocyte diameter data. During the summer, few coelomic gametes are present. Their number increase through the fall and early winter. During late winter and spring they are collected in the storage organ until spawning. Spawning apparently extended from spring until the end of winter.

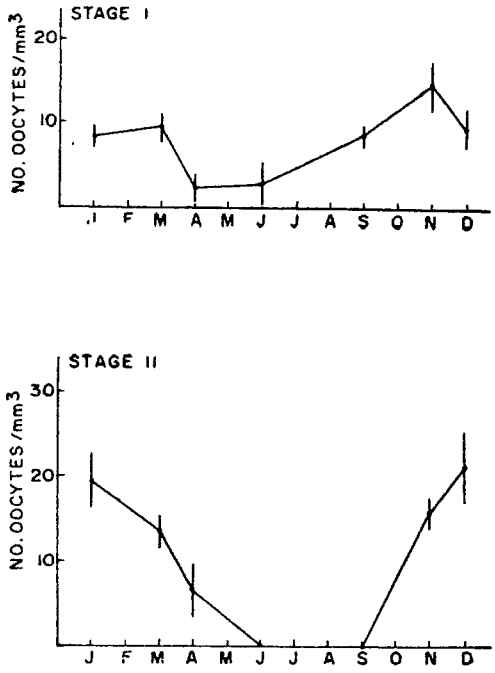

Figure 2. The mean concentration of stage I and stage II coelomic oocytes in *A. maculata*. The bar equals 95% confidence interval.

3.4. *Regression analysis of abiotic parameters with the reproductive cycle*

By regressing the abiotic data from Pirotan Island (DDT, cadmium, organic nitrogen, sulphide, and bottom temperature) against the weighted mean oocyte diameter, it was determined that the environmental parameters did not account significantly for the variation of oocyte size ($P < 0{\cdot}05$). However, temperature, nickle and sulphides accounted for a significant amount of the oocyte size variation during the study period ($R^2 = 0{\cdot}78$, $P < 0{\cdot}01$). Both Backward Elimination and Maximum R^2 Improvement techniques generated the same three-variable model that accounts for 92% of the variation in oocyte diameter over time ($R^2 = 0{\cdot}92$, $P < 0{\cdot}001$). The independent variables selected by these procedures are in order of decreasing importance, bottom water temperature ($P < 0{\cdot}01$), concentrations of nickle ($P < 0{\cdot}01$) and sulphides ($P < 0{\cdot}05$) in the sediment. Adding the remaining independent variables does not significantly improve the predictability of the model.

Regressing all of the independent variables (environmental parameters) against the mean coelomic oocyte concentrations showed no significant contribution ($R^2 = 0{\cdot}85$, $P < 0{\cdot}05$). By variable selection methods, however, it was found that two parameters contribute nearly 81% of the variation ($R^2 = 0{\cdot}79$, $P < 0{\cdot}01$). These are in order of decreasing importance, the concentrations of sulphide ($P < 0{\cdot}01$) and DDT ($P < 0{\cdot}05$). Thus, only a few of the parameters measured are important in determining the number of oocytes produced.

4. Discussion

Unfortunately there is still insufficient information available on the annual reproductive cycles of Echiura to determine how typical the cycle of *A. maculata* is. The smallest sexually mature *A. maculata* encountered in this study is one year old female, 25 mm long, weighing 2·0 g. This is reasonably consistent with the observation of Fisher (1946) who found a 7 mm mature specimen. Baltzer (1931) reported that females of *Bonellia viridis* require two years to reach sexual maturity while the males mature in one or two weeks. *U. caupo* also requires one year to reach sexual maturity.

No studies of annual reproductive cycles in echiurans are available for comparison. However, seasonal gamete production has been reported in the echiurans *Ikedosoma gogshimense* (May and June) (Sawada and Ochi 1962) and *U. unicinctus* (Winter) (Hiraiwa and Kawamura 1936). In direct contrast to this, *U. caupo* produces gametes continuously and contains all oocyte sizes in the coelom simultaneously (Gould-Somero 1975).

The dynamics of oocyte development including the transition from stage I to stage II have been illustrated in diameter frequency polygons. Because *A. maculata* does not produce gametes continuously throughout the year, the frequency of the various oocyte size classes is not proportional to the amount of time the oocyte spends in a particular size class as has been suggested for *U. caupo* (Gould-Somero 1975).

Although the frequency polygons do not provide direct information as to the time course of oogenesis, a rough estimate can be made based on the distribution of the mean diameter of stage I and stage II oocytes over time. The duration of stage I can be estimated by determining the time interval between the onset of increase of stage I mean diameter, which occurs in summer, and the first appearance of stage II oocytes. For instance, at Pirotan Island, after summer the mean diameter of stage I oocyte of *A. maculata* began to increase one month later, stage I oocytes had grown to 22 μm and had become stage II. Thus, it is predicted that stage I lasts from one to two months.

The period of time from the initial appearance of stage II oocytes in the coelom until they reach their maximum diameter provides an estimate of the duration of this phase of growth. Stage II oocytes appeared in coelom after being absent over the summer and reach their maximum diameter after two months, indicating a two-month period of differentiation. Based on the data available for summer, the duration of stage II differentiation is estimated to be $1\frac{1}{2}$ months ($\pm \frac{1}{2}$ month). These data predict, therefore, that stage II lasts from one to two months.

Combining the estimate for stage I and stage II oocyte differentiation gives a range for the time course of oogenesis of three to five months.

Das (1976) has studied the cytochemical and biochemical processes of oogenesis in *Urechis*. By radioactive labelling he has determined that the duration of the period of oocyte differentiation is 135 days. This closely resembles the estimate for *A. maculata* derived from the reproductive cycle data.

Based on the oocyte diameter data, spawning among *A. maculata* population occurred during spring. The data show that the exact time of spawning and the

length of the period preceding resumption of oocyte growth can vary from year to year (Giese 1959a).

The regression analysis demonstrates that temperature plays an important part in determining the gametogenic cycle. Orton's Rule first proposed by Thorson (1946) states that sea temperature is related to the reproductive cycles of marine organisms. While this is important to many animals, other exogenous and endogenous factors may also play vital roles (Giese 1959b ; Giese and Pearse 1974). Controlled laboratory experiments are necessary to define the environmental components essential for determining any gametogenic cycle (Giese and Pearse 1974).

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