Annls Limnol. 32 (2) 1996 : 105-113

Determination of reproduction cycle in a population of *Egeria radiata* (Lam 1804) (Bivalvia: Donacidae) using condition index and histological examination of gonads

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Keywords : Bivalve, *Egeria radiata*, gametogenesis, condition index, Cross River, Nigeria

This study employs histological procedure to elucidate the natural cycles of gonadal development in a population of *E. radiata* in the Cross River, Nigeria. The cycles of condition indices and shell-free dry mass follow a general pattern in which they increased with the sexual maturity of the species till a peak is reached in June when spawning begins. Then there is a gradual decline, as the spawning event progresses, till a minimum is obtained in October when the animal is spent. Thus, the species spawns once in a year during the peak of the rainy season (June to October). The timing of spawning is such that the young larvae stand to profit from the high plankton biomass during the ensuing dry season months.

Détermination du cycle de reproduction d'une population de *Egeria radiata* (Lam 1804) (Bivalva : Donacidae) à l'aide de l'indice de condition corporelle et de l'examen histologique des gonades

Mots clés : Bivalve, *Egeria radiata*, gamétogénèse, indice de condition corporelle, rivière Cross, Nigeria

Cette étude utilise des procédés histologiques pour connaître les cycles de développement des gonades chez une population de *E. radiata* dans la rivière Cross (Nigeria). Les variations des indices de condition corporelle et du poids sec (sans la coquille) des individus suivent un cycle général : ils augmentent lors de la maturation sexuelle de l'espèce jusqu'à un pic atteint en juin lorsque commence la ponte. Puis il y a un déclin progressif tant que dure la ponte jusqu'à un minimum obtenu en octobre lorsque l'animal est épuisé. Ainsi, l'espèce pond une fois l'an pendant le maximum de la saison des pluies (juin à octobre). La période de ponte est telle que les jeunes larves peuvent profiter de la biomasse élevée de plancton pendant les mois de la saison sèche qui suit.

1. Introduction

There are several methods for determining the reproductive season and spawning cycle in bivalves. Some of these are (a) the direct observation of spawning in a laboratory population, or the indirect methods of making inferences from: (b) time of year that larvae appear in plankton (Muus 1973), (c) time of year that juveniles first appear or are most abundant in bottom samples (Britton & Morton 1982), (d) time of year that glochidia are most abundant on fish host (Giusti et al 1975), (e) seasonal changes in the percentage of females incubating larvae within demibranchs (Dudgeon & Morton 1983), (f) seasonal cycle of biochemical composition (Ansell 1974, Etim 1993), (g) temporal variation in condition index and weight of body tissue (Lutz et al 1980, Etim & Taege 1993), (h) seasonal variation in condition index of gonad (Murakoshi & Kawagiti 1986), (i) the recruitment pattern obtained by backward projection of the length frequency data set onto a one-year time scale (Pauly 1987), (j) microscopic examination of gonad smears (Britton & Morton 1982), (k) microscopic studies of the histological preparations of gonadal sections (Dudgeon & Morton 1983).

The observation of spawning in field populations should provide the most reliable approach to the elucidation of natural reproduction, but in practice such da-

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Article available at http://www.limnology-journal.org or http://dx.doi.org/10.1051/limn/1996007

ta are difficult to obtain (Seed 1976). Observation of spawning in laboratory conditions are of limited value and could even lead to spurious conclusions concerning spawning in natural habitat due to the impossibility of simulating the natural condition in laboratory. This holds true whether the laboratory population is «naturally» made or experimentally induced to spawn. Additionally, most of the conditions (e.g. electric current) that could induce spawning in a laboratory population are never found in the wild.

The use of larval appearance in plankton could only be applied to families with veliger (= the free swimming larval stage with ciliated velar lobes employed in swimming and feeding) e.g. in the Order Arcoida is Arcidea, in the Order Mytiloida is Mytilidae, in the Order Veneroida are Dreissenididae, Selenidae, Donacidae and Solecurtidae; in the Order Myoida are Corbiculidae, Erodonidae, Terenidae and in the subclass Anomalodesmata is Lyosiidae. Checking for juveniles in bottom samples is suitable to families with direct development i.e. the juveniles are released directly from the females with no veliger or parasitic form. In the Order Veneroida this is exemplified by the families Corbiculidae and Sphaeriidae. The method of sampling for glochidia (= bivalve larvae which are parasitic on fish) on fish host is suitable only to species with glochidial developmental stages e.g. in the Order Unionida are Unionidae, Margaritiferidae, and Hyriidae.

A major objection to the indirect method of studying the abundance of larval plankton or juveniles in bottom samples is that there is no guarantee that those larvae or spat were spawned in the same locality; they could have been carried from considerable distance by water currents from localities having quite different hydrographic conditions (Seed 1976). Same is true of studying glochidia on fish host; the fish host could have migrated with parasitic larvae from other localities. Another shortcoming of studying bivalve spawning from sampling for larval abundance in plankton is that larval abundance, as was found out in *Anomalocardia squamosa* Lam, could indicate a continuous pattern of larval settlement in a year; giving the impression of a once-a-year spawning season, while histological studies of gonad reveals more accurately a twice-a-year breeding cycle (Morton 1979). Studies of histological preparations and microscopic examination of histological sections of gonad is undisputably the most reliable procedure for elucidating reproductive cycle of bivalve molluscs (Seed 1976, Britton & Morton 1982, Nott 1980). However, since animals are sacrificed, continuous observations can not be made on the same individual.

This study employs condition indices and histological procedure to elucidate the natural cycles of gonadal development in a population of *E. radiata* in the Cross River system, Nigeria. Understanding the reproductive biology of a species is central to the proper management and conservation of the stock, and a prerequisite for its successful cultivation.

2. Material and methods

Each month about ten large specimens (with lengths > 7.00 cm) of *E. radiata* were obtained from the Cross River at Itu (≈ Long. 8° 03" E., Lat. 5° 10" N.). The hydrology and limnology of this water body has been documented (Moses 1979, Etim 1994 and several other references therein). The animals with their shell valves forced opened were transported immersed in formaldehyde solution. In the laboratory, the soft tissue was removed from the shell; a section was cut along the dorso-ventral axis through the anterior end of the gonad close to the anterior retractor muscle. These gonad slivers were fixed in formalin, dehydrated by transferring through graded concentrations of alcohol, cleared in chloroform/xylene mixture, impregnated with paraffin wax at 60° C, embedded and blocked in solid paraffin, trimmed with a knife, cut with a rotary microtome at 5 microns. Sections were then mounted on microscope slides, stained with hematoxyline and counterstained with eosin (see details in Howard & Smith 1983).

Slides were examined with light microscope; first under low power to scan the entire gonadal area, then under high power to assess each follicle. Examination of the slides in this way made it quite possible to device a gametogenic index for the species. Photomicrographs of the sections were made. Using method of Britton & Morton (1982) ctenidial smears were also made and examined under the microscope.

In another but related experiment, monthly samples (10 to 20 specimens with lengths between 7.00 to 7.30 cm) were also obtained and the length (maximum anterior-posterior dimension) of each specimen measured with a pair of callipers. Total mass, shell free wet mass, shell-free dry mass (oven-dried at 60° C for 72 hours) were taken and the internal shell volume determined (by noting the quantity of water from a burette needed to fill each of the shell valves). Ash content of dried body tissue was determined as the residue left after burning the sample in the muffle furnace at 500° C for 12 hours. The condition indices of each specimen was then computed as (a) Ash-free dry body mass/Shell mass, (b) Shell-free wet body mass/Total mass; (c) Ash-free dry body mass/(Length)^3, (d) 100

(Shell-free dry body mass)/ Shell mass, (e) 100 (Shell-free dry mass)/ Shell volume.

The monthly mean of each condition index were then plotted against time (month of collection) to obtain a pattern indicative of their seasonal variation.

3. Results

Except for the areas occupied by the muscular and digestive systems, most of the visceral mass is taken up by reproductive system. The gonad is beige in colour when unriped (November to June) but slightly creamy when riped (August to October). The stomach is located a bit to the anterior end of the visceral mass and is covered anteriorly and dorsally by a mass of dark green digestive diverticular. A translucent crystalline style extends into the floor of the stomach from the conjoined crystalline sac and the midgut. The whole gut is covered by the gonad and its associated connective tissues. In the few hermaphrodite individuals seen, the ovaries were located in the anterio-posterior region of the visceral mass, while the testes were located in the posterio-ventral region.

The hematoxylin/eosin staining rendered the cytoplasmic materials pink and the nuclei violet, thus facilitating the categorisation of the following gametogenic stages.

Inactive phase. The inactive (Manzi et al 1985, Rosenblum & Niesen 1985) or primordial (Morton 1985) stage is characterised by gonads which are in a stage of reproductive quiescence in which follicles are tiny or at times absent. At this stage it was hard (albeit not impossible) to distinguish the male from the female.

Immature phase. In females the oocytes enlarge and grow and are not numerous. In males the primary spermatogonia are seen. They are small and uniform in size.

Mature/Spawning. The oocyte appear as round cells in the lumina of alveoli. They are large and attached by slender stalks which are not easily seen. The large oocytes fill the lumina and they are packed together. In males, masses of spermatozoa are arranged in more or less radial columns inside alveoli. The tails are oriented towards the centre. The heads are bullet-shaped.

In Nigeria, there are two climatic seasons: the rainy (April to September) and dry (October to March) seasons. This work shows that spawning in *E. radiata* occurs once in a year during the peak of the rainy season.

The seasonal patterns of variations in shell-free dry mass, percentage water and other indices of condition are shown in Fig. 1 and 2. Table 1 gives the monthly changes in developmental stages of the gonad while Fig. 3 depicts the different stages categorised.

The mean shell-free dry mass rose from a value of 3.304 g (± 0.465 g) in February 1987 at the beginning of the sampling period to a peak of 5.581 g (± 0.365 g) in June 1987. After, there was a fall in mean shell-free dry mass till a minimum value of 1.68 g (± 0.109 g) was obtained in October 1987. This same pattern repeated itself through the subsequent sampling months of 1988 and 1989. For 1988 peak value of 5.636 g (± 0.760 g) was recorded in June while a minimum value of 1.629 g (± 0.225 g) was recorded in November. For 1989 a peak value of 5.674 g (± 0.368 g) was recorded in June. At the beginning of the sampling in February

Table 1. Monthly changes in developmental stages of the gonad of *E. radiata*

Tableau 1. Modifications mensuelles des stades de développement de la gonade de *E. radiata*.

Month	Stages of gonad development	
	Male	Female
August 1988	Mature/Spawning	Mature/Spawning
September 1988	Mature /Spawning	Mature/Spawning
October 1988	Spent/Inactive	Spent/Inactive
November 1988	Spent/Inactive	Spent/Inactive
December 1988	Inactive	Inactive
January 1989	Inactive	Inactive
February 1989	Immature	Immature
March 1989	Immature	Immature
April 1989	Immature	Immature/Mature
May 1989	Immature/Mature	Immature/Mature
June 1989	Mature/Spawning	Mature/Spawning
July 1989	Mature/Spawning	Mature/Spawning
August 1989	Ripe/Spawning	Ripe/Spawning
September 1989	Mature/Spawning	Mature/Spawning

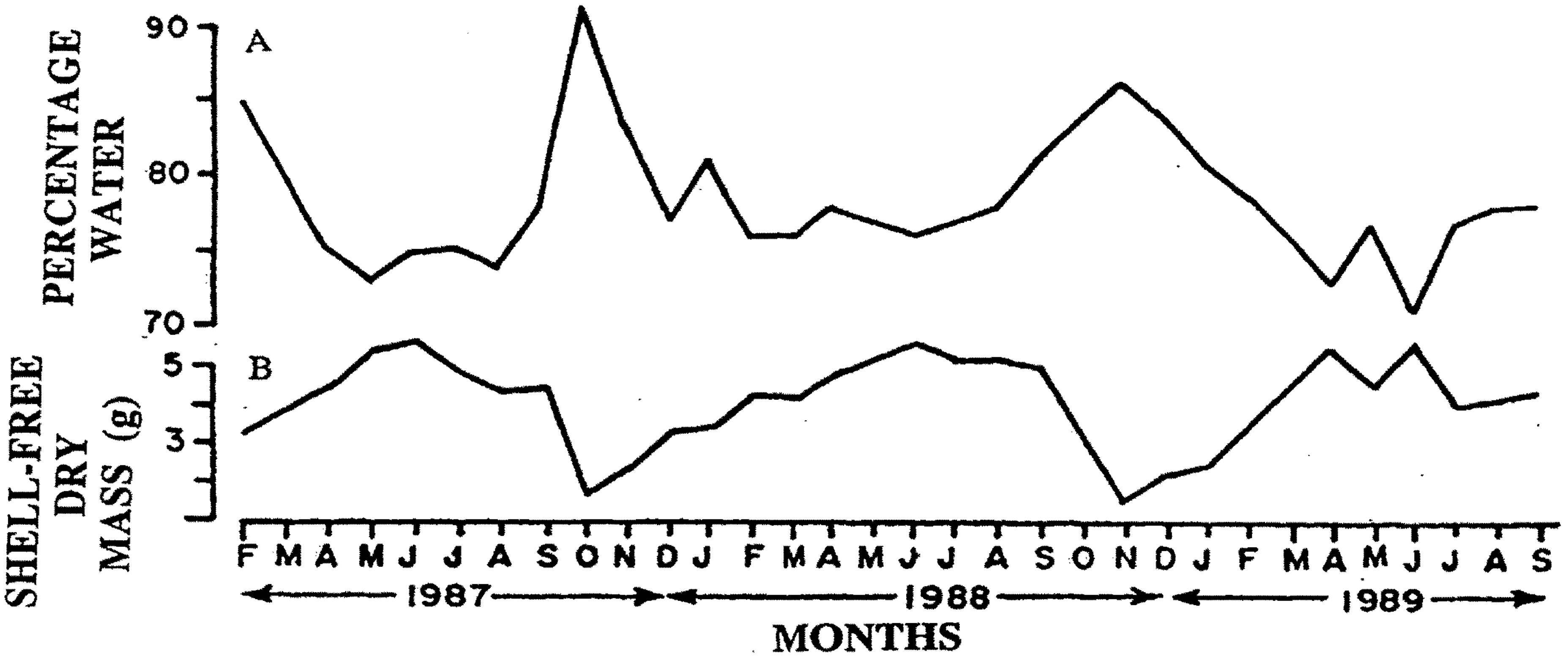

Fig. 1. *Egeria radiata* : seasonal variation in (A) Percentage water and (B) Shell-free dry mass (g)

Fig. 1. *Egeria radiata* : variation saisonnière en (A) teneur en eau et (B) poids sec sans coquille.

1987, the mean percentage water content was 84.887 % (± 1.86 %) and by May 1987 it has fallen to 73.16 % (± 0.69 %). It then rose to 90. 91 % (± 1.58 %) in October 1987. After, it declined to 76.37 % (± 1.19 %) in June 1988 with a subsequent peak value of 86.44 % (± 1.63 %) in November 1988.

4. Discussion

Extant scientific literature shows that gametogenesis in any population of *E. radiata* has not been studied before. However, there are quite a few unsubstantiated references to the spawning of the species in the Volta (Ghana) and Cross (Nigeria) rivers. Generally, these records are fragmentary and often contradictory.

Pople (1966) and Whyte (1982) stated that the population of *E. radiata* in the Volta river spawns during the dry season months when water salinity rises up to 1 ppt. Having been widely cited (e.g. in Beadle 1982 and Sastry 1979) this view has now gained currency. In this work, quite the opposite is obtained - the species in the Cross River spawns from the peak of the rainy season to the beginning of the dry season (June to October). The climatic conditions and the hydrological regimes in the Volta river is quite similar to that of the Cross River system. In which case, a similarity in the spawning season between the Volta and the Cross River populations of *E. radiata* should be expected. The contrary may well mean a lack of evidence for dry season spawning in the Volta. Lawson (1964) observed that in the Volta river marked-recaptured specimens showed reduced growth during flood months of July to December and, *ipso facto*, he concluded that the species was «reproducing at this time.» Moses (1990) thought the species should spawn during April just as he (Moses 1987) found for some finfishes of this flood river. Actually, the above mentioned reports by Pople, Whyte and Moses were more of reasoned speculations than products of empirical researches.

The present investigation reveals interesting and hitherto unknown facts about the sexuality, sexual strategy and reproductive cycle of this species. From its external morphology, the species is not sexually dimorphic, neither could the appearance of the gonad to the naked eye help in differentiating the sexes. The species is gonochöristic with few (about 3.5 %) cases of hermaphroditism. This pattern of sexuality is characteristical of most freshwater bivalves (Fretter & Graham 1964, Purchon 1977, Morton 1982). Although the need for hermaphroditism in freshwater bivalves is not known for certain (Fretter & Graham 1964), but it has been a subject of lively discussion (Dudgeon & Morton 1983, Fretter & Graham 1964, Purchon 1977, Morton 1982). The young larvae has the inert potential to develop into male or female. The suppression of one sex in favour of the other is determined by its genetic constitution under the influence of certain environ-

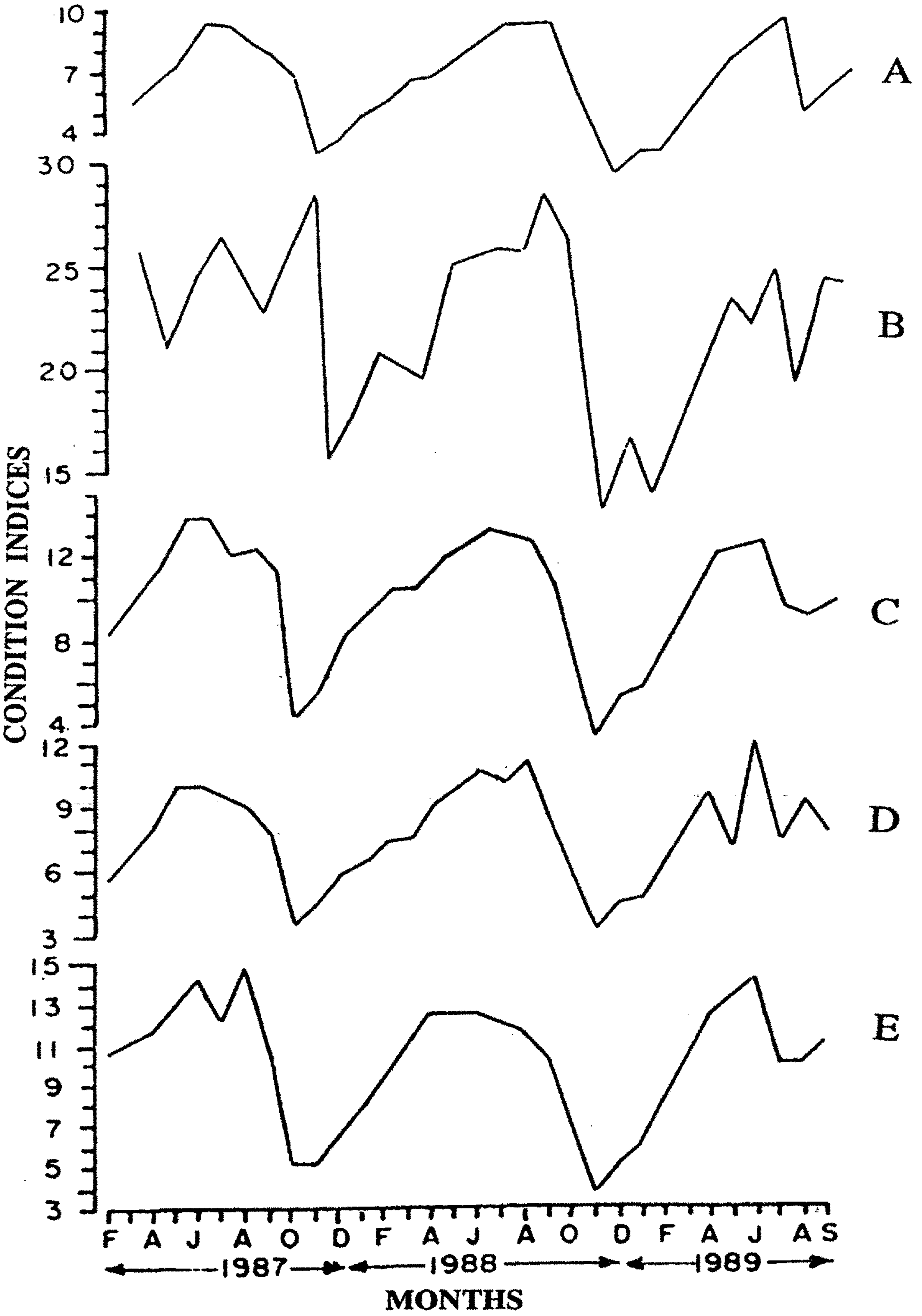

Fig. 2. *Egeria radiata* : seasonal variation in five different morphometric indices of condition (A) Ash-free dry body mass/Shell mass, (B) Shell-free wet body mass/Total mass, (C) Ash-free dry body mass/(Length)^3, (D) 100 x Shell-free dry body mass/Shell mass, (E) 100 x Shell-free dry mass/Shell volume.

Fig. 2. *Egeria radiata* : variation saisonnière de cinq indices morphométriques de condition corporelle différents (A) Poids sec du corps sans cendres/Poids coquille, (B) Poids frais du corps sans la coquille/Poids total, (C) Poids sec du corps sans cendres/(Longueur)^3, (D) Poids sec (x 100) du corps sans coquille/Poids coquille, (E) Poids sec (x 100) du corps sans coquille/Volume coquille.

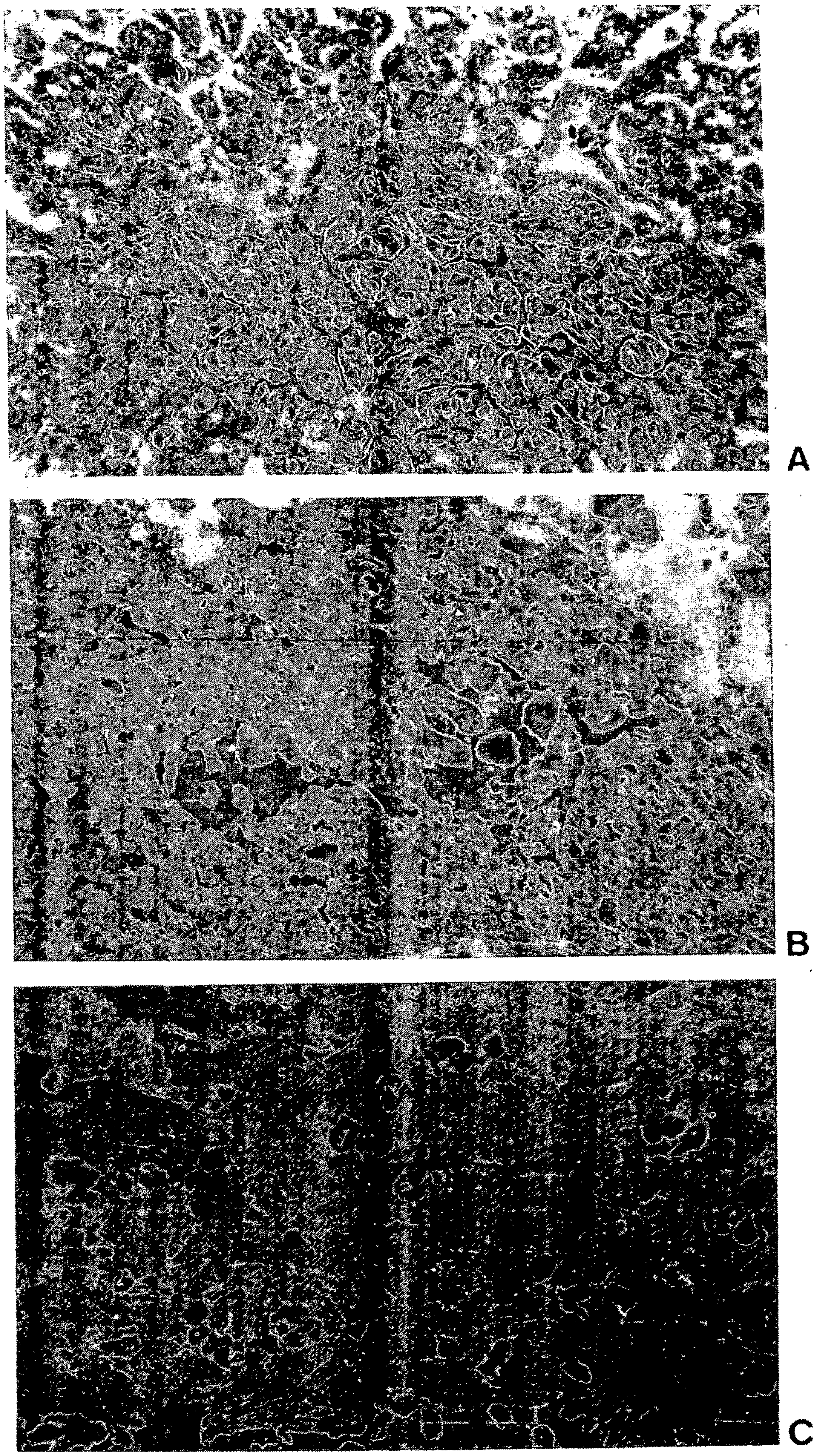

Fig. 3. *Egeria radiata* : gametogenic stages. (A). immaturing female, (B) mature female, (C) partially spent female.
Fig. 3. *Egeria radiata* : stades de gamétogénèse. (A) femelle immature, (B), femelle mature, (C) femelle en cours de ponte.

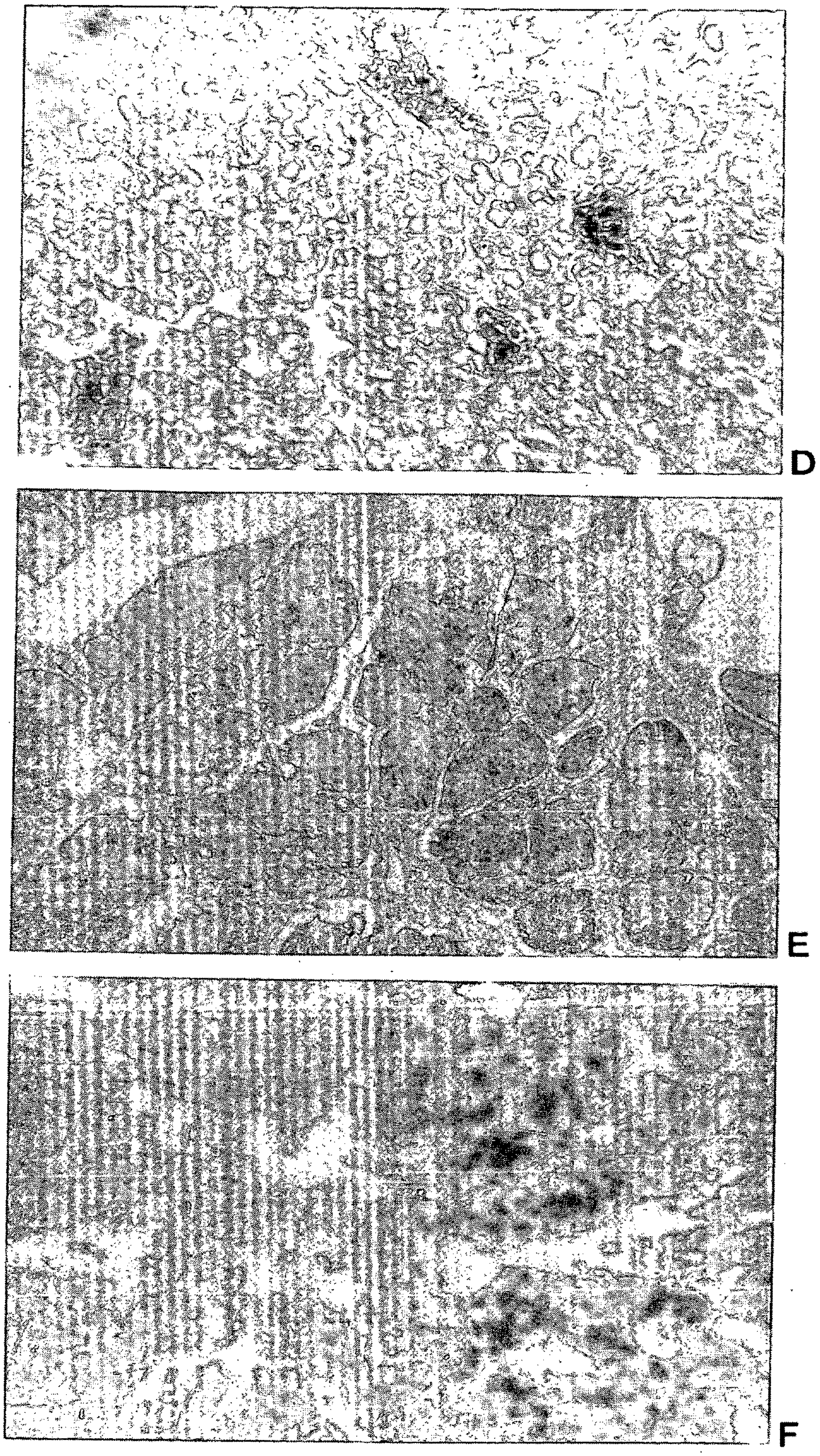

Fig. 3. *Egeria radiata* : gametogenic stages. (D) immature male, (E) mature male, (F) partially spent male.

Fig. 3. *Egeria radiata* : stades de gamétogénèse. (D), mâle immature, (E) mâle mature, (F) mâle au cours de la phase de reproduction.

mental factors. Thus, a genetic and/or environmental change could lead to a change from unisexual to hermaphroditic conditions, or vice versa. Within this context, it may be reasoned that hermaphroditism occurs especially when it proves to be of survival value. In *E. radiata,* it probably confers on some few individuals the potential of self-fertilization so that in adverse conditions they can assume responsibility for the perpetuation of the species. Such adverse conditions might occur when excessive flood results in unusually fast currents sweeping male gametes down to the sea.

For gonochoristic species, the sex ratio is such that females are usually more than males (Fretter & Graham 1964, Dudgeon & Morton 1983). Sex ratio, like expression of sexuality, is to some extent a function of habitat. For instance, the female/hermaphrodite ratio in *Anodaona cygnea* L. has been found to vary from habitat to habitat (Bloomer 1939). According to Morton (1982) *A. cygnea* is dioecious in running water but in stagnant water the percentage of hermaphrodite increases tremendously. Similarly, *Corbicula fluminea* L. is a protandric hermaphrodite in Hong Kong (its natural range) but simultaneous hermaphrodite in North America (its introduced range).

There exist distinct seasonal changes in shell-free dry mass, condition indices and percentage water content in *E. radiata* (Fig. 1). When shell-free dry mass is a maximum, the percentage water is a minimum and vice versa. Such a simultaneous increase in percentage water along with a decline in shell-free dry mass during spawning has been shown to occur in two different species of *Balanus* (Barnes et al 1963), in two different *Chlamys* species (Ansell 1974, Taylor & Venn 1979), and also in two separate *Tapes* species (Beninger & Lucas 1984) and has been interpreted to mean that the animals absorb more water to fill the lumen created in the gonad after the release of gametes (Taylor & Venn 1979). Aquatic organisms subjected to experimental period of starvation in the laboratory exhibit an increase in water content of their body. From this Beninger & Lucas (1984) reasoned that increase in water content of body after spawning is an indicator of a condition similar to nutritional deficiency.

The seasonal variation in shell-free dry mass and condition indices of bivalves in different regions of the world have been shown to follow specific patterns correlated with sexual cycle of the organism e.g. Taylor & Venn (1979) for a European , Nair & Nair (1987) for Asian , Erkom & Griffiths (1991) for African and Lutz et al (1980) for a North American species. The cycles of condition indices and dry tissue mass of *E. radiata* conforms with this general pattern in which they increase with the sexual maturity of the species till a peak is reached, and then falls as the spawning event progresses till a minimum is obtained at the end of the spawning period.

Rainfall is one climatic factor with the most profound effect on the limnology and phenology of the river system (Etim & Taege 1993). The proximate factors which stimulate spawning in the feral population of the species is the sum total of the effect of all environmental changes which result from the twin effect of rain and flooding. During the rainy/flooding season, there is an enormous amount of run-off water which carries nutrients and debris from land into the main river channels. This leads to the overflow of the river and the flooding of the adjacent plains during the peak of the rains. During this period, there is an increase in water depth at the sampling area from 4 to 12 m, a lowering of salinity from 0.25 ppt in April to 0.19 ppt in August, and a fall in water temperature from 32° C in March to 26° C in July. Peak phytoplankton biomass is found in February (about 2500 cells/ml) and May (about 2000 cells/ml) while a minimum value of about 500 cells/ml is found in October (Etim & Taege 1993). The cycles of phytoplankton biomass is such that after February and May abundance, there is a gradual decline till the lowest value is obtained in October. After, there is a rapid increase in abundance from October to February again. The increased insolation and water temperature during the dry season months together with the elevated nutrients levels that persist from the rainy season period lead to a marked increase in phytoplankton biomass during the succeeding dry season period. The species utilizes the abundant phytoplankton for tissue growth during the dry season, and by wet season spawning begins. Thus, the timing of the spawning is such that the young animals stand to profit from the increased productivity of the river during the ensuing dry season months.

Acknowledgements

I appreciate the sponsorship from the Alexander von Humboldt Foundation (AvH), Bonn , and also the support of Prof. Dr. W. Arntz and Dr. Thomas Brey as my scientific hosts. Thanks to Dr. Manfred Taege and Prof. U. K. Enyenihi (both of University of Calabar, Nigeria), and Prof. V. L. A. Yoloye (University of Ilorin, Nigeria) who read and appraised an earlier version of this manuscript in thesis form, and to M. Albert Ekanem, P. Perrara, and A. Essien for help in histological work. Author's Home Institution: Institute of Oceanography, University of Calabar, Calabar, Nigeria.

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